Performance Characteristics of High-Throughput Serologic Assays for Severe Acute Respiratory Syndrome Coronavirus 2 with Food and Drug Administration Emergency Use Authorization: A Review

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KEYWORDS

- SARS-CoV-2 • COVID-19 • Antibody • Serology • High-throughput assays

KEY POINTS

- The currently available high-throughput severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serologic assays with Food and Drug Administration (FDA) Emergency Use Authorization (EUA) are quite heterogeneous, detecting different antibody classes (eg, immunoglobulin M (IgM), IgG, total immunoglobulin) against a variety of SARS-CoV-2 antigens (eg, spike glycoprotein, receptor-binding domain) using a variety of different methods (eg, enzyme-linked immunosorbent assays, chemiluminescent immunoassays, and so forth). Understanding the advantages, limitations, and performance characteristics of each is necessary before implementation in the clinical laboratory.

- Serologic assays with high specificity (ie, ≥99.5%) are preferred in low disease prevalence settings to maximize the positive predictive value of serologic test results. Most of the SARS-CoV-2 serologic assays with FDA EUA have documented specificity ranges approaching 100% among prepandemic and cross-reactivity panels.

- The sensitivity of SARS-CoV-2 serologic assays is affected by multiple factors, including disease severity, patient immunostatus, and timing of sample collection postsymptom onset, among others. Most of the patients with COVID-19 are seropositive after at least 14 days of symptoms.

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INTRODUCTION

Throughout the coronavirus disease 2019 (COVID-19) pandemic, the role of serologic testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been debated among clinicians and laboratorians. In contrast, among both public and government forums, interest in this methodology has oscillated between "none," to headlines suggesting that such testing may help to "reopen economies."[1,2] In reality, the truth lies somewhere in between these 2 dichotomies. Currently, the role of serologic testing for SARS-CoV-2 in the clinical realm remains fairly limited and of note, has not significantly changed since the start of the pandemic. These potential uses, not solely limited to the clinical setting, include the following[3–5]:

- Aid in the diagnosis of COVID-19 in patients with a negative SARS-CoV-2 molecular or antigen detection assay, who present at least 7 days after disease onset and with a prior negative antibody test result
- Aid in the diagnosis of complications associated with COVID-19, including multisystem inflammatory syndrome in children
- Manufacture of COVID-19 convalescent plasma
- Use for SARS-CoV-2 seroprevalence surveys to document incidence of natural infection and/or vaccination
- Use in research (eg, vaccine efficacy, immunity, humoral immune response kinetics, and so forth)

As with any emerging pathogen, the development and deployment of clinical laboratory methods for the detection of SARS-CoV-2, including molecular, antigenic, and serologic tests, occurred in parallel to our growing understanding of virus' pathology and optimal test utilization practices. With respect to serologic methods specifically, more than 200 assays were commercially available in the United States early in the pandemic due to multiple factors, including high consumer interest and limited oversight from the US Food and Drug Administration (FDA), which did not require Emergence Use Authorization (EUA) for SARS-CoV-2 serologic assays, unlike for molecular and antigenic assays, until May 4th, 2020.[6] As of the writing of this article (April 2021), there are currently 75 SARS-CoV-2 serologic assays with FDA EUA, and more than 260 assays that the FDA specifically indicates should not be used or distributed due to either poor performance, lack of EUA receipt, or voluntary removal from the market by the manufacturer.[7]

Among the SARS-CoV-2 serologic tests with FDA EUA, there is significant variability in the methods (eg, enzyme linked immunosorbent assays [ELISAs], chemiluminescent immunoassays [CIAs], lateral flow immunoassays, and so forth.) and design characteristics (eg, SARS-CoV-2 antigen used, targeted antibody class, result reporting, and so forth.) of these assays, which may affect their clinical performance characteristics, sample throughput, and the capability of laboratories to implement them in their local facility settings. This article focuses on discussing the performance characteristics of commonly used high-throughput assays with FDA EUA for detection of SARS-CoV-2 antibodies in the central clinical laboratory. For a discussion of lateral flow assays or alternative means for detection of antibodies to SARS-CoV-2, the reader is referred to Ochola and colleagues' article, "Performance Evaluation of Lateral Flow Assays for COVID-19 Serology," and Patel and colleagues' article, "Alternative Methods to Detect SARS-CoV-2 Antibodies," in this issue.

