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Severe Acute Respiratory Syndrome Coronavirus 2 Serology Testing – A Laboratory Primer

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KEYWORDS

• SARS-CoV-2 serology • COVID-19 • Antibody laboratory testing

KEY POINTS

- There are dozens of EUA serology assays,for SARS-CoV-2 that differ in methodology, antibody class detected, antigenic target, and performance characteristics. Although there are recommendations against using IgM as a standalone and IgA, there are no other specific recommendations with regard to antigenic target or antibody class.
- The vast majority of antibody assays are qualitative and detect binding antibodies which include neutralizing antibodies. There is one EUA serology assay that specifically detects neutralizing antibodies. Multiple studies have demonstrated a positive correlation between binding and neutralizing antibody assays.
- Antibody testing should not be used for diagnosing SARS-CoV-2 infection and utility is currently limited to seroprevalence studies, as an aid in supporting a multisystem inflammatory syndrome in children (MIS-C) diagnosis, or diagnosis in adults presenting late in the disease course, and identifying eligible donors for COVID-19 convalescent plasma (CCP).
- As of May 2021, there are no recommendations from any of the professional societies (IDSA, CDC, AACC) for antibody testing to qualify for vaccine administration postnatural infection or for assessing adequate immune response due to vaccination.

INTRODUCTION

In 2019, a new coronavirus virus, SARS-CoV-2, emerged that would lead to a worldwide pandemic and highlight the importance of laboratory medicine in infectious disease management.[1] In 2021, SARS-CoV-2 remains a priority for laboratory testing. Although diagnostic testing to determine who was infected with the virus was at the forefront of the pandemic, as serology testing became available, public interest in

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Clin Lab Med 42 (2022) 1–13
https://doi.org/10.1016/j.cll.2021.10.003
0272-2712/22/

testing quickly rose and demanded that laboratories offer serology testing, even though antibody testing utility was limited. In the early days of the pandemic, March and April 2020, serology testing was not recommended for clinical purposes and was deemed of limited clinical value.[2,3] Therefore, the FDA did not see a need for strict regulations for antibody testing. This led to a proliferation of SARS-CoV-2 antibody tests, dominated early on by lateral flow assays (LFA) imported from various parts of the world. At the time, the FDA only required that the manufacturer notify the FDA of their intent to bring an antibody assay to market without any data requirements to support the performance characteristics of the assay. The consequence was a rapid and unprecedented proliferation of unvalidated, expensive assays quickly made available to anyone who wanted access. In addition, many were confused about rapid tests and incorrectly assumed that because of the ease of use that these rapid tests could be used in any setting, such as physicians' offices, without laboratory oversight or validation. The combination of public curiosity as to whether they had been infected with the virus and the lack of validated antibody tests used indiscriminately in any setting was accompanied by a considerable amount of bad press because many of the assays were inaccurate. This situation quickly escalated and highlighted the need for quality serology tests, FDA oversight, and the importance of the laboratory in validating serology assays. In early May 2020, the FDA issued new guidance for Emergency Use Authorization (EUA) claims for serology assays, that stated that, although manufacturers could notify the FDA of their intent to bring a serology assay to market as a first step to obtaining EUA, the manufacturer also had to provide supporting data to the FDA within 10 days of the notification. In addition, the FDA instituted an umbrella protocol that allowed for serology assays to be independently evaluated through NIH by agencies such as the National Cancer Institute (NCI), CDC, and Biomedical Advanced Research and Development Authority (BARDA). The FDA has also published templates for test manufacturers with recommendations for the number of samples that should be evaluated to determine performance characteristics and threshold requirements for performance characteristics (please refer to the section on serology assay evaluation).

