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REVIEW

Serodiagnostics for Severe Acute Respiratory Syndrome–Related Coronavirus-2

A Narrative Review

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Accurate serologic tests to detect host antibodies to severe acute respiratory syndrome–related coronavirus-2 (SARS-CoV-2) will be critical for the public health response to the coronavirus disease 2019 pandemic. Many use cases are envisaged, including complementing molecular methods for diagnosis of active disease and estimating immunity for individuals. At the population level, carefully designed seroepidemiologic studies will aid in the characterization of transmission dynamics and refinement of disease burden estimates and will provide insight into the kinetics of humoral immunity. Yet, despite an explosion in the number and availability of serologic assays to test for antibodies against SARS-CoV-2, most have undergone minimal external validation to date. This hinders assay selection and implementation, as well as interpretation of study results. In addition, critical knowledge gaps remain regarding serologic correlates of protection from infection or disease, and the degree to which these assays cross-react with antibodies against related coronaviruses. This article discusses key use cases for SARS-CoV-2 antibody detection tests and their application to serologic studies, reviews currently available assays, highlights key areas of ongoing research, and proposes potential strategies for test implementation.

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Since the initial identification of severe acute respiratory syndrome–related coronavirus-2 (SARS-CoV-2) as the etiologic agent of coronavirus disease 2019 (COVID-19), there have been over 5.3 million confirmed cases and around 340 000 deaths reported worldwide, according to the World Health Organization (WHO) (1). However, given the prevalence of asymptomatic or minimally symptomatic individuals (2, 3), the imperfect sensitivity of molecular assays performed at a single time point (4), and limited molecular testing capacity in several parts of the world, the true number of infections probably exceeds the WHO's estimate by several fold.

In addition to scaling up molecular testing for diagnosis of active disease, several countries have incorporated serologic surveillance studies to their COVID-19 pandemic response. These studies can help elucidate disease transmission dynamics and improve disease burden estimates by identifying persons who were previously infected, even if pauci- or asymptomatic (5); assess transmission within and between subgroups in the population; and provide insight into the kinetics of humoral immunity after infection (6, 7). Serologic testing may also serve as an adjunct to molecular methods for COVID-19 diagnosis in certain clinical scenarios (8).

Despite a rapid increase in the number and availability of serologic assays to test for antibodies against SARS-CoV-2 (9), most have undergone minimal or no external validation or have poorly described validation panels, which hinders assay selection and interpretation of results. In addition, interpretation of serologic assays is limited at present because of critical knowledge gaps. For example, no definite serologic correlates of protection from infection or disease have been identified in humans, and the degree to which these assays cross-react with antibodies against related coronaviruses is poorly described.

We discuss key use cases for SARS-CoV-2 antibody detection tests and their application to serologic studies. We review currently available assays, highlight key areas of ongoing research, and propose potential strategies for test implementation.

METHODS

We searched the MEDLINE Ovid database for articles on SARS-CoV-2 serologic assays (the **Appendix**, available at Annals.org, shows the search strategy). Additional studies were identified by hand-searching references of selected articles, consulting international experts, and searching COVID-19 and SARS-CoV-2 preprints on medRxiv and bioRxiv. This search was last updated on 20 May 2020.

DEFINING KEY USE CASES FOR SEROLOGY IN THE SARS-COV-2 PANDEMIC

Whereas the utility of antibody detection tests for the diagnosis of active COVID-19 is limited (8), serologic assays are crucial for documenting prior infection and the presence of antibodies, which may indicate immunity. **Table 1** (10) shows potential use cases for SARS-CoV-2 antibody testing.

The interpretation of the results of antibody testing for SARS-CoV-2 (**Figure**) can present challenges, owing to uncertainty about 1) whether mild and asymptomatic cases mount a detectable humoral immune response; 2) whether the detection of antibodies correlates with protective immunity; 3) the duration of antibody response and anamnestic responses after infection; and 4) the relative importance of the humoral, cellular, and

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Key Summary Points

Molecular testing on respiratory specimens, the current gold standard for diagnosis of SARS-CoV-2 infection, is hampered by imperfect sensitivity and limited testing capacity.

Antibody testing has potential to aid in particular diagnostic scenarios, such as in RT-PCR negative patients who present later during disease course. Antibody testing should not be used as the sole basis for diagnosis of acute COVID-19.

Appropriately designed seroepidemiologic studies will play an essential part in the public health response to the COVID-19 pandemic by characterizing transmission dynamics, refining disease burden estimates, and providing insight into the kinetics of humoral immunity to SARS-CoV-2.

Validation of novel antibody detection tests for SARS-CoV-2 must pay careful attention to the choice of source populations and reference standards, and to possible cross-reactivity with antibodies to other human coronavirus infections.

Plaque reduction neutralization assays are currently the reference standard for determination of host antibodies capable of inhibiting viral replication, but must be performed in a biosafety level 3 laboratory.

