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Laboratory Diagnosis for SARS-CoV-2 Infection



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KEYWORDS

- SARS-CoV-2 diagnostics • COVID-19 • Nucleic acid amplification testing • Serology
- Antigen testing

KEY POINTS

- Understanding performance characteristics, advantages, limitations, and best clinical uses of SARS-CoV-2 diagnostic assays is important when interpreting test results for clinical management, infection control purposes, and public health decision making.
- Nucleic acid amplification testing (NAAT) such as reverse-transcriptase polymerase chain reaction remains the gold standard for diagnosis of acute SARS-CoV-2 infection.
- Antigen tests can be used to diagnose acute infection in symptomatic individuals when NAAT is unavailable or not easily accessible; testing should be performed within 3 to 7 days of symptom onset to maximize sensitivity. Antigen tests can be used to screen for COVID-19 infection in high-risk congregate or community settings to identify infected individuals quickly to prevent ongoing transmission.
- Antigen tests have the potential for use as a marker of transmissibility in individuals with COVID-19 infection (especially when positive), but the performance of current assays suffers from significant interassay and interuser variability; more data are needed to establish their use in this regard.
- Serologic testing is best used when identifying individuals with prior or late COVID-19 infection and in the diagnosis of multisystem inflammatory syndrome.

INTRODUCTION

Diagnostic testing for SARS-CoV-2 continues to be a critical component of the pandemic response. Numerous SARS-CoV-2 tests that provide rapid, accurate, and reliable results at various stages of COVID-19 infection are now available. Having a clear understanding of test characteristics, advantages, limitations, and best clinical

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uses is critical when interpreting test results for clinical management, infection control purposes, and public health decision making. Here, we provide an overview of SARS-CoV-2 diagnostic testing with a focus on assay types that are commonly used in the clinical setting. Test characteristics and “best use” scenarios are described.

Virological Biomarkers During the Course of COVID-19 Infection

The natural history of COVID-19 infection consists of an acute phase, which can range from asymptomatic to severe illness, followed by a convalescent period that can range from weeks to months. Throughout these phases, specific virological and immunologic biomarkers appear at different time points; these markers serve as targets for diagnostic testing in infected individuals (Fig. 1). SARS-CoV-2 RNA is the first viral marker identified in persons with infection, with detectable levels present in the respiratory tract shortly preceding or around the time of symptom onset.¹ RNA concentrations peak during the first week of symptoms, then decline in levels during the next 2 to 3 weeks.² However, viral RNA can persist at detectable levels for months despite clinical infection resolution, particularly in immunosuppressed individuals.^{3,4} Similarly, SARS-CoV-2 viral antigens also become detectable in the respiratory tract around the time of symptom onset in those who become symptomatic; however, antigen levels decrease faster compared with viral RNA, with a decline in levels approximately 1 week after symptoms.⁵ Viral dynamics are generally similar among asymptomatic persons with SARS-CoV-2 infection, although the time to RNA and antigen clearance tends to be shorter. Due to these characteristics, the detection of viral RNA (via molecular-based testing) and/or antigens in respiratory samples serve as markers of acute infection in symptomatic and asymptomatic individuals.