The pandemic and serology testing have rapidly evolved and today we have a plethora of EUA serology assays available, and the list is still growing every day. There have been 21 new serology assays approved just since January 1, 2021. The good news is that many advances have been made and there are many high-quality assays but there is now increased confusion about test choice, test utility, and test result interpretation. The SARS-CoV-2 EUA serology testing landscape has been recently reviewed by Ravi and colleagues.[4] Confusion is driven not just by a large number of assay options but also by the rapidly evolving science about antibody kinetics, antibody durability, and protective immunity in the context of SARS-CoV-2 infection and now, vaccination.

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 HUMORAL RESPONSE

Not All Antibodies Are Created Equal

One concept that is not typically highlighted for other infectious diseases as far as the choice of serologic assay that has become critical to our understanding of SARS-CoV-2 infection is the different categories of antibodies and their role in the adaptive immune response. All antibodies bind to an antigen and serve a role in clearing infection. However, binding antibodies consist of both nonneutralizing and neutralizing antibodies (Nabs). Non-Nabs typically develop before Nabs and may function in viral clearance but do not extinguish infective virus. In contrast, there is a category of

binding antibodies that are referred to as Nabs that can be of various antibody classes and have the unique ability to prevent cellular infection, potentially limiting initial infection and disease severity, as well as possibly preventing reinfection. For example, in the case of SARS-CoV-2 infection, Nabs develop that bind to the receptor-binding domain (RBD) region of the virus, thereby interfering with the virus's ability to interact with the angiotensin-converting enzyme 2 (ACE2) cellular receptor on the cell surface and thereby preventing cellular infection.[5–10] The typical laboratory antibody assays measure binding antibodies, without distinguishing between neutralizing and non-Nabs. Although Nab assays provide a functional indication of the immune system and may correlate with protective immunity, it is not established what concentration of Nabs confers protective immunity due to natural infection or vaccination.

Due to the role of Nabs, many studies have investigated a correlation between commercial serology assays that measure binding antibodies and neutralization assay results. There is a general qualitative agreement and a positive correlation between binding and Nab assays. Studies also show that not surprisingly, there is a higher degree of correlation between Nab assays and binding antibody assays that use the spike protein as a target.[11–13] Nab concentrations provide important information about levels of functional antibody and have been used in vaccine development; however, there are currently no recommendations for the clinical use of neutralization assays to specifically assess vaccine response, determine infection risk or predict disease severity.[14–16]

Antibody Kinetics and Durability

Although we continue to learn about the fine details of the humoral response against SARS-CoV-2 as the pandemic unfolds, we do have a basic understanding of the antibody response in SARS-CoV-2 infection. The majority of studies indicate that infected individuals mount a SARS-CoV-2 specific antibody response in the acute stage of the disease and over 90% of infected individuals have detectable antibodies 3-weeks postsymptom onset. For IgM, the time to seroconversion ranges from 4 to 14 days.[17] Mean time to seroconversion for IgG is 12 to 15 days, and generally detectable 7 to 14-days postsymptom onset. For IgA, most studies suggest seroconversion within 4-days postsymptom onset. IgM and IgG develop almost simultaneously without a significant delay between detectable IgM and IgG.[18] The majority of studies demonstrate that IgM peaks 2 to 5-weeks postsymptom onset, and rapidly declines thereafter. IgA is less well studied but also seems to decline within a few weeks postinfection.

Early studies suggested that IgG antibody responses waned rapidly during the convalescent stage[18] and that IgG may not be durable, particularly in individuals who experienced mild forms of COVID-19.[19] More recent studies suggest that the IgG antibody response postnatural infection is detectable during the convalescent stage, and although IgG levels decline over time and may vary with disease severity, an IgG response can remain detectable up to several months, with at least one study reporting detection of RBD-spike IgG seropositivity in 88% of individuals at 8-months postinfection.[17,20–22] Studies also indicate that 4% to 10% of infected individuals may have undetectable or a delayed antibody response following SARS-CoV-2 infection.[22]

