

Review of SARS-CoV-2 Antigen and Antibody Testing in Diagnosis and Community Surveillance



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KEYWORDS

- COVID-19 • SARS-CoV-2 • Antigen • Antibody • Serology • Vaccine • Pfizer • Moderna

KEY POINTS

- SARS-CoV-2 antigen tests offer short turnaround time and high specificity but the lower sensitivity relative to nucleic acid testing increases the risk of further transmission by patients with false-negative antigen test results.
- Although numerous SARS-CoV-2 antibody detection methods exist, the lack of harmonization and correlation with neutralizing antibodies limits their clinical usefulness.
- SARS-CoV-2 antibody titers are higher after vaccination than after a natural infection, but antibody longevity and the frequency at which vaccine re-immunization will be needed remain unknown.

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), identified as the cause of the coronavirus disease 2019 (COVID-19) global pandemic, is a single-stranded RNA virus belonging to the coronavirus family. It consists of structural spike proteins that interact with angiotensin-converting enzyme 2 receptors to infect host cells, nucleocapsid protein that encapsulates the RNA, and envelope protein that surrounds the nucleocapsid¹. Commercially available antibody assays have predominantly been developed to target antibodies to either the spike or nucleocapsid

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proteins. Although the nucleocapsid protein is highly conserved and less susceptible to genetic variation, the spike protein is the target of neutralizing antibodies, which are hypothesized to correlate with immunity²⁻⁵.

Despite public health efforts to encourage masking, social distancing, and surveillance testing, the SARS-CoV-2 virus continued to spread at an alarming rate. As a result, significant effort was dedicated to the development of vaccines against SARS-CoV-2. After rapid development and deployment, several manufacturers began clinical trials on their vaccine within just months of the sequencing of the SARS-CoV-2 virus⁶. All vaccines available at the time of this publication target the spike protein, and immunocompetent individuals who receive the vaccine develop only antispikes antibodies. In contrast, after a natural infection, both antispikes and antinucleocapsid antibodies are detectable. Additional longitudinal studies are required to determine the longevity of antibodies after a natural infection or vaccination.

The gold standard for diagnosing a SARS-CoV-2 infection is nucleic acid amplification testing with throat or nasopharyngeal swabs⁶. However, difficulty of sample collection, slow turnaround time owing to batch mode testing and limited instrument availability, and supply chain shortages for reagents and consumables limited the ability to produce quick diagnostic results in many laboratories. As a result, several manufacturers developed rapid antigen-based assays for the diagnosis of SARS-CoV-2. Although these tests offer several logistical advantages, including rapid identification of infected individuals and ease of implementation in a nonlaboratory setting, antigen testing is considered less sensitive than molecular diagnostic techniques. Furthermore, the performance may vary considerably depending on whether it is used to diagnose symptomatic individuals, or to screen for asymptomatic individuals⁷.

The essential role of rapid and accurate clinical laboratory testing has been highlighted during the SARS-CoV-2 global pandemic. In this review, we discuss the design and performance characteristics of commercially available antibody platforms. We then review antibody response after natural infection and after vaccination, with an emphasis on development of the 3 vaccines currently authorized for use in the United States (Pfizer-BioNTech, Moderna, and Janssen Biotech, Inc). Finally, we consider the use of antigen testing as an alternative diagnostic tool to nucleic acid testing. Taken together, we emphasize the essential contributions of laboratory medicine professionals in the global effort to detect, contain, and eradicate SARS-CoV-2.

ANTIBODY TESTING

The appearance of and subsequent spread of SARS-CoV-2 has challenged health care systems on a global scale. The accurate and rapid detection of the SARS-CoV-2 virus has propelled the laboratory community, particularly molecular pathology and microbiology laboratories, into the spotlight. As the pandemic has grown and evolved, new assay modalities focusing on the human host's adaptive immune response to SARS-CoV-2 have become available.

By April of 2020 the US Food and Drug Administration (FDA) began to grant emergency use authorization (EUA) for a limited number of immunoassays designed to detect the presence of antibodies specific to the SARS-CoV-2 virus⁸. Importantly these serology tests are not designed to detect current infection with the SARS-CoV-2 virus, because the immunoglobulins specific to viral proteins may not have developed in the time between infection and symptom onset. Consequently, a diagnosis of active SARS-CoV-2 infection is best achieved using nucleic acid techniques, or via specific detection of SARS-CoV-2 viral proteins.

Despite these limitations, numerous *in vitro* diagnostic companies and clinical laboratories devoted considerable resources to develop serologic methods to detect SARS-CoV-2 specific IgM and IgG antibodies with the expectation that these assays may fill an unmet laboratory testing need.

Assay Format

At the time of this report, 52 assays have attained EUA from the FDA, with the exclusion of point-of-care lateral flow immunoassays and laboratory developed tests. These EUA SARS-CoV-2 serology assays fall into 2 general methodologic categories, antibody isotype specific, and nonspecific or total immunoglobulin assays (**Fig. 1**, **Table 1**). Within the isotype-nonspecific and -specific categories, there are also methodologic differences in the antigen immobilization scheme used, with assays using microparticle or paramagnetic/magnetic particles predominating over the more traditional microwell- or plate-based formats.