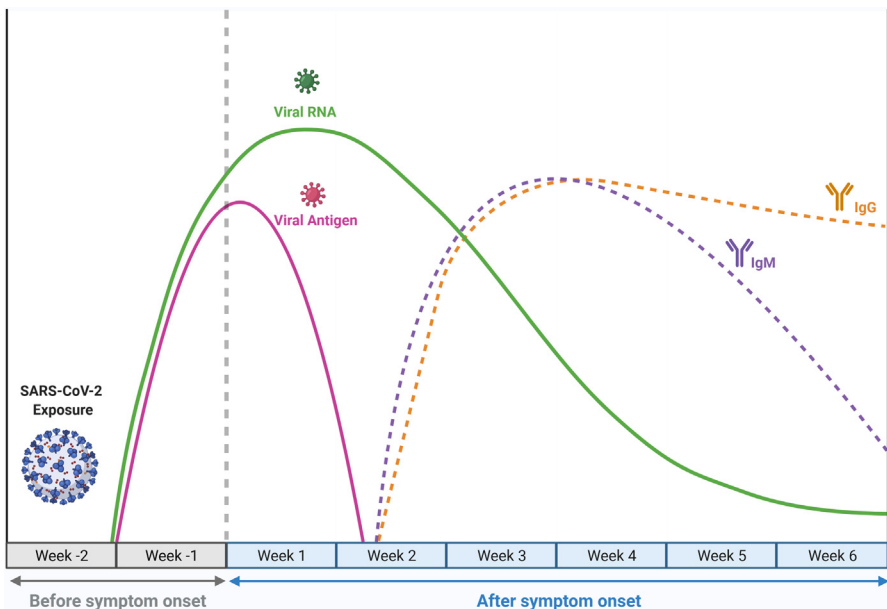


Fig. 1. Timing of virological and immunologic biomarkers during COVID-19 infection. (Adapted from “time course of COVID-19 infection and Test positivity”, by BioRender.com (2021). Retrieved from <https://app.biorender.com/biorender-templates>.)

In contrast, SARS-CoV-2 immunoglobulins are not reliably detectable until 2 or more weeks after symptom onset at which time both IgM and IgG isotypes appear at approximately the same time interval.⁶ IgM antibodies begin to decline by 2 months postsymptom onset, whereas IgG antibodies persist beyond that time frame.⁶ Based on these characteristics, detectable antibodies are indicators of recent or past SARS-CoV-2 infection and are of limited value during acute infection.

Common Molecular, Antigen, and Antibody Targets

SARS-CoV-2 is an enveloped single-stranded RNA virus within the *Coronaviridae* family.^{7,6} The genome contains open reading frames (ORFs) that encode both nonstructural and structural proteins (Fig. 2). Key structural proteins include spike (S), envelope (E), membrane (M), and nucleocapsid (N), which play roles in viral entry or assembly (Fig. 3).² Molecular-based tests often target regions of the genome that encode ORF1ab (including RNA-dependent RNA polymerase [RdRp]), S, E, M, and N proteins; common antigen and antibody targets include the N and S proteins.

Molecular-Based Testing

Overview

Molecular diagnostics is the analysis of genetic material and the products they encode in an effort to identify disease-causing sequences or microbes. One type of molecular diagnostic testing, nucleic acid amplification testing (NAAT), is used for the detection of SARS-CoV-2 RNA, most commonly from upper respiratory tract samples and is the reference method for COVID-19 diagnosis in both symptomatic and asymptomatic individuals, particularly during acute infection.

SARS-CoV-2 nucleic acid amplification testing methodologies

The most common NAAT method used for SARS-CoV-2 identification is reverse-transcriptase polymerase chain reaction (RT-PCR). SARS-CoV-2 RNA extracted from an individual sample is reverse transcribed using reverse transcriptase into complementary DNA (cDNA), which is then denatured by heat to create 2 single-stranded pieces of DNA.^{7,8} Using the original strands as templates, DNA polymerase synthesizes cDNA, resulting in the duplication of the original DNA. The cycle of denaturing and synthesizing continues for 30 to 45 cycles, depending on the assay, amplifying any target genes present in the sample. The number of cycles necessary to produce the predetermined detectable level of viral genes is called the cycle or crossing threshold (Ct; Fig. 4).^{7,8} Ct values are semiquantitative and inversely proportional to the level of viral RNA present in a sample.

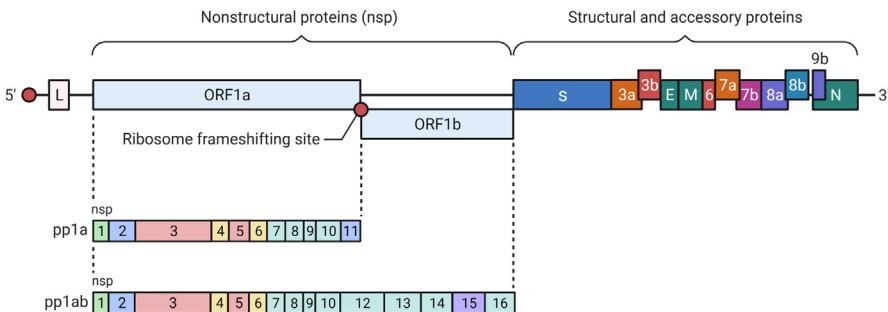


Fig. 2. SARS-CoV-2 genome. (Reprinted from "Organization of SARS-COV genome", by BioRender.com (2021). Retrieved from <https://app.biorender.com/biorender-templates>.)