Nab titers have been shown to correlate with disease severity, and individuals with a more severe form of disease had higher titers of Nabs.[17,23–25] Most studies demonstrated that Nabs are detectable between 7 and 15-days postsymptom onset and most individuals were positive by 21-days postdisease onset.[26] Although asymptomatic individuals had lower antibody titers, Nab titers varied considerably between individuals.[24] Furthermore, although disease severity affected the magnitude of the Nab

response, some studies suggest that the kinetics of the response were not impacted.[27] For example, in one study, individuals with more severe disease had higher Nab titers than individuals with milder forms of disease but the number of days to peak neutralization titers did not differ based on disease severity.[27] Although Nab titers plateau within a few weeks, Nab titers may be detectable for months.[20] The humoral response in the context of SARS-CoV-2 has been reviewed by multiple groups[5,8,17,28]

Antibody durability has also been studied in response to vaccination. Although there was a slight decline over time, both binding and Nabs were detectable and remained elevated at least 6-months postvaccination with the Moderna vaccine.[29] Postvaccine studies for the Pfizer vaccine yielded similar results, demonstrating sustained antibody durability at least in response to mRNA vaccines. Studies are ongoing to determine when antibodies wane to levels that may warrant a booster dose of these vaccines.

In conclusion, individuals who are infected with SARS-CoV-2 and are symptomatic develop SARS-CoV-2 specific antibodies. IgM rises quickly and peaks 2 to 5-weeks postsymptom onset and then rapidly declines to undetectable levels within another 3 to 5 weeks. In contrast, IgG peaks 3 to 7-week postdisease onset, then plateaus and moderately declines for the next few weeks but can persist and be detected for several months postinfection.[20,22] Because there is no significant delay between IgM and IgG seroconversion, serology should not be used to diagnose SARS-CoV-2 infection, and there is no substantial benefit for using IgM standalone assays. In addition, for assessing exposure weeks after symptom onset IgG is useful as it is more durable. Vaccine-induced antibodies are detectable at least 6-months postvaccine administration of either of the 2 mRNA vaccines, Moderna and Pfizer.[14,30] Ongoing studies will further refine these findings.

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 SEROLOGY TESTING LANDSCAPE

As of April 2021, the FDA site lists 75 serology assays that have received EUA in the United States. Currently available commercial serology assays vary in methodology, antibody class detection, and antigen targets. There are 3 general types of methodologies: ELISA, LFA that provide rapid results and chemiluminescent immunoassays (CIA) Often, an individual major manufacturer may have multiple assays that have received EUA. For example, a single manufacturer may have an IgG, an IgM, and a total antibody assay. In addition, some vendors also have the same antibody class for a different target, such as a nucleocapsid IgG assay and a spike IgG assay. A few assays detect antibodies to more than one viral protein target. The vast majority of the assays are approved for use in high and moderate complexity settings. Only 5 of the many rapid, LFA are CLIA-waived. Sample types include plasma and serum, fingerstick whole blood, and the most recent addition, dried blood spot for home collection. Only a handful of the assays are semiquantitative, and one has EUA claim for specifically detecting Nabs. The following link (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-serology-and-other-adaptive-immune-response-tests-sars-cov-2) to the FDA site is a helpful reference as it lists the current EUA serology assays available and general overview of the assay. Another useful link is: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance. At this site, one can not only read the instructions for use (IFU), instructions for health care providers and test recipients for each assay but can also quickly ascertain the performance characteristics of a serology

assay based on the data the manufacturer provided and additional findings if the assay was independently evaluated by NCI, CDC, or BARDA. Needless to say, the sheer number of serology assay options for a single infectious agent is not only unprecedented but makes navigating the testing landscape increasingly difficult.

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 SEROLOGY ASSAY DESIGNS

Binding Antibody Assays

The vast majority of commercial assays are geared toward detecting the IgG isotype, but there are several total antibody assays, IgM & IgG combination (particularly for LFA), and a few IgM standalone assays that have received EUA. Although IgA assays have been developed, they are not in use in the United States, as studies have shown that they lack specificity. Professional guidelines do not recommend IgA assays or the use of a standalone IgM assay but do not otherwise express a preference for assays based on antibody class(es) detected.[15,16,31] IgG and total antibody assays have become the most commonly used assays because antibody testing is not recommended for diagnosis. Therefore, assays that detect IgG or total antibodies can be used to determine exposure and are the most widely used.