Given the close homology of SARS-CoV-2 proteins with those of other coronaviruses, including the SARS-CoV-1 virus that caused the more limited SARS outbreak between 2002 and 2004, there was concern that serology assays would be subject to frequent false-positive results owing to prior exposure to related human coronaviruses (HCoV). The spike proteins of the related betacoronaviruses infecting humans display varying degrees of sequence homology (SARS-CoV-1 = 76%, Middle Eastern respiratory syndrome [MERS]-CoV = 42%, HCoV-OC43 = 30%, and HCoV-HKU1 = 29%). Although HCoV-OC43 and HCoV-HKU1 are endemic and seroprevalence is high, the sequence differences in spike proteins between them and SARS-CoV-2 are sufficient to prevent measurable or significant cross-reactivity⁹. Although there is predicted to be intermediate cross-reactivity between spike antibodies to SARS-CoV-1 and MERS-CoV for SARS-CoV-2 spike serology assays, the low seroprevalence for these 2 coronaviruses cause them to be less of a concern¹⁰.

The low cross-reactivity for spike proteins from human betacoronaviruses was a welcomed discovery, and this feature also extends to assays targeting the SARS-CoV-2 nucleocapsid protein. Although the nucleocapsid protein of the closely related SARS-CoV-1 virus and MERS-CoV display homology with SARS-CoV-2, the amino acid sequence differences between SARS-CoV-2 nucleocapsid and the other endemic coronaviruses^{11–13} are sufficient to limit predicted cross-reactivity.

Data from subsequent publications examining available serologic assays from *in vitro* diagnostic manufacturers has confirmed these predictions. These publications demonstrated very low false-positive rates across multiple test manufacturers and platforms by testing sera from either well-characterized SARS-CoV-2 polymerase chain reaction (PCR)-negative patients, or samples collected before December 2019, the generally accepted date associated with SARS-CoV-2 spread^{14–17}.

These articles also characterized the persistence of antibody up to 200 days after a positive SARS-CoV-2 PCR test result¹⁵. When the data of these publications are normalized to the number of days after a positive SARS-CoV-2 PCR test, the *in vitro* diagnostic platforms examined (**Table 2**) display sensitivities ranging from 75% to 99%^{14–17}.

Further, these articles also provide indirect evidence as to the clear lack of usefulness in diagnosing active SARS-CoV-2 infection. The sensitivity for detecting a specific antibody response at approximately 7 days after a positive SARS-CoV-2 PCR result ranged from 58% to 96%. This wide range in sensitivity is surprising, particularly in that the same platform in one publication displayed a sensitivity of 59% between 3 and 7 days post positive PCR result, whereas in another the sensitivity was reported as 96% for samples collected less than 7 days after a positive PCR test^{15,17}. One

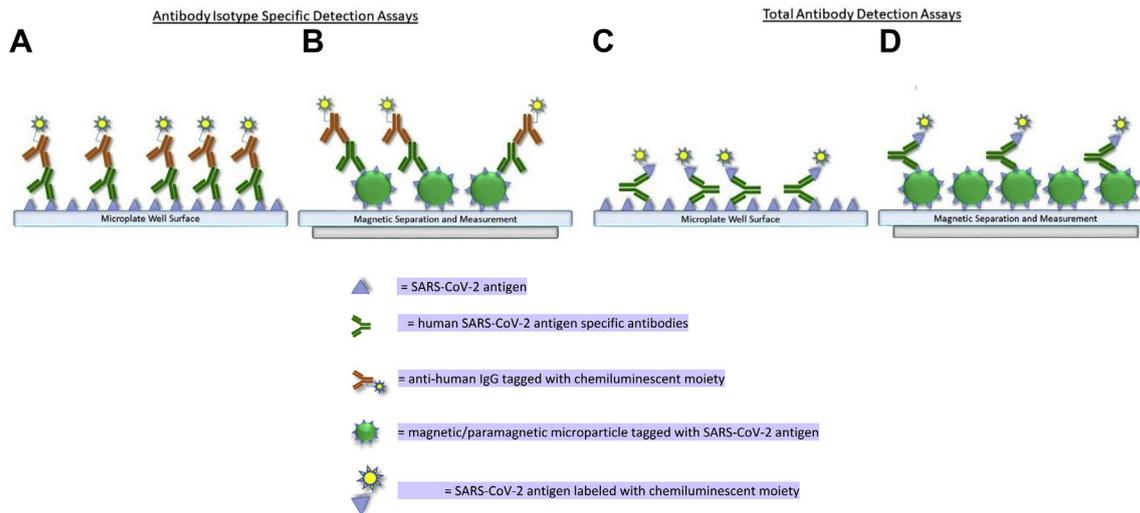


Fig. 1. SARS-CoV-2 serology assays fall into 2 distinct groups, those that are antibody isotype specific (**A**, **B**), and those that are total antibody assays that cannot distinguish between IgA, IgG, and IgM antibody isotype (**C**, **D**). The assays also differ in regards to separation technology with SARS-CoV-2 antigen immobilized on a microwell or microtiter plate (**A**, **C**), or a paramagnetic or magnetic microparticle (**B**, **D**).