Table 1

Summary of select high-throughput, automated serologic assays with FDA EUA for detection of antibodies to SARS-CoV-2

Assay Name (Manufacturer)	Method	Antibody Class Detected	SARS-CoV-2 Antigen	Platform/Analyzer	Result Output	FDA-Reported Sensitivity[b] (95% CI)	FDA-Reported Specificity[b] (95% CI)
AdviseDx SARS-CoV-2 IgM (Abbott Laboratories Inc.)	CMIA	IgM	S	Architect i or Alinity i	Qualitative	95% (89.9%–100%)	99.6% (94.6%–99.8%)
AdviseDx SARS-CoV-2 IgG II (Abbott Laboratories Inc.)	CMIA	IgG	RBD	Architect i or Alinity i	Semiquantitative	NA	NA
SARS-CoV-2 IgG Assay (Abbott Laboratories Inc.)	CMIA	IgG	NC	Architect i or Alinity i	Qualitative	100% (89.9%–100%)	≥ 99% (94%–99.8%)
Access SARS-CoV-2 IgG (Beckman Coulter, Inc.)	CIA	IgG	RBD	Access 2, Dxl 600, Dxl 800	Qualitative	96.8% (91.1%–98.9%)	99.6% (99.2%–99.8%)
Access SARS-CoV-2 IgM (Beckman Coulter, Inc.)	CIA	IgM	RBD	Access 2, Dxl 600, Dxl 800	Qualitative	96.7% (92.5%–98.6%)	99.9% (99.5%–100%)
Access SARS-CoV-2 IgG II (Beckman Coulter, Inc.)	CIA	IgG	RBD	Access 2, Dxl 600, Dxl 800	Semiquantitative	NA	NA
Platelia SARS-CoV-2 Total Ab (Bio-Rad Laboratories Inc.)	ELISA	Total	NC	Microplate washer/reader[a]	Qualitative	98% (89.5%–99.6%)	99.3% (98.3%–99.7%)
VIDAS SARS-CoV-2 IgM (bioMerieux SA)	ELFA	IgM	RBD	VIDAS, MINI VIDAS, VIDAS 3	Qualitative	100% (85.7%–100%)	99.4% (97.7%–99.8%)
VIDAS SARS-CoV-2 IgG (bioMerieux SA)	ELFA	IgG	RBD	VIDAS, MINI VIDAS, VIDAS 3	Qualitative	100% (88.3%–100%)	99.9% (99.4%–100%)