Antibody isotype is just one of the SARS-CoV-2 serology assay attributes that a laboratory must consider when choosing which SARS-CoV-2 assay to implement. Another important consideration is the viral target of the assay. SARS-CoV-2 consists of a single-stranded positive-sense RNA genome which encodes for nonstructural and 4 structural proteins, including the spike (S) and the nucleocapsid (N) proteins. The spike glycoprotein, S1 subunit is a surface protein present on the virion that contains the RBD which binds the angiotensin-converting enzyme 2 (ACE2) receptor and mediates entry into the host cell. The RBD and spike protein are the primary targets for Nabs in SARS-CoV-2 infection. Nabs prevent viral infection of the cell by interfering with the ability of the virus to interact with the ACE2 cell surface receptor.[32,33] Assays may contain different spike regions as targets, including S1 & S2, S1 only, or RBD only. The N protein is the most abundantly expressed viral protein and encapsulates viral RNA. It is well established that antibody responses against the nucleocapsid and spike proteins of the SARS-CoV-2 virus are readily detected in individuals who have been infected with SARS-CoV-2 and have also become the favored targets for serology assays.[25] There are some assays that use both the S and N proteins as antigenic targets.[4,34,35] Although both of these targets have been used extensively in developing serology assays for determining exposure to SARS-CoV-2, recent attention has turned to IgG antibody assays against the spike protein, as a possible tool for assessing immune response due to vaccination.

Severe Acute Respiratory Syndrome Coronavirus 2 Nab Assays

Although Nabs play a crucial role in SARS-CoV-2 infection, there is only one assay that has received EUA that specifically detects Nabs. This is in part because developing a Nab assay that can be adapted to a clinical laboratory is difficult to achieve. The gold standard for measuring Nabs is the plaque reduction neutralization test (PRNT). A classic PRNT assay determines the serum dilution that inhibits viral growth (50% or 90% inhibition) in cell culture and can therefore provide a titer. However, these assays require expertise in cell culture, are labor-intensive and require live virus, which in the case of SARS-CoV-2 would necessitate a biosafety level 3 (BSL3) facility. Another methodology is the pseudovirus-based live cell neutralization assay. This

methodology uses a pseudoviral vector to express the protein target of interest, such as the spike for SARS-CoV-2, therefore eliminating the need for live SARS-CoV-2 virus and a BSL3 facility. However, this method still requires viral and cell culture expertise and is not amenable to high throughput settings and rapid turnaround time (TAT), as needed for implementation in a clinical laboratory. These classical methods that use live or pseudotyped virus and determine the serum dilution that inhibits virus growth maybe the gold standard for measuring Nab concentrations but are really only suited for research.[24,36–38] More recently, surrogate viral neutralization tests (sVNT) have been developed. sVNT have a percent inhibition cut-off that allows for a qualitative determination of presence or absence of Nabs.[39] The Nab assay that has received EUA uses the spike protein as a target because the primary target of Nabs is the spike protein. The assay does not detect a particular antibody class. The role of Nab assays in the clinical laboratory remains to be determined.