Table 1
SARS-CoV-2 Serology Assays

Test Name	Antibody Target	Antibody Isotype(s)	Assay Format^d	Qualitative (Q) or Semiquantitative (SQ)
Abbott AdviseDX SARS-CoV-2 IgG II	RBD	IgG	B	SQ
Abbott AdviseDX SARS-CoV-2 IgM	Spike	IgM	B	Q
Abbott SARS-CoV-2 IgG ^a	Nucleocapsid	IgG	B	Q
Beckman Coulter Access SARS-CoV-2 IgG	RBD	IgG	B	Q
Beckman Coulter Access SARS-CoV-2 IgG II	RBD	IgG	B	SQ
Beckman Coulter Access SARS-CoV-2 IgM II	RBD	IgM	B	Q
bioMerieux VIDAS SARS-CoV-2 IgG	RBD	IgG	A	Q
bioMerieux VIDAS SARS-CoV-2 IgG	RBD	IgM	A	Q
Bio-Rad Platelia SARS-CoV-2 Total Ab	Nucleocapsid	IgA, IgG, IgM	C	Q
DiaSorin Liaison SARS-CoV-2 IgM	RBD	IgM	B	Q
DiaSorin Liaison SARS-CoV-2 S1/S2 IgG	S1 and S2	IgG	B	Q
Diazyme DZ-Lite SARS-CoV-2 IgG	Nucleocapsid and spike	IgG	B	Q
Diazyme DZ-Lite SARS-CoV-2 IgM	Nucleocapsid and spike	IgM	B	Q
Euroimmun Anti-SARS-CoV-2	S1	IgG	A	Q
IDS SARS-CoV-2 IgG	Nucleocapsid and spike	IgG	B	Q
Inova Diagnostics, QUANTA Flash SARS-CoV-2 IgG	Nucleocapsid and spike	IgG	B	SQ
Luminex xMAP SARS-CoV-2 Multi-Antigen IgG Assay	S1, RBD, nucleocapsid	IgG	B	Q
Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 IgG	Spike	IgG	A	Q
Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 Total	Spike	IgA, IgG, IgM	C	Q
Phadia AB ELIA SARS-CoV-2-Sp1 IgG	Spike	IgG	A	SQ
Roche Elecsys Anti-SARS-CoV-2	Nucleocapsid	IgA, IgG, IgM	D	Q
Roche Elecsys Anti-SARS-CoV-2 s	Spike	IgA, IgG, IgM	D	SQ

(continued on next page)

Table 1
(continued)

Test Name	Antibody Target	Antibody Isotype(s)	Assay Format ^d	Qualitative (Q) or Semiquantitative (SQ)
Siemens Healthcare Diagnostics SARS-COV-2 IgG ^b	RBD	IgG	B	SQ
Siemens Healthcare Diagnostics SARS-CoV-2 Total ^b	RBD	IgA, IgG, IgM	D	SQ
Siemens Healthcare Diagnostics Dimension/Dimension EXL SARS-CoV-2 IgG ^c	RBD	IgG	Other	SQ
Siemens Healthcare Diagnostics Dimension/Dimension EXL SARS-CoV-2 Total ^c	RBD	IgA, IgG, IgM	Other	Q
Thermo Fisher OmniPATH COVID-19	Spike	IgA, IgG, IgM	C	Q
Zeus Scientific ELISA SARS-CoV-2 IgG	Spike and nucleocapsid	IgG	A	Q
Zeus Scientific ELISA SARS-CoV-2 Total	Spike and nucleocapsid	IgA, IgG, IgM	C	Q

Abbreviations: RBD, receptor binding domain; S1, S1 domain of SARS-CoV-2 spike protein; S2, S2 domain of SARS-CoV-2 spike protein.

^a Abbott Alinity i SARS-CoV-2 and Abbott ARCHITECT SARS-CoV-2 use the same assay format and are shown here in one row.

^b Siemens ADIVA Centaur and Atellica assays utilize the same assay format and are shown here in one row per assay format (IgG vs Total Immunoglobulin).use

^c Siemens Dimension and Dimension EXL SARS-CoV-2 assays use the LOCI (luminescent oxygen channeling assay) technology not depicted in [Fig. 1](#).

^d Assay format described in Figure 1.

Assay Manufacturer	Sensitivity (%)	References
Abbott SARS-CoV-2 IgG	81 ^a –99	(Poore et al, ¹⁵ 2021; Tang et al, 2020)
Beckman Coulter Access SARS-CoV-2 IgG	78	(Poore et al, ¹⁵ 2021)
Diazyme DZ-Lite SARS-CoV-2 IgG	96	(Suhandynata et al, ¹⁶ 2020)
Euroimmun Anti-SARS-CoV-2	75 ^a	(Tang et al, ¹⁷ 2020)
Roche Elecsys Anti-SARS-CoV-2	87–96	(Poore et al, ¹⁵ 2021; Suhandynata et al., 2020)

^a Sensitivity defined as positive serology result at either ≥ 14 d or ≥ 15 d after a positive SARS-CoV-2 PCR result.

possible explanation for the discrepancy in sensitivity is a difference in time between symptom onset and performance of the PCR test.