Urgent research is needed to determine the serologic correlates of immunity against SARS-CoV-2.

innate responses. Of note, infection prevalence in the population being tested must always be considered. In patients with clinical features of COVID-19, a highly specific test, such as SARS-CoV-2 polymerase chain reaction (PCR), has a high positive predictive value for true infection. Conversely, if testing asymptomatic individuals when the true seroprevalence of a population is only 5%, an assay with a specificity of 95% would produce a false-positive rate of 50%. Low specificity is particularly problematic in cases where incorrectly identifying an individual as immune could place them at significant risk—for instance, if they were to enter settings with high risk for exposure without appropriate personal protective equipment.

Validating Serologic Tests for a New Pathogen

The sensitivity of a serologic assay can be established by testing sera from patients who have been identified as infected on the basis of a reference standard. However, a single estimate of sensitivity to describe test performance can be difficult to interpret when samples are collected at different time points since infection. Sensitivity estimates will vary according to time since infection in the validation cohort. Early (<7 days since symptom onset) and mid-stage (8 to 14 days) PCR-confirmed cases of COVID-19 will have lower rates of seroconversion than in the later stage (>14 days); thus, antibody tests will have lower sensitivity to detect infection in earlier phases. Likewise, antibody responses may be more easily detectable in severe cases (hospitalized patients) than in mild or asymptomatic infections (11).

Establishing the analytic specificity of SARS-CoV-2 seroassays presents a challenge because of potential for cross-reactivity with antibodies to related coronaviruses (11, 12). To address this, test reactivity thresholds used to define a positive result can be adjusted to optimize the tradeoff between sensitivity and specificity (13). With higher thresholds, sensitivity decreases as cases with low serum antibody levels are categorized as negative, but specificity improves as low amounts of nonspecific antibody are no longer considered positive. Physicochemical assay variables can also be modified so that less specific antibodies, with less "avidity" for the antigen, are excluded. This also improves specificity at some expense to sensitivity. Tests that target IgM, which by its nature can be nonspecific, will probably have increased risk for false-positive results.

Validation of the clinical specificity of a serologic assay requires sera from different types of sources. In the case of COVID-19, sera collected before the end of 2019 are presumed to be seronegative for SARS-CoV-2 (14). The samples chosen should be representative of the population of interest. In addition, individuals known to have been infected with various common pathogens, including other human coronaviruses, but who could not have been infected with SARS-CoV-2, should be evaluated to demonstrate the absence of cross-reactivity. Finally, patients with illnesses known to stimulate high levels of polyclonal antibodies, such as Epstein–Barr virus infection, malaria, or conditions associated with production of rheumatoid factor, can be evaluated for cross-reactivity (15–17). Without these validations, assay specificity will be difficult to establish. Once a particular assay is shown to have high sensitivity and high specificity, this assay can serve as a surrogate "gold standard" for the validation of other assays, as well as a standard for quantitative assays.

The Role of a Reference Standard

To date, most published SARS-CoV-2 serologic assay validations have classified patient sera according to SARS-CoV-2 PCR results (18). Polymerase chain reaction assay is an imperfect comparator for SARS-CoV-2 diagnosis because of variable analytic performance across assays (19), and because PCR sensitivity depends on sample type, quality of sampling, and timing relative to illness onset (4, 20). This can lead to unpredictable directions of bias for seroassay accuracy esti-

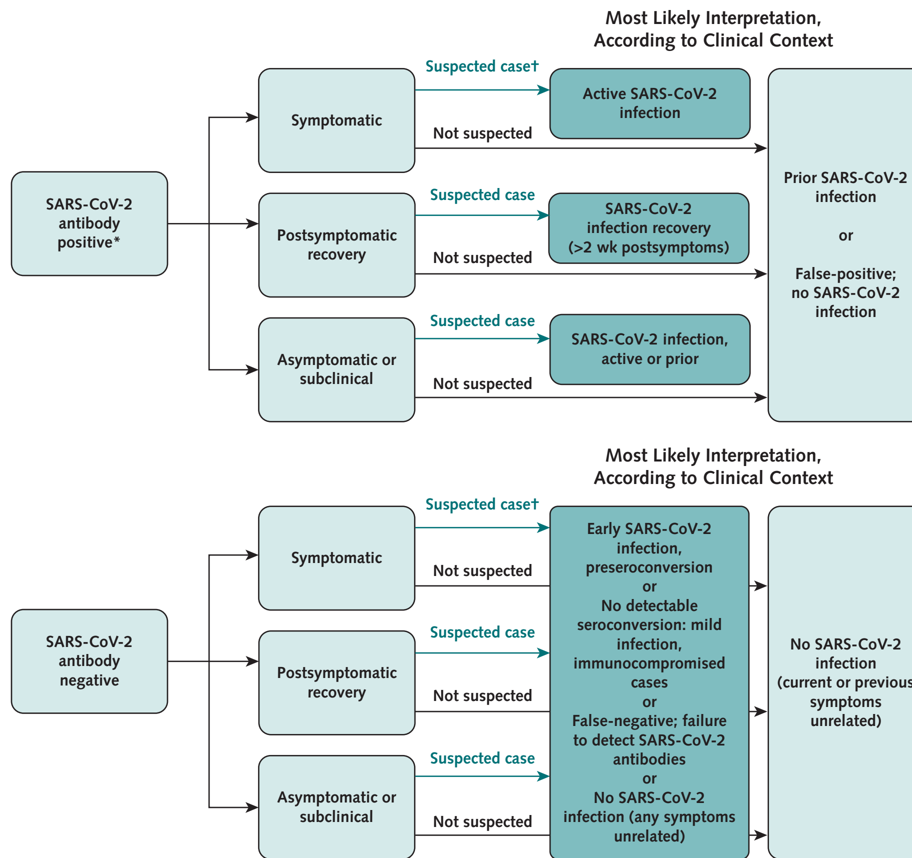
mates. There is an urgent need for validation studies to provide more detail on PCR comparators and on study populations, especially regarding disease severity and timing in the illness course. Furthermore, to enable a better understanding of the diagnostic accuracy of various SARS-CoV-2 serologic tests, the development of reference panels, including seroconversion panels, by using well-characterized sera is necessary.