EVALUATION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 SEROLOGY ASSAYS

As mentioned above, the FDA now requires that manufacturers of SARS-CoV-2 antibody assays submit supporting data to FDA within 10 days of notifying the FDA of the intent to bring an antibody assay to market and has published specific templates with sample size and performance threshold recommendations for EUA submission for serology assays. Although there are many caveats in the template depending on whether the assay is designed to detect individual or combined SARS-CoV-2 antibody classes, there are some general rules. Evaluation of at least 75 unique samples, preferably collected from subjects before December 2020, is recommended for specificity studies. Furthermore, if the 75 samples were tested from a population that has a high prevalence of vaccination against, and/or infection with common viruses and the observed percent positive agreement (PPA) is greater than 95% then specific cross-reactivity studies are not required. Evaluation of sensitivity requires a minimum of 30 unique samples collected from individuals with RT-PCR confirmed SARS-CoV-2 infection. Clinical performance data for sensitivity is stratified by days postsymptom onset and the typical timeframes suggested are 0 to 7 days, 8 to 14 days, and $\geq$ 15 days. For IgG and total antibody assays, 30 samples collected at day 15 or later postsymptom onset, are recommended. Therefore, for SARS-CoV-2 serology assays, generally, the minimum PPA required is 90% and the minimum negative percent agreement is 95%.[40]

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 SEROLOGY TESTING RECOMMENDATIONS

SARS-CoV-2 serology testing is recommended by a number of professional societies for the following applications: (1) seroprevalence and epidemiologic studies, (2) as an aid in diagnosing multisystem inflammatory syndrome in children (MIS-C), (3) support a diagnosis in individuals with symptoms consistent with SARS-CoV-2 infection who repeatedly test negative by NAAT, and (4) identifying eligible COVID-19 convalescent plasma (CCP) donors.

Serology assays have been used extensively for seroprevalence studies.[41] Given that a large proportion of adults have now been immunized in the United States, serology-based seroprevalence studies are more difficult to interpret. Careful consideration must be given to the choice of the assay and respective antigenic target used for this type of investigation (please see below).

Serologic testing can also be helpful clinically for the diagnosis of both MIS-C and adults who present late in the disease course. MIS-C develops in some children infected with SARS-CoV-2, often after the viral infection is no longer detectable by NAAT.[42] Serology testing for MIS-C is now a criterion included in the case definition..[31] For adults who have symptoms consistent with SARS-CoV-2 infection or have been exposed to SARS-CoV-2 infection but are repeatedly NAAT negative, antibody testing can also be used as the confirmation of SARS-CoV-2 infection. Generally, the use of either an IgG or a total antibody assay at 3 to 4 weeks (no sooner than 14 days) post-symptom onset for optimal accuracy, when using serology assays as an adjunct for the confirmation of SARS-CoV-2 infection is recommended.[15,31,34]

The use of convalescent plasma to treat patients with COVID-19 was implemented early during the pandemic. Passive antibody transfer as a therapy has been used for a number of infectious diseases in the past, including influenza.[43] Initially, only one commercial assay was approved for the selection of individuals considered to have "high SARS-CoV-2 antibody titers" and who were eligible for COVID-19 convalescent donations. However, in recent months, the FDA has updated the guidelines and has now established individual manufacturer-dependent cut-offs for several commercial assays that measure binding antibodies that can be used for the qualification of high antibody titer samples that can be used for CCP donations.

One serology testing application that has been used in the research setting but has yet to be used clinically, is to monitor vaccine response. As of May 2021, there are no recommendations for determining who should qualify for vaccination or what is considered an appropriate or protective immune response postvaccine administration based on serology results.[16] This is due to both the way vaccine efficacy was assessed during the vaccine clinical trials and the lack of standardization for both binding and Nab assays. Vaccine trials evaluated vaccine efficacy by comparing how many individuals became infected with SARS-CoV-2 in the control and vaccinated groups during the course of the clinical trials. And although various binding and Nab assays were used to determine if individuals mounted an immune response there was no cut-off on any assay that was evaluated for protective immunity.[14] In fact, 100% of vaccinated individuals developed robust levels of binding and Nabs in response to vaccination with the Moderna mRNA vaccine.[14] Although currently there are no recommendations for monitoring or assessing appropriate immune response due to vaccination using serology testing, many individuals who have been vaccinated have sought serology testing postvaccination. And although a detectable immune response postvaccination indicates that the individual has mounted an immune response to the vaccination, it is imperative to emphasize that there is no threshold antibody level associated with protective immunity on any platform, including Nab assays.