Serology Correlation with Neutralizing Antibody Titer

Of the numerous hurdles limiting the clinical usefulness of SARS-CoV-2 serology testing are the lack of assay harmonization or international standardization, as well as the currently sparse data correlating serology antibody result with neutralizing antibody titer. In 2 recent publications, this question of correlation of serology result to neutralizing titer was examined^{18,19}. Both publications demonstrated that a positive correlation exists between automated serology assay signal and neutralizing antibody titer. The limitation of these studies is that neither article contains data from quantitative or semiquantitative serology assays, but rather rely on the ratio of assay signal to a positive calibrator or cut-off signal.

In the publication by Tang and colleagues¹⁹, the authors demonstrate that at a neutralizing titer of 1:64, the positive percent agreement (PPA) for the Abbott, Roche, and Euroimmun assays are 96%, 100%, and 92%, respectively, when using the manufacturers' specified positive ratio thresholds. At the manufacturer specified positive thresholds the negative predictive agreement of the 3 were 50%, 70%, and 47%, respectively. The authors also calculated assay-specific ideal ratios for the 3 assays which slightly decreased the PPA, but increased the negative predictive agreement for each assay.

In an article by Suhandynata and colleagues²⁰, the authors examined the PPA between 3 commercially available serology assays as well as a neutralization assay titer of 50. Samples testing positive on both the Diazyme and Roche assays or the Diazyme and Abbott assays had PPA values of 79.2% and 78.4%, respectively. Unfortunately, owing to the methodologic differences between the 2 articles, a direct comparison of data is not possible.

These early studies correlating automated serology assay signal with the neutralization titer are encouraging; however, additional studies using semiquantitative or quantitative assays are needed to determine if a universal threshold indicating immunity is possible.

ANTIBODY RESPONSE

Early publications supported a classic viral response pattern after infection with SARS-CoV-2^{21,22}. In this model, IgM antibodies are first detected within 1 week after infection, and IgG antibodies develop several days after that. Instead, IgG antibodies

to SARS-CoV-2 appear before or at the same time as IgM antibodies^{23,24}. In the classic viral response model, IgG antibodies were predicted to increase over time, peak around 1 month after an infection, and remain detectable for 1 to 6 years²². Although antibody longevity may not be confirmed for several years, many research groups have investigated the time to initial antibody detection and ongoing studies are monitoring antibody response over time.

Determining antibody seroconversion rates after a SARS-CoV-2 infection can be difficult because the exact day of infection is often unknown. The window period between infection and the presence of detectable antibodies ranged from less than 4 days to several weeks after a confirmed infection^{16,23–27}. Seroconversion kinetics may also depend on disease severity. People with mild or asymptomatic infections generally had a weaker immune response than those with symptomatic or severe infection^{23,26}. However, it has also been reported that asymptomatic patients seroconvert more quickly compared with those who are symptomatic²⁷. Interindividual variation combined with the novelty of the SARS-CoV-2 virus have contributed to an incomplete understanding of the humoral response.

As described elsewhere in this article, the antibody response pattern over time varies widely with the analytical platform being used^{14–17}. However, differing antibody trends are at least partially attributed to whether antinucleocapsid or antispike antibodies are being monitored. Previous reports have demonstrated that, although nucleocapsid antibodies continue to increase over time, antibodies to the spike protein begin to decrease within 1 to 4 months after symptom onset^{15,28,29}. This phenomenon has been observed on several different test platforms. Despite this finding, antibodies to both the spike and nucleocapsid antibodies remain detectable for several months after infection. Future studies should continue to monitor the longevity of antibodies to both the nucleocapsid and spike proteins after natural infection.

Neutralizing antibodies can block infection by the SARS-CoV-2 virus. Because the spike protein engages angiotensin-converting enzyme 2 receptors to initiate infection, antibodies to the spike protein have been theorized to correlate with antibody neutralization^{5,19,20}. After a natural infection, spike antibodies were moderately associated ($R^2 = 0.46$) with neutralizing antibody titers but suffered from a poor negative percent agreement¹⁹. Interestingly, the correlation between antinucleocapsid antibodies with neutralizing antibodies was assay dependent, but ranged from a coefficient of determination of 0.29 to 0.47. After vaccination, antibodies to the spike protein also exhibited a moderate association ($R^2 = 0.39$) with neutralizing titers²⁰. Current vaccinations only elicit a humoral response against the spike protein of SARS-CoV-2 and, therefore, would not have any association with nucleocapsid antibody response.