Table 1. Possible Use Cases for Antibody Detection Tests

Use Case*	Advantages	Limitations	Considerations
Diagnosis			
Aid diagnosis of suspect cases, especially when PCR-negative but radiography or CT is suggestive	May improve overall sensitivity of diagnosis Diagnosis of patients presenting late or for postinfectious syndromes (low viral load) Diagnosis of patients when lower respiratory tract sampling not available	Unlikely to catch early-stage infection (<7 d) May not detect asymptomatic cases Negative test cannot rule out infection IgM appears early, but is less specific	Total antibody may have best sensitivity Should be confirmed by PCR, where possible Rising titers and seroconversion can improve clinical sensitivity and specificity
Aid diagnosis of suspect cases when PCR is not available†	May improve overall sensitivity of diagnosis Diagnosis of patients presenting late or for postinfectious syndromes (low viral load) Diagnosis of patients when lower respiratory tract sampling not available Could enable decentralized or community testing in settings where the availability of PCR testing is limited	Unlikely to catch early-stage infection (<7 d) May not detect asymptomatic cases Negative test cannot rule out infection IgM appears early, but is less specific	Total antibody may have best sensitivity Should be confirmed by PCR, where possible Rising titers and seroconversion can improve clinical sensitivity and specificity
Identification of individuals with protective immune status‡			
Identify convalescent plasma donors	Treatment of critically ill patients	Ideal timing of collection unknown to optimize efficaciousness	Preferentially, patients recovered from moderate to severe disease (high titer); in theory, may be derived from vaccinated donors
Health care and essential worker immunity	Potential to expedite health care (essential) worker resumption of activities after recovery. Self-testing potentially possible	Antibody-positive individuals may still be infectious/shedding virus Antibody neutralization and protection unknown Duration of protection unknown	High-prevalence population Without knowledge of duration of immunity, retesting interval unknown
Individual assessment of immunity	Could enable individuals to understand their personal exposure history and potentially their risk	Antibody-positive individuals may still be infectious/shedding virus Antibody neutralization and protection unknown Duration of protection unknown	Potentially low prevalence population and low positive predictive value Without knowledge of duration of immunity, retesting interval unknown
Public health response and planning			
Situational analysis or seroepidemiologic surveillance to estimate seroprevalence and seroconversion	Estimation of number of people previously infected to inform public health measures Can inform more accurate estimates of infection fatality rate Serial sampling to estimate seroincidence	May require high numbers of tests Choice of population studied and source of serum samples is important to avoid bias in estimates	General population or targeted populations Can be coupled with case-based surveillance programs or to sentinel sites to monitor trends or identify hotspots
Community-based contact tracing§	Objective marker to define chains of transmission or to connect case clusters	May have lower sensitivity in asymptomatic cases Negative test cannot rule out past infection	Negative contact should still self-quarantine for 14 d and monitor for symptoms Less useful as seroprevalence rises in the community
Management of exposed individuals	Potential to expedite allowing general population to return to work or general activities if deemed to be immune Self-testing potentially possible	Serologic correlates of immunity remain to be determined	Requires very high specificity, and possibly confirmatory testing Ethical and legal consequences
Monitoring of essential workers for exposure (with priority given to health care workers)	Decentralized testing Rapid results	Antibody-positive individuals can still be infectious or shedding virus	Requires repeated testing at regular intervals for high-contact/high-risk populations
Assessment of vaccine immunogenicity	Aid vaccine development	May need to be antigen-specific	Specific to vaccination, challenge antigens

CT = computed tomography; PCR = polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome–related coronavirus-2.
* Use cases might evolve over time to include serologic tests as part of clinical management, as knowledge of the SARS-CoV-2–specific immune response grows.
† Requires careful development of interpretive guidelines.
‡ Conditional on identifying correlates of protection for SARS-CoV-2.
§ See reference 10.

Figure. Possible interpretation of antibody test results, based on symptomatology.