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Table 1
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Assay Name (Manufacturer)	Method	Antibody Class Detected	SARS-CoV-2 Antigen	Platform/Analyzer	Result Output	FDA-Reported Sensitivity[b] (95% CI)	FDA-Reported Specificity[b] (95% CI)
LIAISON SARS-CoV-2 S1/s2 IgG (DiaSorin Inc.)	CIA	IgG	S1/s2	LIAISON XL	Qualitative	97.6% (87.4%–99.6%)	99.3% (98.6%–99.6%)
LISAISON SARS_CoV-2 IgM (DiaSorin Inc.)	Indirect CIA	IgM	RBD	LIAISON XL	Qualitative	91.8% (85.6%–95.5%)	99.3% (98.9%–99.5%)
Anti-SARS-CoV-2 ELISA (IgG) (EUROIMMUN US Inc.)	ELISA	IgG	S1	Microplate washer/reader[a]	Qualitative	90% (74.4%–96.5%)	100% (95.4%–100%)
cPass SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript USA Inc)	Blocking ELISA	nAb	RBD	Microplate washer/reader[a]	Qualitative	100% (87.1%–100%)	100% (95.8%–100%)
SCoV-2 Detect IgG ELISA (InBios International Inc.)	ELISA	IgG	Not Indicated	Microplate washer/reader[a]	Qualitative	100% (88.7%–100%)	100% (95.4%–100%)
SCoV-2 Detect IgM ELISA (InBios International Inc.)	ELISA	IgM	Not indicated	Microplate washer/reader[a]	Qualitative	96.7% (83.3%–100%)	98.8% (93.3%–100%)
xMAP SARS-CoV-2 Multi-Antigen IgG (Luminex Corp)	FMIA	IgG	S1/RBD/NC	FlexMap 3D, MAGPIX, Luminex 200	Qualitative	96.2% (89.8%–98.7%)	99.3% (98.3%–99.7%)
VITROS Anti-SARS-CoV-2 Total (Ortho Clinical Diagnostics, Inc.)	CIA	Total Ab	S1	VITROS 5600/XT 7600, VITROS ECi/ECiQ/3600	Qualitative	100% (92.7%–100%)	100% (99%–100%)

Assay	Method	Antibody	Antigen	Platform	Type	Sensitivity % (95% CI)	Specificity % (95% CI)
VITROS Anti-SARS-CoV-2 IgG (Ortho Clinical Diagnostics, Inc.)	CIA	IgG	S	VITROS 5600/XT 7600, VITROS ECi/ECiQ/3600	Qualitative	90% (76.9%–96%)	100% (99.1%–100%)
Elecsys Anti-SARS-CoV-2 (Roche Diagnostics)	ECLIA	Total Ab	NC	cobas e411/e602/e801	Qualitative	100% (88.3%–100%)	99.8% (99.7%–100%)
Elecsys Anti-SARS-CoV-2 s (Roche Diagnostics	ECLIA	IgG	RBD	cobas e411/e602/e801	Semiquantitative	96.6% (93.4%–98.3%)	100% (99.9%–100%)
SARS-CoV-2 Total (Siemens Healthcare Diagnostics)	CIA	Total Ab	RBD	Atellica IM Analyzer, ADVIA Centaur, Dimension Vista System	Qualitative	100% (91.6%–100%)	99.8% (99.3%–99.9%)
SARS-CoV-2 IgG (Siemens Healthcare Diagnostics)	CIA	IgG	RBD	Atellica IM Analyzer, ADVIA Centaur, Dimension Vista System	Semiquantitative	100% (91.6%–100%)	99.9% (99.6%–100%)
OmniPATH COVID-19 Total Antibody (Thermo Fisher Scientific)	ELISA	Total Ab	RBD	Microplate washer/reader[a] or Dynex Agility	Qualitative	96.7% (83.3%–99.4%)	97.5% (91.3%–99.3%)
SARS-CoV-2 IgG Test System (Zeus Scientific Inc)	ELISA	IgG	RBD/NC	Microplate washer/reader[a]	Qualitative	93.3% (78.7%–98.2%)	100% (94.8%–100%)

Abbreviations: CI, confidence interval; CIA, chemiluminescent immunoassay; CMIA, chemiluminescent microparticle immunoassay; ELFA, enzyme-linked fluorescence assay; ELISA, enzyme-linked immunosorbent assay; FDA, US Food and Drug Administration; FMIA, fluorescent microbead immunoassay; nAb, neutralizing antibody; NC, nucleocapsid; RBD, receptor binding domain; S, spike glycoprotein; S1, spike glycoprotein subunit 1; S2, spike glycoprotein subunit 2.

[a] Laboratories may alternatively validate these on fully automated ELISA processors.

[b] Data from FDA EUA Authorized Serology Test Performance: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance.