As a result of the COVID-19 global pandemic, vaccine development through multinational collaborations has occurred at an unprecedented rate. Antibody response after vaccination will be an important consideration when establishing reimmunization intervals. At the time of this publication, all vaccines currently granted EUA by the World Health Organization target the spike protein and, therefore, only elicit antispike antibodies. In these cases, vaccination status should be monitored with an antispike assay. However, there are vaccines currently under development that contain live, attenuated, or inactivated virus that would induce both a nucleocapsid and spike antibody response, similar to a natural infection³⁰. In these cases, vaccination antibody response could be monitored with either an antispike or antinucleocapsid assay and would remain indistinguishable from a natural infection. Currently available vaccines result in antibody titers that exceed those seen after a natural infection^{31–33}. Although this robust response is promising, antibody longevity after vaccination remains unknown and may vary with vaccine technology. Understanding how each

vaccine works will be an important consideration when monitoring antibody titers to determine reimmunization frequency.

COVID-19 VACCINES

After the COVID-19 outbreak in December 2019, the entire genomic sequence of SARS-CoV-2 was published in January 2020. By March 2020, vaccines were developed, shipped to appropriate testing sites, and early phase clinical and preclinical trials were underway. As of June 2021, nearly 300 vaccine candidates were under clinical or preclinical development³⁰. These vaccines use a variety of technologies, including adenovirus vectors, viral-like particles, inactivated or attenuated virus, and synthetic DNA or RNA. Dosing schemes range from 1 to 3 doses given over zero to 56 days. Although more than a dozen vaccines have already been cleared for emergency use by the World Health Organization, only 3 vaccines were granted FDA EUA status for use in the United States. These vaccines are manufactured by Pfizer-BioNTech, Moderna, and Janssen Biotech, Inc.

Pfizer-BioNTech Vaccine

Pfizer-BioNTech produced 2 RNA-based vaccines formulated in lipid nanoparticles. The first, BNT162b1, encoded a trimerized SARS-CoV-2 receptor-binding domain of the spike glycoprotein whereas the second, BNT162b2, encoded a membrane-anchored full-length spike glycoprotein stabilized in the prefusion conformation^{31,34,35}. During phase I clinical trials, immunogenicity and safety were monitored for various dosing paradigms of BNT162b1 and BNT162b2. Although both vaccines were found to be effective and exhibited tolerability profiles similar to other messenger RNA (mRNA)-based vaccines³⁶, BNT162b2 had a milder systemic reactogenicity profile while exhibiting a similar antibody response to BNT162b1. Both vaccines exhibited a strong dose-dependent response, and the 2-dose series of 30 µg BNT162b2 vaccine was selected as the candidate to advance to phase II and III clinical trials³¹.

The Pfizer-BioNTech BNT162b2 vaccine was submitted for EUA to the FDA on November 20, 2020³⁷. The vaccine series consisted of 2 doses given 21 days apart. The submission included data from ongoing clinical trials consisting of 44,000 participants and the vaccine was found to be 95% effective at preventing SARS-CoV-2 infection with no major safety concerns identified. On December 11, 2020, this became the first COVID-19 vaccine to receive EUA clearance from the FDA in the United States. In May 2021, the FDA expanded the EUA to allow for vaccination in children as young as 12 years of age.

Moderna Vaccine

Similar to Pfizer-BioNTech BNT162b2, the Moderna mRNA-1273 vaccine is also a lipid nanoparticle-encapsulated mRNA-based vaccine encoding a stabilized prefusion trimer of the spike glycoprotein. In early clinical trials, participants received 2 injections of either a 25, 100, or 250 µg dose 28 days apart^{32,38}. Two doses were deemed necessary to elicit sufficient pseudovirus neutralizing activity. The median antibody response was similar between the 100 µg and 250 µg dose groups but the 100 µg dose group had a more favorable reactogenicity profile. Therefore, the 100 µg dose was chosen to advance into additional clinical trials.

Moderna submitted the mRNA-1273 COVID-19 vaccine (2 doses of 100 µg, administered 1 month apart) to the FDA for EUA on November 30, 2020, for adults 18 years of age or older³⁹. The phase III clinical trials consisted of 30,400 participants and demonstrated a greater than 94% efficacy at preventing COVID-19⁴⁰. Adverse reactions were

reported frequently, but were considered mild, with injection site arm pain, fatigue, and headache being the most common. One week after Pfizer-BioNTech, the Moderna mRNA-1272 vaccine was issued EUA from the FDA for use in adults on December 18, 2020.

Janssen Biotech, Inc, Vaccine

The Janssen Biotech, Inc (Johnson & Johnson) Ad26.COVS vaccine is an adenovirus vector encoding a variant of the SARS-CoV-2 spike protein. Beginning in July 2020, Janssen conducted multicenter phase I clinical trials with doses of 5×10^{10} or 1×10^{10} viral particles per milliliter given as a single dose or on a 2-dose schedule^{33,41}. All dosing schemes had an acceptable safety and reactogenicity profile and more than 90% of participants demonstrated the presence of both S-binding and neutralizing antibodies after a single dose of either potency vaccine. Interestingly, adverse events were more common after the first dose, a finding that contrasted both the Pfizer-BioNTech and Moderna mRNA-based vaccines³³. Although a second vaccine dose exhibited slightly increased immunogenicity, Janssen recognized the logistical advantages of a single-dose vaccine and decided to proceed with a single dose of the 1×10^{10} viral particles per milliliter vaccine in phase III clinical trials. This decision was further supported by nonhuman primate studies that demonstrated complete or near-complete protection against SARS-CoV-2⁴². More recently, it was discovered that the Ad26.COVS vaccine may offer some protection against other SARS-CoV-2 variants of concern⁴³.