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 SEROLOGY TEST REPORTING AND INTERPRETATION

SARS-CoV-2 antibody test result interpretation is complex. Although in its simplest form, a negative antibody result indicates no SARS-CoV-2 exposure or vaccination, and a positive antibody result suggests exposure or possibly vaccination, all results must be interpreted in context. Variables that impact interpretation include: timing of the sample collection, patient clinical history, antigen target, and performance characteristics of the assay used.

Timing of sample collection in serology testing is crucial for appropriate test interpretation. Most notably, suboptimal timing due to the early collection of sample

postsymptom onset can result in a false-negative result. A false-negative result in someone who was exposed to SARS-CoV-2 is also possible in patients who are immunocompromised or individuals who had asymptomatic infection.[44]

Antigenic targets further complicate the interpretation. Due to the mass vaccination success in the United States, the antigen target has become a recent conundrum. Clinicians and epidemiologists may want to determine who has been exposed to infection and who has been vaccinated. Because the spike protein is the target of the vaccines that have been approved to date in the United States, it is reasonable to think that one can distinguish between these 2 scenarios by testing for spike and nucleocapsid antibodies. For example, individuals who are positive by nucleocapsid assays must have had a natural infection because the vaccines do not use nucleocapsid as the antigen for antibody stimulation. Indeed most recent updates from the CDC reflect this approach and test interpretation.[16] However, caution must be taken because, in the absence of clinical history, this approach is predicated on the assumption that the nucleocapsid and spike assays used in a laboratory have the same sensitivity and specificity which is not likely. There have been reports of known, confirmed SARS-COV-2 cases that subsequently tested positive by a spike assay but negative by a nucleocapsid assay.[45,46] Clinical history is crucial to correct test result interpretation, otherwise, test results could translate in misclassifying an individual's status. Seroprevalence studies and reference laboratories may be particularly challenged by the lack of clinical history to assist in test interpretation. The merits of using nucleocapsid and spike assays to distinguish between vaccinated and previously infected individuals is an active area of research and publications are forthcoming.[47]

In addition, assay performance characteristics not only vary between assays, but even small differences in specificity and sensitivity between assays can translate to substantial differences in positive predictive value (PPV) and negative predictive value (NPV) depending on disease prevalence. For example, an assay that has 98.1% sensitivity and 99.6% specificity that translates into 99.9% NPV and only 92% PPV when disease prevalence is 5.0%. If the disease prevalence is 10% the NPV only drops to 99.8% but the PPV increases to 96.1%. It is understandable that PPV was of particular concern during the early days of the pandemic, when disease prevalence was low. Therefore, the CDC made the following recommendation to increase PPV: (1) test only individuals who have a high likelihood of exposure to SARS-CoV-2, (2) test with an assay that has greater than 99.5% specificity, and (3) if not possible to test with an assay that has greater than 99.5% specificity then implement an orthogonal approach to testing.[16]

The orthogonal approach to testing is based on testing with one serology assay and if the sample is positive by the first assay, then the sample is tested by a second assay. Ideally, the assay with the highest specificity should be used first to minimize discrepant results between the 2 assays used in an orthogonal testing approach. Otherwise, the assays used in this type of algorithm can be the same antigenic target but different method (ELISA spike and CIA spike), or the same method but different antigenic targets (CIA nucleocapsid, CIA spike). If both test results are positive, then the PPV is very high, assuring that the result is a true positive. However, if the second test is negative interpretation is less clear. Although at first glance this would suggest a false-positive result with the first assay, it may be that the discrepant results are due to differences in sensitivity between the assays and not a reflection of the accuracy of the first test. Discrepant results must be interpreted with caution and considered in the context of the patient's clinical history. Orthogonal testing has also been applied for seroprevalence studies.[41] Today, the prevalence of disease has increased across the country and assays with greater than 99.5% specificity are more readily available;

therefore, the need for orthogonal testing to increase PPV is no longer a priority for most laboratories.