KEY DESIGN DIFFERENCES AMONG SARS-CoV-2 HIGH-THROUGHPUT SEROLOGIC ASSAYS WITH FDA EUA

Although not an exhaustive list, the more commonly used, automated SARS-CoV-2 serologic assays are listed in **Table 1**, alongside defining method and format characteristics. Most of these assays are based on either chemiluminescent or enzymatic reactions, with fewer assays using fluorescence as the output marker. Although the CIAs and fluorescence-based immunoassays are typically designed to be performed on specific automated platforms, ELISAs may be semiautomated using microplate washers and readers, or may be fully automated using open-system ELISA processors, following completion of laboratory validation/verification studies. In addition, although most of the assays, regardless of format, provide qualitative results only, an increasing number of assays are receiving FDA EUA as semiquantitative methods, reporting out results in "arbitrary units (AU)" per mL, alongside a qualitative interpretation. Importantly, however, although there is now a SARS-CoV-2 immunoglobulin G (IgG) standard available through the World Health Organization (WHO), widespread standardization among the high-throughput semiquantitative assays has not yet occurred.[8] Therefore, the reported values are not interchangeable across platforms, and significant variability has been observed between methods.[9,10]

Another key differential feature of serologic assays is the SARS-CoV-2 antigen they are based on, which varies between epitopes of either the viral spike (S) glycoprotein or the nucleocapsid (NC) protein (see **Table 1**). The S glycoprotein decorates the surface of SARS-CoV-2 and mediates binding to and fusion with the human angiotensin-converting enzyme 2 (ACE2) for cellular entry and is also the primary target for neutralizing antibodies to the virus.[11] More specifically, each monomer of the S trimer is composed of 2 subunits—S1 and S2. Within the S1 subunit lies the receptor binding domain (RBD), which specifically interacts with the ACE2 receptor for binding. Most of the high-throughput serologic assays have been designed to detect antibodies to recombinant versions of the RBD, followed by recombinant S or S1 antigens (see **Table 1**). Less frequently, manufacturers have used recombinant SARS-CoV-2 NC as the target antigen, which is also highly immunogenic but is involved with viral RNA replication, packaging, and viral particle release.[12] Although there are some reports suggesting differential sensitivity of serologic assays based on whether SARS-CoV-2 S or NC components are targeted, knowing which antigen the serologic assay is based on is increasingly important from the perspective of result interpretation in the setting of increasing SARS-CoV-2 vaccination rates. Currently, all of the SARS-CoV-2 vaccines with FDA EUA are designed to induce a humoral and cellular immune response to the S glycoprotein.[13] Therefore, although anti-S/RBD/S1/S2-based serologic assays may result as positive in either vaccinated or naturally infected individuals, anti-NC assays will only be positive in those who have had a prior natural infection with SARS-CoV-2.

The final key differential feature among high-throughput SARS-CoV-2 serologic assays is the antibody type and the class or classes of antibodies detected (see **Table 1**). Currently all but one of the serologic assays with FDA EUA detect *binding* antibodies to the virus, with a single assay specifically detecting *neutralizing* antibodies (nAb; cPass GenScript USA Inc.). Although binding antibodies will recognize different immunogenic viral epitopes, their binding antibodies may either not affect infectivity or they may recruit other components of the immune system to help inactivate the virus (eg, via opsonization or complement fixation). In contrast, nAb recognize and specifically bind to viral epitopes involved with cellular entry and replication, resulting in the

inhibition of viral infectivity, independent of other elements of the immune system. The presence of nAb has typically been used as a correlate for protective immunity for a variety of infectious diseases and has been shown to increase alongside binding antibodies following both natural infection with and vaccination against SARS-CoV-2.[14–17]