The figure shows a decision tree for interpreting antibody test results by symptomatology (symptomatic, postsymptomatic, asymptomatic or subclinical) and whether the patient is a suspected case. It is presumed herein that antibody tests with the highest possible sensitivity and specificity are used, and that the symptomatology is occurring early in the pandemic, when seroprevalence is low and before the availability of a vaccine. For SARS-CoV-2, the accuracy of antibody test results and the appropriate test interpretation both depend on clinical context. In some situations, the clinical context does not enable a single interpretation of the antibody test result. For example, a positive antibody test in a low-risk population could be the result of prior infection, or it could be a false-positive result. Similarly, a negative antibody test in a high-risk population cannot a priori differentiate among preseroconversion, undetectable seroconversion, a false-negative result, or the absence of infection. SARS-CoV-2 = severe acute respiratory syndrome–related coronavirus-2.
* The relationship between positive antibody results and protective immunity will vary among assays and must be validated individually.
† Includes high exposure, high risk, hot spots, and contact tracing.

Assays for the Detection of Host Antibodies to SARS-CoV-2

Coronavirus spike (S) and nucleocapsid (N) envelope proteins are highly immunogenic and constitute important antigenic targets for the development of serologic assays (11, 21). As with SARS-CoV-1, the S protein of SARS-CoV-2 binds to the cell surface angiotensin-converting enzyme 2 (ACE2) receptor (21–23). Host neutralizing antibodies (nAbs) appear to be predominantly directed at the S protein (24). The N protein plays crucial roles in viral replication and assembly, is highly conserved, and induces antibodies sooner than S during infection (6, 25, 26). Commercial SARS-CoV-2 serologic assay development has focused on enzyme immunoassays, such as laboratory-based enzyme-linked immunosorbent assays (ELISAs) and rapid lateral flow assays (LFAs). More complex serum neutralization assays are important as a reference standard and to assess immunity.

Neutralizing Antibody Titers

Only a subset of antibodies raised against a specific antigen have the property of neutralizing viral replication. Neutralization assays, such as plaque reduction and microneutralization methods, provide essential data for the validation of candidate diagnostic tests and to define correlates of protective immunity. The primary drawback of functional assays of SARS-CoV-2 neutralization is that they can only be performed by experienced staff in a biosafety level (BSL) 3 laboratory owing to the need to culture live virus, which increases complexity and cost. Thus, efforts to circumvent these obstacles have converged on finding surrogates of traditional neutralization titers. Live pseudotyped viruses have been developed that incorporate the S protein of SARS-CoV-2, can be cultivated in BSL-2 conditions, and express a reporter enzyme when infecting cells through binding to the ACE2 receptor, thereby allowing for automated quantification (27). Such reporter virus sys-

tems would offer substantial advantages in terms of speed, cost, and scalability while providing a quasi-functional assessment of the host neutralizing antibody response (18). Other groups are striving to create surrogates of neutralization that bypass the need for viral culture through the use of blocking ELISA formats (28).

Laboratory-Based Immunosorbent Assays

For high-throughput and inexpensive (after initial capital outlay) screening in clinical laboratories, relevant antigenic targets can be purified or synthesized, and 1 or more can be incorporated into an ELISA test platform. Specific antibody–antigen reactivity is detected by using enzyme conjugates that produce color changes or other detector labels that can be objectively measured (29). The ELISAs detect antibodies directed at the chosen antigen without regard for their ability to elicit viral neutralization. Thus, interpretation of immune status from ELISA results requires rigorous characterization of the assay with respect to a reference standard. For the moment, this work has not been done for SARS-CoV-2. Furthermore, universal standards for reporting are lacking (some assays produce semiquantitative results, others are qualitative), and assays have variable test detection limits and reproducibility and use different analytes (IgG, IgM, IgA) or combinations thereof, with unclear effect on performance (30). It is thus not surprising that estimates of ELISA test sensitivity and specificity vary widely across assays and even within assays evaluated by different investigators (**Table 2**) (31–47).

Lateral Flow Assays

The LFAs leverage the same capture agents as an ELISA in a lateral flow strip format (48). The lateral flow format enables a simple and fast time to result (10 to 30 minutes), but with tradeoffs in detection that is several-fold less sensitive than their ELISA counterpart, a higher cost per test, and lower throughput (49). For LFAs, follow-up confirmatory testing is typically recommended. Most provide qualitative, visual results subjectively interpreted by the operator. The use of a small instrument reader can increase test sensitivity and may permit quantitative and more reproducible results (50, 51). To enable community-based and home testing, LFAs should be paired with minimally invasive samples, such as finger-prick or oral fluid or swabs, and minimal sample processing.

These tests are ideal for near-patient testing and low infrastructure settings, such as the lower levels of the public health system in low- and middle-income countries (52), where they have been used to effectively screen and triage cases of epidemic and nonepidemic diseases. Particularly where resources are constrained, inexpensive LFAs may be useful to expand diagnostic test capacity. Many SARS-CoV-2 LFA antibody tests are available; however, the performance of these tests is still under evaluation, and their value needs to be carefully weighed depending on the use case. A recent large study found heterogeneous and inconsistent results among 10 LFAs and identified signal interpretation as a major obstacle (41).