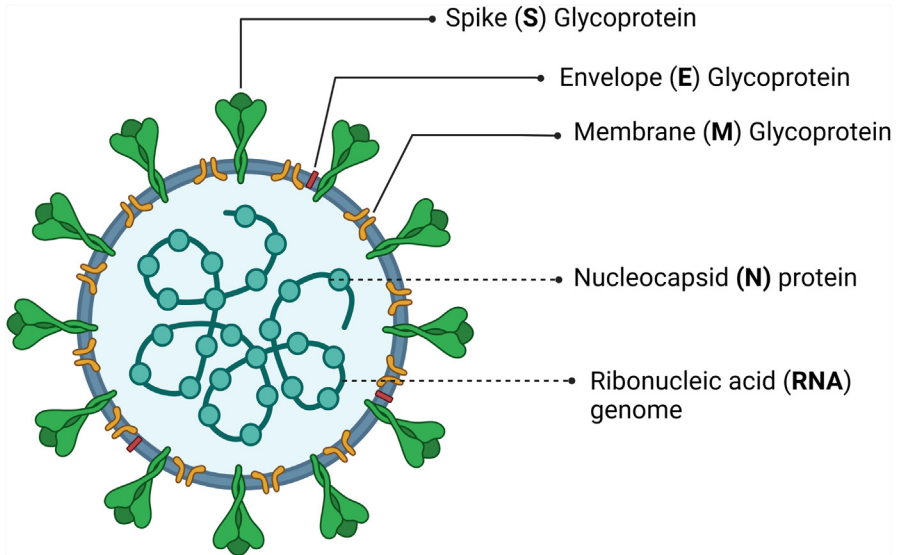


Fig. 3. SARS-CoV-2 structure. (Adapted from “human coronavirus Structure”, by BioRender.com (2021). Retrieved from <https://app.biorender.com/biorender-templates>.)

In addition to RT-PCR, isothermal nucleic acid amplification (NAA), including loop-mediated or transcription-mediated amplification, is a common alternative method used by clinical laboratories for SARS-CoV-2 nucleic acid detection. Isothermal NAA achieves enzymatic amplification at a constant temperature, as opposed to PCR where temperature cycling is required. Isothermal NAA therefore eliminates the need for thermal cyclers for high-temperature DNA denaturation, decreasing the device footprint and making technology more amenable to bench-top, point-of-care (POC) testing.

SARS-CoV-2 target genes vary between assays and include the RdRp ORF1ab, E, N, S, and M genes (see Fig. 2).^{7,9–11} Importantly, the E gene is not specific to SARS-CoV-2 and may result in cross-reactivity with other Sarbecoviruses, including SARS-CoV-1.¹⁰ Because of the potential for cross-reactivity and for genetic reassortment in any individual gene target, most assays use at least two targets to maximize test performance.

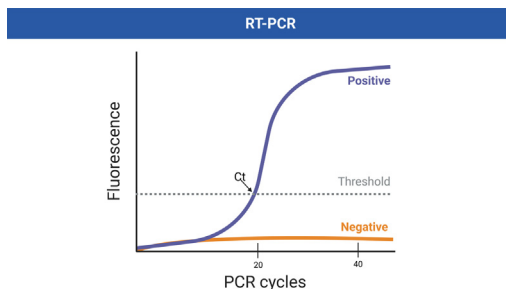


Fig. 4. RT-PCR. (Adapted from “COVID-19 diagnostic Test through RT-PCR”, by BioRender.com (2021). Retrieved from <https://app.biorender.com/biorender-templates>.)