PERFORMANCE CHARACTERISTICS AMONG THE COMMONLY USED HIGH-THROUGHPUT SARS-CoV-2 SEROLOGIC ASSAYS

The selection of which high-throughput SARS-CoV-2 serologic assay to implement in the central laboratory depends on multiple factors beyond just the reported performance of the assay, including factors such as which platforms are available in the laboratory and have excess capacity, reagent cost, and workflow considerations, among others. Following selection, the assay must be verified before clinical use, which early during the pandemic was challenging for multiple reasons, including limited availability of well-characterized specimens, lack of a reference standard method and an unclear understanding of what is required for verification of assays with FDA EUA. In an effort to provide guidance to laboratories both during the current and for future pandemics, multiple organizations, including the American Society for Microbiology and the American Association for Clinical Chemistry, put forth detailed documents for verification of serologic assays with EUA during a pandemic, to which the reader is referred.[18,19]

With the rapid development and deployment of SARS-CoV-2 serologic tests and increasing unease regarding the accuracy associated with several of these assays, the FDA put forth an easily accessible summary of assay-specific performance characteristics, which were used to base the decision for EUA authorization.[20] These data were provided by the assay manufacturer as part of the EUA application process and/or from testing performed at the National Cancer Institute and are summarized in **Table 1**. Overall, sensitivity and specificity values for these assays were high, ranging from 90% to 100% and 97.5% to 100%, respectively. However, these data do not provide a complete picture, as sensitivity can vary depending on time of testing postsymptom onset, disease severity, and patient immunostatus among other factors. As a result, over the past year, a steady influx of independent evaluations of these high-throughput assays have been published, although it is important to recognize that these studies are quite heterogeneous, varying dramatically in the types of sample sets used for accuracy analysis, including differences associated with time to sample collection relative to symptom onset, patient disease severity and age distribution, the reference method, and so on. Although this somewhat limits the ability to do direct cross-study comparisons, a high-level summary of the reported performance characteristics of commonly evaluated high-throughput assays is presented in the following section.

SARS-CoV-2 Serologic Assay Specificity

SARS-CoV-2 is the third coronavirus in the last 20 years to have spilled over from an animal reservoir to cause human disease, following SARS-CoV in 2002 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012. Although SARS-CoV and MERS-CoV are no longer widely circulating, 4 endemic CoVs continue to cause typically mild, upper respiratory tract infections worldwide, including HKU1, OC43, 229E, and NL63. Therefore, a primary concern among both manufacturers and laboratorians considering implementing SARS-CoV-2 serologic tests was what level of specificity could such assays achieve given that greater than 65% of children and greater than 90% of adults older than 50 years are seropositive for antibodies to at

least one of the commonly circulating CoVs.[21,22] At the amino acid level, the endemic CoVs share 28.4% to 34.6%, 21% to 31.2%, and 33.1% to 42.3% identity with the SARS-CoV-2 NC, S1 and S2 proteins, respectively, indicating that antibody cross-reactivity is possible among these viruses.[23,24]

Several approaches have been taken to assess assay specificity, including evaluation of samples collected before the pandemic, and in samples collected from patients with confirmed, alternative CoV or other respiratory pathogen infections. Using the Abbott SARS-CoV-2 IgG chemiluminescent microparticle immunoassay (CMIA; NC-based assay), Brecher and colleagues showed 100% specificity of this method among 20 sera collected from patients at least 30 days from polymerase chain reaction–confirmed infection with one of the 4 endemic CoVs.[25] Subsequent, larger studies using cross-reactivity and prepandemic healthy donor panels have likewise documented somewhat more variable, although still high, specificity levels for the Abbot IgG CMIA (97.5%–100%). Interestingly, using prepandemic samples collected from patients in Nigeria, a recent study showed a 6.1% false positivity rate for the Abbott IgG CMIA among 212 samples with high levels of antimalaria antibodies.[26] This unexpected cross-reactivity, which can be substantially minimized using urea treatment, has not yet been replicated with other assays. The other frequently used anti-NC based assay is the Roche total antibody anti-NC electrochemiluminescence immunoassay (ECLIA), for which studies have consistently reported exceedingly high specificity ranges greater than 99% (**Table 2**).[10,27–29] Among the NC-based ELISAs, the Bio-Rad Platelia assay has been most frequently evaluated, with slightly lower overall specificity reported, ranging from 95% to 100% using similar panels (see **Table 2**).[28,29]