DESIGN OF SEROEPIDEMIOLOGIC STUDIES

Population-based seroepidemiologic studies are an important source of evidence about SARS-CoV-2 transmission dynamics and will be critical for informing interventions to mitigate the effects of the COVID-19 pandemic (53). Whereas reports of clinical cases identify persons with acute disease, seroepidemiologic studies identify those who were infected previously, including those who experienced mild disease or subclinical infections and thus may not be subject to biases due to health care–seeking behavior and limitations on eligibility for testing during acute disease. These assessments of seroprevalence overall and in specific groups can be used to estimate important characteristics of the pandemic (54–56). Serologic surveillance studies can also assess the accumulation of persons with antibody responses over time to estimate incidence of SARS-CoV-2 infection (57, 58) and can track age- and jurisdiction-specific disease susceptibility and identify at-risk populations (59). Utilizing standard protocols for the design and implementation of serologic studies (60) and making protocols publicly available can improve scientific rigor and ensure comparability across studies undertaken in different populations. Of note, the WHO Unity studies aim to combine worldwide seroepidemiologic study data (61).

STUDY DESIGN CONSIDERATIONS

Cross-sectional serologic surveillance studies are a key first step toward determining the proportion of a population that has been infected with SARS-CoV-2. When estimating age-specific seroprevalence is the primary aim, the gold-standard study design is the conduct of appropriately powered, cross-sectional, age-stratified, population-representative, randomly sampled, serologic studies in each population of interest. This study design, when implemented appropriately, ensures that the estimates obtained are representative of the population of interest and minimizes the potential that the results may have common sources of bias (62). In addition, many variations of this design are also valuable for estimating age-specific seroprevalence, especially when statistical methods are used that can account for alternative design elements and sources of uncertainty (63). Layering seroprevalence surveys onto other existing observational or interventional studies or utilizing residual sera from blood donors or from routine lab tests can increase feasibility and timeliness of estimating seroprevalence at some risk to generalizability.

To determine SARS-CoV-2 seroincidence, or the proportion of the population seroconverting over a certain time frame, longitudinal studies can be conducted among cohorts of individuals who are at high risk for exposure (such as health care workers) or among those for whom little is known about the risk for infection (such as children). Furthermore, longitudinal serologic surveillance can be implemented to provide insight in situations where prevention and control measures are

Table 2. ELISAs With an FDA EUA as of 20 May 2020, or With Publicly Available Performance Characteristics*

Company and Location	Assay	Target Analyte	Regulatory Status	Sensitivity and Specificity (95% CI), %	Population in Which Assay Was Evaluated	Reference
Abbott Laboratories, United States	Architect SARS-CoV-2 IgG	IgG	FDA EUA	Sensitivity: 100 (95.8–100) Specificity: 99.6 (99.0–99.9)	NA	34
				Sensitivity: 53.1 (39.4–66.3) for 1–7 d since onset to 100 (95.1–100) at 17 d since onset	689 sera from 125 RT-PCR–positive patients	35
				Specificity: 99.9 (NA)	1020 prepandemic sera (from 2018–2019)	
				Sensitivity: 0.0 (0.00–26.47) at <3 d since onset to 93.8 (82.80–98.69) at ≥14 d since onset	103 sera from patients with confirmed SARS-CoV-2 infection	36
				Specificity: 99.4 (96.41–99.98)	153 prepandemic sera collected in 2015	
				Sensitivity: 29.3 (23.7–35.6) at ≤7 d since onset to 96.8 (95.5–97.9) at ≥22 d since onset; overall sensitivity, 80.4 (78.9–81.7)	423 sera from patients with confirmed SARS-CoV-2 infection	37
				Specificity: 99.8 (99.3–100)	1013 sera from prepandemic U.S. blood donors	
Beijing Wantai Biological Pharmacy Enterprise, China	Wantai SARS-CoV-2 Ab ELISA	Total Ab	CE-IVD	Sensitivity: 93.3 (77.9–99.2)	30 sera from hospitalized patients with SARS-CoV-2 infection	38
				Specificity: 100 (95.6–100)	82 prepandemic sera (blood donors or other diseases)	
				Sensitivity: 97.5 (91.3–99.7)	Plasma from 80 hospitalized patients with confirmed SARS-CoV-2 infection	39
				Specificity: 100 (98.8–100)	300 healthy community members	
Bio-Rad Laboratories, United States	Platelia SARS-CoV-2 Total Ab	Total Ab	FDA EUA	Sensitivity: 92.2 (81.5–96.9) Specificity: 99.6 (98.7– 99.9)	NA	34
DiaSorin, United States	LIAISON SARS-CoV-2 S1/S2 IgG	IgG	FDA EUA	Sensitivity: 94.4 (88.8–97.2)	304 sera collected during local epidemic.	40
				Specificity: 97.8 (94.1–99.1)	Compared with SARS-CoV-2 microneutralization assay	
				Sensitivity: 97.6 (87.4–99.6) Specificity: 99.3 (98.6–99.6)	NA	734
Epitope Diagnostics, United States	EDI Novel Coronavirus COVID-19 IgG ELISA kit	IgG	CE-IVD	Sensitivity: 39.3 (21.5–59.4) for 1–5 d since onset, to 90.9 (58.7–99.8) >20 d since onset	128 sera from 79 patients with SARS-CoV-2 infection	41
				Specificity: 90.7 (83.6–95.5)	108 blood donors pre–July 2018	
				Sensitivity: 100 (84.6–100) Specificity: 88.7 (77.0–95.7)	75 sera from hospitalized patients	42
Epitope Diagnostics, United States	EDI Novel Coronavirus COVID-19 IgM ELISA kit	IgM	CE-IVD	Sensitivity: 17.9 (6.1–36.9) for 1–5 d since onset, to 81.8 (48.2–97.7) >20 d since onset	128 sera from 79 patients with SARS-CoV-2 infection	41
				Specificity: 97.2 (92.1–99.4)	108 blood donors pre–July 2018	
EUROIMMUN, Germany	Anti–SARS-CoV-2 ELISA (IgA)	IgA	CE-IVD	Sensitivity: 20.8 (9.3–32.3)	95 hospitalized patients with suspected COVID-19	43
				Sensitivity: 70.0 (34.7–93.3)	10 sera from 3 patients with SARS-CoV-2 infection	44
				Specificity: 94.6 (90.5–97.3)	203 sera from patients with other infections	
				Sensitivity: 93.3 (77.9–99.2)	30 sera from hospitalized patients with SARS-CoV-2 infection	38
				Specificity: 92.7 (84.7–97.3)	82 prepandemic sera (blood donors or other diseases)	
				Specificity: 73.0 (NA)	37 sera from patients with other respiratory infections	45
EUROIMMUN, Germany	Anti–SARS-CoV-2 ELISA (IgG)	IgG	CE-IVD; FDA EUA	Sensitivity: 14.6 (4.6–24.5)	95 hospitalized patients with suspected COVID-19	43
				Sensitivity: 60.0 (26.2–87.8)	10 sera from 3 patients with SARS-CoV-2 infection	44
				Specificity: 96.1 (92.4–98.3)	203 sera from patients with other infections	
				Sensitivity: 66.7 (47.2–82.7)	30 sera from hospitalized patients with SARS-CoV-2 infection	38