In summary, many variables, including patient history, have an impact on the accuracy of the test result and interpretation. Both the FDA and best practices require that clinical serology results must be accompanied by clear footnotes on the patient chart that state the limitations of serology testing. Most, importantly, serology testing should not be used for diagnosing SARS-CoV-2 infection. Other important limitations include that a negative SARS-CoV-2 antibody result does not rule out current or past infection and a positive SARS-CoV-2 antibody test can be due to cross-reaction with other commonly circulating human coronaviruses. The clearer and more comprehensive yet concise information a laboratory can provide in the test order recommendations and/or chart comments regarding the details of the assay used (such as the antigenic target, antibody class detected) and specific limitations, the more helpful it is for clients, clinicians, and patients.

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 ANTIBODY TESTING PERSPECTIVE

Although many studies have been conducted to address immunity postinfection and postvaccination, some aspects of humoral immunity in response to SARS-CoV-2 infection are still being defined. Studies have often yielded conflicting results about various aspects of SARS-CoV-2 infection humoral response. It is important to note that many of the studies, particularly early in the pandemic, were limited in patient numbers, patient demographics, and temporal follow-up. More recent studies have had access to larger and more diverse cohorts and extended study duration. Another complicating factor that can affect the result of studies attempting to address the fundamental serology questions in the context of SARS-CoV-2 infection is that the assay used for these studies may also have an impact on the findings. Fundamentally, it is still not known what constitutes a protective immune response when assessing antibody response, in the context of natural infection or vaccination.

Another challenge to making a meaningful interpretation for SARS-CoV-2 antibody test results is the lack of standardization for both binding and Nab assays. Substantial test performance variation and therefore choice of assay can have a significant impact on the overall conclusion of a study or clinical test interpretation.

The lack of standardization between any of the EUA serology assays, neutralizing, and binding antibody assays makes it difficult to interpret results obtained with different serology assays. This is the case for both clinical interpretation and a confounding factor in research studies. Semiquantitative assay results have no commutability and cannot be used interchangeably between assays, even if the assays are semiquantitative. Although the need for standardization is undeniable, the first step is to determine what constitutes humoral protective immunity. Antibodies as a correlate of protective immunity and accompanying standard threshold have been developed for other infectious diseases such as hepatitis B, whereby hepatitis B surface antibody levels more than 10 mIU/mL indicate protective immunity.[43] It is, therefore, possible that someday there will be SARS-CoV-2 antibody manufacturer-specific cut-offs, as has been established for SARS-CoV-2 antibody assays in the context of CCP, or a standard that can be used to firmly establish what constitutes a protective antibody response in the context of SARS-CoV-2.

In summary, the SARS-CoV-2 pandemic continues to dominate the world and US health care. Laboratory testing, including serology testing, remains at the forefront of the public health response. Current antibody testing is not limited by technology

or supply chain issues, but important limitations do exist. The limitations consist of rapidly changing understanding of the immune response to natural infection with SARS-CoV-2, evolving knowledge regarding vaccine response to a new form of vaccine technology, and the lack of standardization for serology assays. Although antibody tests are widely available, there is a need for standardization to increase the clinical utility of antibody testing in the future.

The laboratory must remain vigilant in staying current with advancing knowledge, rapid developments in testing methods, and updated recommendations. The laboratory remains critical to ensuring a quality result by validating/verifying the test and implementing appropriate quality control measures.[15] Finally, the laboratory is crucial to educating clinicians, patients, and the public alike about the complexity and limitations of SARS-CoV-2 antibody testing.

CLINICS CARE POINTS

- SARS CoV-2 serology assays are not standardized.
- Clinical utility remains limited.
- If a serology assay is used as an adjunct to nucleic acid amplification tests (NAATs) for supporting a clinical diagnosis in MIS-C or in adults with suspicion of SARS-CoV-2 who are NAAT negative, IgG, and total antibody assays should be used 3 to 4 weeks postsymptom onset for optimal accuracy.

DISCLOSURE

The author has nothing to disclose.

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