Most of the high-throughput SARS-CoV-2 serologic assays are based on recombinant spike protein components, either S1 or RBD, or a combination. The reported specificity range for CIA-based anti-S assays is similarly high as that observed for NC-based methods, typically greater than 99% (see **Table 2**). Two frequently evaluated S-based assays for which lower specificities have more been reported include the DiaSorin S1/S2 IgG CIA and the Euroimmun S1 IgG ELISA.[10,27–32] Among the studies evaluating the DiaSorin IgG CIA, Turbett and colleagues demonstrated statistically significant ($P<.005$) lower specificity of this assay in more than 1200 prepandemic samples, as compared with the Abbott IgG CIA and the Roche Total antibody ECLIA (ie, 97.8% vs 99.3% and 99.7%, respectively).[31] Jääskeläinen and colleagues report the lowest specificity for both the DiaSorin IgG CIA and the Euroimmun IgG ELISA at 91.4% and 87.7%, respectively.[10] Although these data are based on a small cross-reactivity panel set (N = 81 samples), this study is among the few to attempt to identify the potential causes of cross-reactivity, although no consistent cause was determined for these 2 assays.

Implementing highly specific SARS-CoV-2 serologic assays is essential in order to maximize the positive predictive value of results. Although this concept remains important today, this was essential early during the pandemic when prevalence of SARS-CoV-2 in the community was low—low prevalence, coupled with lower assay specificity characteristics, is associated with low positive predictive values, leading to higher rates of false-positive results.[19] Minimum serologic assay specificity thresholds have been recommended by the Centers of Disease Control and Prevention and FDA (ie, $\geq$99.5%) and the WHO (ie, $\geq$97%).[5,20,33]

SARS-CoV-2 Serologic Assay Sensitivity

The sensitivity of any serologic assay for detection of antibodies to an infectious pathogen is affected by several factors, including the limit of detection of the assay itself for

Table 2
Summary of peer-reviewed performance characteristics of select high-throughput serologic assays for SARS-CoV-2

Manufacturer/ Method	Antibody Targeted/ SARS-CoV-2 Antigen	Reported % Sensitivity		Reported % Specificity Prepandemic Samples and/ or Other Infections	Reference
		≤14 d PSO	15–150 d PSO		
Abbott/CMIA[a]	IgG/NC	40.5%–81.8%	64%–100%	97.5%–100%	10,27–32,43,46–50
Bio-Rad/ELISA	Total Ab/NC	67.8%–83%	86.7%–100%	95%–100%	28,29,46,50
Beckman/CIA	IgG/RBD	29.7%–56%	58.3%–86.7%	99.8%–100%	28,29,43
DiaSorin/CIA	IgG/S1:S2	38.5%–70.8%	54.2%–90.9%	91.4%–100%	9,10,28–31,46,50,51
EUROIMMUN/ELISA	IgG/S1	27.5%–48%	73%–100%	87.7%–100%	10,27–32,46,52
Ortho Clinical/CIA	IgG/S1	38.5%–84.8%	79.3%–97.0%	98%–100%	9,27,29,30,48
Ortho Clinical/CIA	Total Ab/S1	61.4%–100%	90%–100%	99%–100%	9,29,30,48
Siemens/CIA	Total Ab/RBD	32.4%–73.3%	84%–97%	99%–99.5%	29,30,43,50
Roche/ECLIA[a]	Total Ab/NC	37.8%–72.3%	73%–100%	99.1%–100%	9,28–30,32,43,46–53
Roche/ECLIA	Total Ab/RBD	61.5%–90.9%	83.3%–100%	100%	9,48,53