Continued on following page

Table 2–Continued

Company and Location	Assay	Target Analyte	Regulatory Status	Sensitivity and Specificity (95% CI), %	Population in Which Assay Was Evaluated	Reference
				Specificity: 96.3 (89.7–99.2)	82 prepandemic sera (blood donors or other diseases)	
				Sensitivity: 90.0 (74.4–96.5) Specificity: 100.0 (95.4–100.0)	NA	34
				Specificity: 91.9 (NA)	37 sera from patients with other respiratory infections	45
				Sensitivity: 86.4 (65.1–97.1) Specificity: 96.2 (87.0–99.5)	75 sera from hospitalized patients	42
				Sensitivity: 0.0 (0.00–26.47) at <3 d since onset to 85.4 (72.24–93.93) ≥14 d since onset	103 sera from patients with SARS-CoV-2 infection	36
				Specificity: 94.8 (89.96–97.72)	153 prepandemic sera collected in 2015	
Guangzhou Darui Biotechnology, China	2019 Novel Coronavirus IgG Test (ELISA)	IgG	RUO	Sensitivity: 23.1 (13.5–35.2)	65 patients with SARS-CoV-2 infection	46
				Specificity: 100 (94.4–100)	64 healthy persons or patients with TB	
Guangzhou Darui Biotechnology, China	2019 Novel Coronavirus IgM Test (ELISA)	IgM	RUO	Sensitivity: 46.1 (33.7–58.9)	65 patients with SARS-CoV-2 infection	46
				Specificity: 78.1 (66.0–87.5)	64 healthy persons or patients with TB	
Mount Sinai Laboratory, United States (17)	COVID-19 ELISA IgG Antibody Test	IgG	FDA EUA	Sensitivity: 92.5 (80.1–97.4) Specificity: 100 (95.1–100)	NA	34
Ortho Clinical Diagnostics, United States	VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Reagent Pack	Total Ab	FDA EUA	Sensitivity: 100 (92.7–100) Specificity: 100 (99.0–100)	NA N/A	34
Roche Diagnostics	Elecsys Anti-SARS-CoV-2	Total Ab	FDA EUA	Sensitivity: 100.0 (92.7–100.0) Specificity: 100 (99.0 –100)	NA	34
				Sensitivity: 83.9 (74.8–90.7)	93 sera from patients with confirmed SARS-CoV-2 infection	47
				Specificity: 100 (99.1–100)	387 retrospective control sera	

Ab = antibody; CE-IVD = Conformité Européenne-In Vitro Diagnostics; COVID-19 = coronavirus disease 2019; EUA = Emergency Use Authorization; FDA = U.S. Food and Drug Administration; NA = not available; RT-PCR = real-time polymerase chain reaction; RUO = research use only; SARS-CoV-2 = severe acute respiratory syndrome–related coronavirus-2; TB = tuberculosis.
* Information from references 31 and 32. A Cochrane collaboration protocol for review of diagnostic assays has been published (33), and data are forthcoming.

changing over time and to evaluate the impact of such measures on the incidence of infection. Studies on the dynamics of humoral responses over time also require longitudinal evaluation.

Numerous other seroepidemiologic study designs can fill in gaps in our knowledge about SARS-CoV-2. For instance, household- or workplace-based serologic studies can aid in the determination of secondary attack rates, especially when the proportion of asymptomatic infections may be high. In addition, well-designed seroepidemiologic studies are critical for informing mathematical models and forecasting tools to guide prevention and control strategies.