Janssen Biotech, Inc, submitted the Ad26.COVS single dose vaccine consisting of 5×10^{10} viral particles for EUA by the FDA on February 4, 2021⁴⁴. Phase III clinical trials of more than 40,000 participants demonstrated that this vaccine was both safe and at least 66% effective in preventing the development of COVID-19 in adults 18 years of age or older. On February 27, 2021, the FDA issued an EUA for the Janssen the Ad26.COVS single dose vaccine, making it the third approved for use in the United States. However, on April 23, 2021, the FDA amended the EUA to include information about the rare occurrence of cerebral venous sinus thrombosis in women after vaccination.

As more vaccines are developed, it will become increasingly important to monitor reactogenicity and immunogenicity in addition to efficacy. Differences in immune response and frequency of adverse events may help to personalize vaccine selection, with potent vaccines preferred in individuals who may produce a suboptimal immune response, and single-dose vaccines preferred for those who are prone to adverse reactions after vaccination.

SARS-CoV-2 ANTIGEN TESTING

Nucleic acid amplification tests detecting the presence of SARS-CoV-2 RNA are considered the gold standard for the diagnosis of symptomatic patients by the Centers for Disease Control and Prevention⁴⁵. Although these assays offer optimal sensitivity and specificity, their implementation can be hindered by the limited availability of reagents or other consumables, they require capital investment in the necessary instrumentation, and they must be performed by specialized and highly trained laboratory personnel. Owing to the centralized nature of this testing model, results are often returned several hours or days after specimen collection, complicating efforts to limit further viral spread.

To overcome these limitations, several manufacturers have developed rapid, lateral flow devices that detect SARS-CoV-2 antigen (typically the nucleocapsid protein) in

nasal or nasopharyngeal swabs with results available in 15 to 30 minutes. Briefly, viral proteins present on the test swab are suspended by mixing in a buffer solution, which is transferred to the test cartridge. Labeled antibodies bind to the viral protein of interest and the buffer-suspended antibody–antigen complex migrates through an internal membrane toward the test and control lines. The test line consists of a capture antibody attached to the solid phase that recognizes a different epitope on the viral protein, immobilizing the antigen detector–antibody complex and forming a visible line. Any unbound detector antibody flows past the test line and accumulates at the control line, indicating a valid test. Result interpretation varies by device, but typically follows 1 of 2 models. In the first, after the introduction of patient sample, each device is placed into a reader that evaluates the signal intensity at the test and control lines and produces a digital “detected” or “not detected” result. In the second, test and control line signal intensity is evaluated visually after a manufacturer-defined incubation period. Result interpretation outside of this window can lead to erroneous results.

Although these rapid antigen devices are not considered the gold standard for SARS-CoV-2 diagnosis, they may offer several advantages, including limited expense, capacity for rapid implementation without extensive infrastructure, short turnaround time, and high specificity in populations with a high prevalence of disease. At the time of release for clinical use, the performance of these devices was largely unknown beyond the manufacturers’ validation studies described in the package inserts. However, after implementation in a variety of clinical settings, several recent publications have described the performance characteristics of rapid antigen tests relative to concurrently performed nucleic acid testing as the reference method.

BinaxNOW

The Abbott BinaxNOW COVID-19 antigen card is a lateral flow device granted EUA by the FDA in August 2020. Results are interpreted visually between 15 and 30 minutes after the introduction of the resuspended patient sample to the test card. Analytical sensitivity has been estimated to fall between 4 and 8×10^4 copies per swab, roughly approximating a generic reverse transcriptase (RT)-PCR cycle threshold value of 29 to 30⁴⁶.

One study compared the performance of the BinaxNOW lateral flow antigen test to the Thermo Fisher TaqPath COVID-19 Combo kit in 2645 asymptomatic students at the University of Utah using 2 concurrent nasal swabs self-collected by study participants under the supervision of trained, nonmedical personnel⁴⁷. Antigen testing was performed at the collection site by trained nonmedical personnel while RT-PCR testing was performed at a reference laboratory. SARS-CoV-2 RNA was detected by RT-PCR in 1.7% of the study participants. Relative to RT-PCR, the BinaxNOW antigen test demonstrated a sensitivity of 53.3% and a specificity of 100%.

A second study summarized the performance of the BinaxNOW antigen device relative to the Clinical Research Sequencing Platform (CRSP) SARS-CoV-2 RT-PCR assay in specimens collected at a drive-through community testing site in Massachusetts⁷. Two nasal swabs were collected from each participant by trained medical personnel, with 1 swab used to perform BinaxNOW testing on-site in a dedicated testing tent while the other swab was sent to an off-site reference laboratory for RT-PCR testing. In this study population, 974 of 1380 adults (71%) and 829 of 928 children (89%) were asymptomatic. Among symptomatic participants, the BinaxNOW device demonstrated a sensitivity of 96.5% in adults and 84.6% in children (ages 7–17 years) and a specificity of 100% in both age groups. Among asymptomatic participants, the sensitivity was 70.2% in adults and 65.5% in children and the specificity was 99.6% in adults and 99.0% in children.