Abbreviations: CIA, chemiluminescent immunoassay; CMIA, chemiluminescent microparticle immunoassay; ELISA, enzyme-linked immunosorbent assay; NC, nucleocapsid; PSO, postsymptom onset; RBD, receptor binding domain; S, spike glycoprotein; S1, spike glycoprotein subunit 1; S2, spike glycoprotein subunit 2.
  [a] Qualitative assay.

the target analyte, the ability of the patient to produce antibodies, and the timing of patient sampling following infection. Focusing on the latter, the temporal evolution of a humoral immune response to SARS-CoV-2 follows that of most other viral pathogens and has been discussed in detail elsewhere.[24] Using various serologic methods (most without FDA EUA), which were based on a variety of SARS-CoV-2 target antigens to detect either IgM- or IgG-specific antibodies, the growing body of evidence indicates that most of the infected individuals will develop a humoral immune response to SARS-CoV-2 within 7 to 14 days of symptom onset, with detectable IgM and IgG class antibodies evolving concurrently or closely following one another.[18,19,24] Although the duration of antibody positivity continues to be assessed, as most other viral infections, anti-SARS-CoV-2 IgM antibodies begin to decline 20 to 30 days following symptom onset. With respect to durability of anti-SARS-CoV-2 IgG antibodies, there have been conflicting reports, with some studies showing rapid decline in titers 3 to 4 months postsymptom onset, whereas others report persistent detection for 6 months or longer.[34–37] Although some of this discrepancy is likely due to variability in the assays themselves, it is increasingly apparent that immune responses are also affected by disease severity, immunocompetence, age, and sex, among other factors.[38,39]

The sensitivity of high-throughput SARS-CoV-2 serologic assays has been independently assessed for multiple different platforms, and an overarching summary, separated based on days postsymptom onset (PSO), is presented in **Table 2**. In samples from acutely symptomatic patients, collected within 14 days PSO, regardless of the SARS-CoV-2 antigen target and antibody class detected, the sensitivity of these assays is low and variable. Intraassay variability was also notable among studies evaluating the same method; this was particularly apparent in acute phase samples (eg, Abbott IgG CMIA range 42.5%–81.8%, DiaSorin IgG CIA range 38.5%–70.8%, Ortho Clinical IgG CIA range 38.5%–84.8%). As mentioned earlier, aside from method differences, assay sensitivity is also affected by the severity of disease experienced by the patient, with data showing that hospitalized patients in general develop antibodies sooner and at higher titers as compared with mildly ill or asymptomatic individuals.[36,40–42] Regardless of method, however, sensitivity increases dramatically among samples collected at least 15 days PSO, with most assays approaching 100% sensitivity at these later time points (see **Table 2**). Interestingly, the one assay that did not reach at least 90% sensitivity among samples collected 15 days or longer PSO was the Beckman IgG CIA. Although based on a low to moderate number of samples (N = 24, 30 or 176) from symptomatic patients, 3 separate studies evaluating the Beckman IgG CIA, an anti-RBD assay, reported sensitivities of 74%, 79%, and 86.7% among samples collected at later timepoints PSO[28,29,43]; this is notably discordant with the 96.8% to 100% positive percent agreement indicated by the manufacturer and as published on the FDA Website for this assay.[6] In an effort to optimize the performance characteristics of the assay, Therrien and colleagues proposed an alternative cut-off threshold for the Beckman IgG CIA, which improved sensitivity to 100% without substantially affecting specificity.[29] Although further study of the Beckman IgG CIA is warranted, this underscores the importance of independent clinical assessment of these assays in local hospitals and health care systems before assay implementation.