Current Knowledge Gaps

The Dynamics and Kinetics of Antibody Responses Over Time

A critical aspect in the interpretation of serologic tests is an understanding of the dynamic nature of the humoral response to SARS-CoV-2 infection. A few studies have defined the kinetics of antibody formation in patients with disease ranging from mildly symptomatic to critically ill. These studies have consistently shown that most patients seroconvert by 2 weeks after the onset of symptoms, and almost all patients have detectable antibodies by day 28 (6, 7, 20, 64, 65). Antibodies can be detected as early as 1 day after illness onset, with peak IgM and IgA titers occurring in the ensuing 7 to 14 days and waning thereafter. The IgG response appears to peak simultaneously in some cases, or slightly later in others (66), and plateaus between 15 and 21 days (6). In some cases, IgG titer declines significantly within weeks (67). Some patients appear to have weak or undetectable seroconversion (44, 66). Illness severity probably affects antibody responses. Critically ill patients had a delayed but more robust formation of IgM and IgG in one study (7). Anti–SARS-CoV-2 responses in subclinical infections have yet to be characterized. Finally, the suitability of alternative specimen types to serum, such as saliva or dried blood spots (68, 69), must be established for SARS-CoV-2 serodiagnostics.

Serocorrelates of Protection

Correlates of protection are empirically derived, specific immune markers associated with protection against infection or disease (53). Seropositivity is often a useful correlate for clinical immunity, though cell-mediated immunity is known to be essential and antibody production is not the sole mechanistic contribution to protection (70). The relationship between seropositivity and immune protection has not yet been established for coronaviruses.

A recent report on 175 patients who recovered from COVID-19 showed that nAb titers were moderately correlated with antibodies binding to S protein domains (24). Surprisingly, 30% of patients developed only low titers of nAbs after recovery, with younger patients (15 to 39 years of age) having significantly lower anti–SARS-CoV-2 and nAb titers. This suggest that innate and adaptive cellular immunity are also likely to play a significant role in viral clearance and immunity to coronaviruses (71).

Little is known regarding seropositivity and risk for reinfection to coronaviruses. In a challenge study with HCoV-229E, healthy volunteers who had lower specific IgG titers at baseline were more likely to develop clinically overt infection (72). After the challenge, specific IgG and nAb peaked at 3 weeks and fell considerably at 12 weeks. One year later, 6 out of 9 previously infected participants became infected after a rechallenge, though they were asymptomatic and the duration of viral shedding was shorter than during the first challenge—suggesting at least partial protection induced by the first infection. Of note, the immune response dynamics after SARS-CoV-1 and Middle East respiratory syndrome-coronavirus (MERS-CoV) infection differ substantially from what was seen with HCoV-229E challenge. Values for IgG and nAb peaked 4 months after SARS-CoV-1 and decreased after 16 months. After MERS-CoV infection, 86% of patients had detectable IgG and nAbs for at least 34 months (11).

Cross-Reactivity With Other Coronaviruses

Evaluations of SARS-CoV-2 serologic assays must account for potential cross-reactivity with other coronaviruses, including the 4 endemic human coronaviruses: HKU1, OC43, NL63, and 229E. A systematic review of antibody-mediated immunity to coronaviruses found that studies of serologic responses to human coronavirus N proteins suggest cross-reactivity within human alphacoronaviruses (229E and NL63) and human betacoronaviruses (OC43 and HKU1), but not between human alpha- and betacoronaviruses (11). The available evidence suggests that natural infections with endemic coronaviruses produce little cross-reactivity to emerging coronaviruses SARS-CoV-1 and MERS-CoV.

Regarding SARS-CoV-2 ELISA using S1 protein epitopes, several pilot studies report positive results with sera from patients with SARS-CoV-1, and a lack of significant cross-reactivity when using sera from small numbers of patients seropositive for the endemic human coronaviruses (18, 44). Data regarding SARS-CoV-2 ELISA based on the N protein are more limited. The specificity of 2 ELISA and 10 lateral flow assays has also been assessed against 108 pre–COVID-19 sera from U.S. patients collected in July 2018, and ranged from 84.3 to 100% (41). Finally, in keeping with these results, a surrogate assay of SARS-CoV-2 viral neutralization tests was found to be highly specific among sera positive for endemic human coronaviruses antibodies but showed some degree of cross reactivity with SARS-CoV-1 positive sera (28). Thus, cross reactivity of SARS-CoV-2 serologic assays may be a concern in areas where SARS-CoV-1 and MERS-CoV circulated widely. Overall, serologic tests based on S protein appear to distinguish between emerging and endemic coronaviruses. Assays based on the N protein can serve as a marker of recent infection but might be expected to cross react more with endemic coronaviruses.

Informing Donor Plasma Studies

Convalescent plasma therapy, as a means of providing "passive" immunity to susceptible individuals and as early therapy after infection, has been used for many viral infections (73). This approach was used in a small number of patients with SARS-CoV-1 and MERS-CoV and has shown promise in a few case series of SARS-CoV-2 infection (74–77). Use of COVID-19 convalescent plasma has been approved in several jurisdictions under the category of an emergency investigational new drug (78).