A third study compared the BinaxNOW antigen device to either the CDC or Fosun SARS-CoV-2 RT-PCR assay using concurrently collected nasal (antigen) and nasopharyngeal (RT-PCR) samples from participants in 2 community testing centers in Pima County, Arizona⁴⁸. Antigen testing was performed on site according to the manufacturer's instructions and RT-PCR testing was performed within 24 to 48 hours at an off-site laboratory. Of the 3419 participants, 827 (24.2%) reported at least 1 symptom consistent with SARS-CoV-2 infection. SARS-CoV-2 RNA was detected in 161 participants (4.7%): 113 of 827 were symptomatic (13.7%) and 48 of 2592 were asymptomatic (1.9%). In symptomatic participants presenting within 7 days of symptom onset, the sensitivity and specificity of the BinaxNOW device were 71.1% and 100%, respectively. In asymptomatic participants, the sensitivity and specificity were 35.8% and 99.8%, respectively.

Sofia

The Quidel Sofia SARS-CoV-2 lateral flow antigen assay was granted EUA in May 2020 and is intended for use within 5 days of symptom onset. Results are introduced into a device reader that reports digital results as positive or negative between 15 and 30 minutes.

One study performed on 2 college campuses in Wisconsin compared the performance of the Sofia antigen device to either the Centers for Disease Control and Prevention or Thermo Fisher TaqPath COVID-19 Combo RT-PCR assays⁴⁹. At University A, all persons tested for screening or diagnostic purposes were eligible to participate in the study. At University B, participation was limited to only those quarantined after a known COVID-19 exposure. Two concurrent nasal swabs were collected from participants in both groups (medical personnel collect at University A, self-collect at University B). Limited information was provided regarding the logistics of antigen test performance, with the authors indicating only that testing was performed according to the manufacturer's instructions. Of the 1098 participants, 227 (20.7%) reported at least 1 symptom; 871 (79.3%) were asymptomatic. Overall RT-PCR positivity was 5.2% (40 symptomatic and 17 asymptomatic participants). The sensitivity, specificity, positive predictive value, and negative predictive value were 80.0%, 98.9%, 94.1%, and 95.9%, respectively, in the symptomatic group and 41.2%, 98.4%, 33.3%, and 98.8%, respectively, in the asymptomatic group.

A second study evaluated the performance of the Sofia antigen device in the emergency department of a tertiary medical center in Los Angeles, California⁵⁰. Paired nasal (antigen) and nasopharyngeal (RT-PCR) specimens were collected by medical personnel for all patients admitted to the hospital through the emergency department. RT-PCR testing was performed using the Fulgent COVID-19 assay and antigen testing was performed on-site in the emergency department. Of the 2039 participants, 307 (15.1%) reported at least 1 symptom. SARS-CoV-2 RNA was detected in 68 of 307 symptomatic participants (22.1%) and 81 of 1732 asymptomatic participants (4.7%). Relative to RT-PCR, the sensitivity and specificity of the Sofia antigen test were 72.1% and 98.7%, respectively, in the symptomatic group and 60.5% and 99.5%, respectively, in the asymptomatic group.

A third study evaluated the performance of the Sofia antigen device relative to the Hologic Aptima SARS-CoV-2 TMA assay in symptomatic individuals presenting to an urgent care center in West Bend, Wisconsin⁵¹. Concurrently collected nasal (antigen) and nasopharyngeal samples (TMA) were collected by medical staff in the urgent care center, with both specimens sent to a clinical laboratory for testing. SARS-CoV-2 RNA was detected in 18% of symptomatic patients seen in the clinic in the month before the implementation of antigen testing and this positivity rate remained constant

throughout the study period. Of the 298 patients tested within 5 days of symptom onset, the sensitivity, specificity, positive predictive value, and negative predictive value were 82.0%, 100%, 100%, and 96.5%, respectively. Of the 48 patients tested more than 5 days after symptom onset, antigen test performance decreased, with a sensitivity, specificity, positive predictive value, and negative predictive value of 54.5%, 97.3%, 85.7%, and 87.8%, respectively.

Becton Dickinson Veritor

The Becton Dickinson (BD) Veritor lateral flow antigen device was granted EUA in July 2020 and is intended for use with nasal swabs collected from patients suspected of SARS-CoV-2 infection within 5 days of symptom onset. Results are generated by a cartridge reader at least 15 minutes after introduction of the sample to the test device and are reported qualitatively as positive or presumptive negative.