FUTURE OUTLOOK FOR SARS-CoV-2 SEROLOGIC TESTING

Despite initial excitement for SARS-CoV-2 serologic assays, their clinical role and utilization in the laboratory has remained minimal, as evidenced by the relatively low

volume of test requests (personal observation, E. S. Theel, 2020). The reason behind this is multifold, including the lack of a well-defined correlate or surrogate of protective immunity, the inherent heterogeneity among currently available serologic assays, and as a result, the limited clinical actions that can be taken based on results from these tests. Although a positive anti-SARS-CoV-2 result indicates previous infection or vaccination (depending on the assay used), what remains undefined is the minimal antibody "threshold" associated with long-term protective immunity, as exists for other vaccine preventable diseases.[17,44] It is clear that both natural infection and vaccination are associated with protection for at least 6 to 9 months; however, it is also evident, as discussed earlier, that antibody titers can be quite variable among individuals, in both their levels and persistence.[15,16,34,45] Once such a threshold is established and the availability of SARS-CoV-2 vaccines stabilizes, results from SARS-CoV-2 serologic tests may provide more value—for example, they may be used to identify individuals who may benefit from revaccination.

Alongside the identification of a "minimum" antibody threshold associated with protective immunity, there will be a need to standardize SARS-CoV-2 serologic assays. Most of the current assays are qualitative; however, increasingly, manufacturers are developing semiquantitative assays, which provide a quantitative value typically in AU/mL. Although these values provide a general sense of antibody "levels", these semiquantitative assays are not standardized (as of the writing of the article) to the WHO SARS-CoV-2 immunoglobulin reference and therefore are not standardized to each other.[8] Such standardization will be necessary in the future to increase the clinical value of SARS-CoV-2 serologic test results.

SUMMARY

Serologic assays for SARS-CoV-2 emerged exceedingly rapidly in the early days of the pandemic, from both established and new *in vitro* diagnostic manufacturers. The ability to perform independent clinical evaluations of these assays was initially challenging due to limited reagent availability, lack of sufficient patient samples, and an unclear understanding of both how to verify assays with FDA EUA and how the humoral immune response to SARS-CoV-2 evolves, ultimately leading to the absence of an ideal reference standard for assay/result comparison. Over the past 6 months, however, more detailed, independently performed studies have been published outlining the performance characteristics of the commonly implemented high-throughput SARS-CoV-2 serologic assays (see **Table 2**). As this review has summarized, the reported specificity for most of these assays is exceedingly high, greater than 98% among both prepandemic and cross-reactivity panels. As with most other anti-viral serologic assays, sensitivity is poor to low during the first week PSO for most of the patients, increasing dramatically among samples collected at least 14 days after disease onset. Although most high-throughput assays approached 100% sensitivity at these later time points, some assays did not (eg, Beckman IgG CIA, DiaSorin IgG CIA), further underscoring the importance of clinical validation of assays before implementation (see **Table 2**). The role of serologic testing for SARS-CoV-2 in the future remains difficult to predict. The clinical utility of these assays will likely remain limited, given that most individuals will have been naturally infected or vaccinated. However, there may be a future role of SARS-CoV-2 serologic assays comparable with what is currently the case for other vaccine preventable diseases. Once a correlate of immunity is identified, testing individuals to determine whether a vaccine booster is necessary, especially if individuals are unable to provide documentation of vaccination, or to confirm immunity as is done for

other vaccine-preventable diseases before arriving on campus or starting a job in health care may become a key role for these assays. In this case, high-throughput, specific SARS-CoV-2 serologic tests, primarily targeting detection of spike protein components, will be key to fill this need.

CLINICS CARE POINTS

- Despite the circulation of 4 endemic coronaviruses, most of the commonly implemented high-throughput SARS-CoV-2 serologic assays have exceedingly high specificity, minimizing the risk of false-positive results.
- The sensitivity of high-throughput SARS-CoV-2 serologic assays is best after at least 14 days postsymptom onset.
- The clinical utility of these assays is currently minimal, as they cannot be used to routinely establish a diagnosis of active COVID-19.
- Future optimization of current and new SARS-CoV-2 serologic tests should focus on standardizing these assays to an international reference standard and on the identification of a correlate of immunity against reinfection and/or disease manifestation.

DISCLOSURE

The author has participated on advisory boards for Roche Diagnostics and Accelerate Diagnostics and is on the scientific advisory board for Serimmun Inc.

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