As a general principle, the efficacy of plasma therapy is a function of several factors, including timing of plasma donation (plasma obtained a few weeks after recovery during convalescence is considered more immunogenic, with higher titers of polyclonal neutralizing antibodies), dosage, and timing of administration in relation to onset of disease in the recipient. For COVID-19, identifying "optimal" donors will prove to be an additional challenge, given the heterogeneity in antibody titers during convalescence and the lack of an established correlation between specific antibody titers and clinical efficacy (79). As an example, in the treatment of influenza, plasma with high nAb titers collected from a nonconvalescent general population did not show efficacy (80, 81), suggesting that donor selection should not be based solely on serologic titers. Eventually, antibody derived from vaccinated donors may deserve further study.

SERODIAGNOSTICS AND VACCINATION

Serologic tests are essential to better understand the determinants of SARS-CoV-2 immunity and to guide vaccine development. For SARS-CoV-1 and MERS-CoV, the S protein was shown to be the most important antigen leading to production of nAbs and inhibition of viral entry into the host cells (82). Since then, S protein has been the major target for vaccine candidates. Previous experience using SARS-CoV-1 subunit vaccine based on the full-length S protein showed potent nAb responses and protective immunity in animal models. However, some of these vaccines were also associated with a harmful immune enhance-

ment, as seen in vaccine candidates for dengue or respiratory syncytial virus, leading to a potentially more severe disease in vaccinated individuals (83). Antibody-dependent enhancement has also been seen among SARS-CoV-1–infected macaques injected with anti-spike IgG (84). For SARS-CoV-1 and MERS-CoV, the receptor-binding domain (RBD) of the S protein was shown to be the major immunodominant region. Subunit vaccines targeting RBD specifically elicited high nAb titers but were not associated with immune enhancement (82, 85).

In SARS-CoV-2–infected patients, among the binding antibodies to the different regions of the S protein (S1, S2, RBD), RBD-specific IgG correlated best with nAbs, suggesting that RBD is be a promising target for SARS-CoV-2 vaccine candidates (24). However, because RBD is the most variable region of the genome (86), there is still a theoretical risk for immunologic "escape," as well as immune enhancement development (87). The N protein, a more conserved region of the genome, has been of interest for SARS-CoV-1 and MERS-CoV vaccine candidates and was thought to be at lower risk for immune enhancement; however, it was not shown to elicit nAbs (82). The role of the N protein in SARS-CoV-2 immune response is still unknown.

In conclusion, the COVID-19 pandemic has revealed several gaps in our diagnostic arsenal and is highlighting the essential role of serodiagnostics as part of our public health response. With the use of carefully validated assays, appropriately designed serologic studies will help characterize transmission dynamics and refine disease burden estimates. Urgent scientific research is needed to link specific serologic variables with immunity against SARS-CoV-2.

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Appendix: Search Strategy

The MeSH search terms used for this review were "Coronavirus" [MeSH]; "Coronavirus Infections" [MeSH]; "Antibodies, Viral" [MeSH]; "Immunoglobulin G" [MeSH]; "Immunoglobulin M" [MeSH]; "Immunoglobulin A" [MeSH]; "Immunity, Humoral" [MeSH]; "Cross Protection" [MeSH]; "Cross Reactions" [MeSH]; "Antibody-Dependent Enhancement" [MeSH]; "Betacoronavirus" [MeSH]; "Coronavirus OC43, Human" [MeSH]; "Coronavirus 229E, Human" [MeSH]; "Coronavirus NL63, Human" [MeSH]; "Severe Acute Respiratory Syndrome" [MeSH]; "SARS Virus" [MeSH]; "Middle East Respiratory Syndrome Coronavirus" [MeSH]; "Seroepidemiologic Studies" [MeSH]; "Serology" [MeSH]; "Serologic Tests" [MeSH]; "Complement Fixation Tests" [MeSH]; "Immunoassay" [MeSH]; "Enzyme-linked immunosorbent assay" [MeSH]; "Fluorescent Antibody Technique" [MeSH]; "Fluoroimmunoassay" [MeSH]; "Blotting, Western" [MeSH]; "Hemagglutination Inhibition Tests" [MeSH]; "Neutralization Tests" [MeSH]; "Sensitivity and Specificity" [MeSH]; "Point-of-Care Testing" [MeSH]; "Antigens" [MeSH]; "Diagnosis, Differential" [MeSH]. Non-MeSH search terms used were "covid", "SARS", "SARS-CoV2", "point-of-care test", "antigen", "analyte", "diagnos", "turn around time", "HCoV-229E", HCoV-HKU1", "HCoV-OC43", "HCoV-NL63", "MERS-CoV", "correlates of protection", "seroprevalence", "seroincidence", "seroepidemiology", "complement fixation", "immunofluorescence assay", "hemagglutination inhibition", "western blot", "plaque reduction neutralization assay", "surrogate virus neutralization test".

We screened the results of the search strategy first by title then by abstract for relevant data. This search was last updated on 20 May 2020.