One study evaluated the performance of the BD Veritor device relative to the Simplexa COVID-19 Direct EUA RT-PCR assay in paired nasal (antigen) and nasopharyngeal (RT-PCR) samples from 1384 patients with known SARS-CoV-2 exposure within 5 days of symptom onset presenting to a hospital system in Winston-Salem, North Carolina⁵². Antigen testing was performed at the site of collection according to the manufacturer's instructions, whereas RT-PCR testing was performed in a central laboratory. SARS-CoV-2 RNA was detected in 116 of 1384 specimens (8.4%). Relative to RT-PCR, the BD Veritor demonstrated a sensitivity, specificity, positive predictive value, and negative predictive value of 66.4%, 98.8%, 83.7%, and 97.0%, respectively.

A second study with 2 parts evaluated the BD Veritor relative to the Lyra RT-PCR assay in concurrently collected nasal (antigen) and nasopharyngeal or oropharyngeal swabs (RT-PCR) in 251 symptomatic individuals within 7 days of symptom onset presenting to 21 geographically diverse study locations (part 1)⁵³. RT-PCR testing was performed at a commercial reference laboratory and antigen testing was performed at a laboratory operated by the device manufacturer. SARS-CoV-2 RNA was detected in 38 of 251 part 1 study participants (15.1%). In participants with 2 or more symptoms, the sensitivity, specificity, positive predictive value, and negative predictive value were 88%, 100%, 100%, and 97.3%, respectively. In participants with 1 symptom, values were 67%, 100%, 100%, and 97.7%, respectively. In part 2, concurrently collected nasal swabs from 377 symptomatic participants at 5 study sites were tested at an off-site commercial reference laboratory on the BD Veritor and Sofia devices according to the manufacturer's instructions. Using the Sofia device as the reference method, the BD Veritor demonstrated a sensitivity and specificity of 97.4% and 98.1%, respectively.

Effectiveness of Antigen Testing in Controlling Viral Spread

Many have advocated for the implementation of serial SARS-CoV-2 antigen testing to facilitate rapid identification of infected individuals and permit timely self-quarantine to prevent further viral spread. Although antigen testing is consistently less sensitive than molecular diagnostic techniques, the short turnaround time and capacity for repeated testing may support efforts at viral containment more effectively than single sample nucleic acid testing with a long turnaround time. To date, relatively few studies have tested this hypothesis.

One publication describing the implementation of the Sofia antigen device in routine monitoring of intercollegiate athletes documented 2 separate SARS-CoV-2 outbreaks attributed to false-negative antigen test results⁵⁴. In outbreak A, 32 confirmed cases were traced to contact during a team meeting with a single infectious individual whose

antigen test result was negative on the morning of the meeting. Viral RNA sequences were closely related, supporting transmission from a single individual to the other team members. The authors note that viral transmission was not interrupted until the implementation of RT-PCR testing, which led to the identification of an additional 21 confirmed SARS-CoV-2 infections, 18 of which were not detected by concurrent antigen testing. In outbreak B, 12 confirmed cases were documented in 2 teams competing against each other, all of whose participants received negative antigen test results on the day of competition. Viral RNA sequences were closely related and distinctly different from strains circulating in one of the teams' communities, supporting transmission from one team to the other.

Antigen Conclusions

To decrease the rates of viral transmission, SARS-CoV-2 diagnostic test methods must be analytically accurate, accessible, and reported in a timely and effective manner. Antigen methods generate rapid results and their high specificity and positive predictive value allows SARS-CoV-2-positive individuals to quickly self-isolate, minimizing the risk of further viral spread. However, the lower sensitivity of antigen testing relative to nucleic acid methods increases the likelihood of further transmission in high interaction environments by individuals with false-negative antigen results. With this limitation in mind, confirmation of negative antigen results by nucleic acid testing is recommended, particularly in patient populations with a high disease prevalence.

In addition to the test method used, the environment in which testing is performed is a primary determinant of the effectiveness of SARS-CoV-2 testing efforts. The majority of studies evaluating antigen test performance described dedicated testing spaces staffed by trained operators with no other competing responsibilities. Little is known about how antigen devices perform when implemented in patient care settings with testing performed by clinical personnel who are also actively caring for patients. The limited available data using the BD Veritor device suggest improved performance in a controlled laboratory setting relative to an active patient care environment^{52,53}. However, this observation is complicated by differences in reference method and disease prevalence in the study populations.

SUMMARY

Laboratory medicine professionals play an integral role in the global response to SARS-CoV-2 through the development and implementation of test methods to identify infected individuals and monitor the immune response to vaccination and natural infection. SARS-CoV-2 antibody test methods can be used to confirm past infection and, pending further correlation with neutralizing antibody assays, may help to guide personalized vaccine selection or the establishment of revaccination intervals. Antigen test methods offer rapid turnaround time and improved access to testing as well as high specificity, but their limited sensitivity requires confirmation of negative results by nucleic acid testing, particularly in populations with high disease prevalence.

CLINICS CARE POINTS

- Antigen tests exhibit lower sensitivity relative to nucleic acid testing and increase the risk of further transmission by patients with false-negative antigen results.
- SARS-CoV-2 antibody test methods lack harmonization and correlation with neutralizing antibodies, limiting their clinical usefulness.

- Current vaccines result in higher antibody titers than natural infection, and antibodies made after vaccination should be monitored with antispike antibody assays.

DISCLOSURE

The authors have nothing to disclose.

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