An indeterminate result occurs when only one of the two (or more) gene targets detected by NAAT is identified.⁹ This scenario may occur when the amount of genetic material in the specimen is low such as in cases of early or late infection, poor specimen collection, or cross-contamination. Repeat testing may be helpful to confirm the result, although these results can be considered presumptively positive.⁹ In contrast, an inconclusive result occurs when repeat testing fails to determine the presence or absence of viral RNA.

Molecular testing approaches

Although the mechanism of detecting SARS-CoV-2 viral RNA is the same, RT-PCR and other NAAT-based assays differ in a variety of ways including (1) the testing protocol (one vs two steps), (2) testing prioritization (rapid vs batched), (3) testing strategy (individual vs pooling), (4) testing location (laboratory vs point of care [including home testing]), and (5) the number of detectable viral targets in a given assay (single SARS-CoV-2 target assays vs small multiviral target panels vs extended multiviral target panels). Examples of these options as well as their advantages and limitations are described in [Table 1](#).

Molecular test characteristics

In ideal settings, SARS-CoV-2 NAATs are generally highly sensitive and specific for the diagnosis of COVID-19.^{7,12,13} However, several factors influence the performance of SARS-CoV-2 RNA detection including (1) specimen handling, (2) specimen type, (3) timing of testing in relation to viral acquisition and symptom onset, and (4) presence of symptoms/disease severity. These factors may negatively affect the sensitivity of testing, resulting in false-negative results. They are discussed below:

Specimen handling. The most common source of errors in laboratory testing is in the preanalytical phase, which encompasses specimen collection, adequacy, storage, and transport and precedes test preparation, analysis, reporting, and interpretation. Poorly collected specimens may not contain sufficient viral RNA to attain the threshold of detection for molecular methods, while prolonged transport time and inadequate storage may accelerate degradation of chemically unstable viral RNA in a specimen, leading to false-negative results. Unfortunately, unlike respiratory cultures and direct/indirect fluorescent antibody testing in which specimen adequacy can be ascertained through assessment of the number of columnar epithelial cells in the specimen, molecular testing does not include a similar step. CDC provides best practice recommendations for specimen collection, which varies across specimen types.¹⁴ For specimen storage, CDC recommends storage at 2 to 8° C for up to 72 hours after specimen collection and at $\leq -70^{\circ}\text{C}$ if transport is not possible within 72 hours of collection.

Specimen type. Testing sensitivity varies substantially across specimen types with lower respiratory tract specimens (LRTS) generally yielding higher sensitivities than upper respiratory tract specimens (URTS; [Table 2](#)). The use of LRTS for initial testing is impractical because patients cannot often expectorate a sample; additionally, many of the commercially available SARS-CoV-2 NAATs are not authorized for use with LRTS. Because of these factors, the Infectious Diseases Society of America (IDSA) recommends initially obtaining an URTS for NAAT testing.¹⁵ If testing is negative but suspicion for COVID persists, IDSA recommends pursuing LRTS testing. Among URTS, nasopharyngeal (NP) swabs are considered the gold standard specimen type; alternative acceptable specimen types include saliva, midturbinate (MT), and anterior nasal (AN) swabs. Although still considered an accepted sample type for molecular testing, IDSA no longer recommends collecting oropharyngeal (OP) specimens

Table 1
Overview of molecular diagnostics

Testing Protocol	Definition	Advantages	Limitations
One-step RT-PCR	Assay where nucleic acid extraction, amplification, and detection are performed in a single reaction	Relatively fast depending on the assay Relatively automated Reduced laboratory errors Reduced potential for contamination Potential for high-throughput application	Less flexibility related to use of reagents and consumables More difficult to troubleshoot potential errors related to test process
Two-step RT-PCR	Assay where nucleic acid extraction is performed in a separate reaction from amplification and detection	Improved flexibility related to use of reagents and consumables Easier to troubleshoot errors related to test process	More labor intensive than one-step processes Longer result turnaround time
Testing Prioritization	Definition	Advantages	Limitations
Rapid testing	Single use test that can provide results in <60 min	Rapid result turnaround time Relatively easy to perform Can be performed at the POC depending on the assay	Variable sensitivity and specificity depending on the assay Lower throughput
Batched testing	Test where multiple individual specimens can be processed and analyzed simultaneously in parallel, typically with a result time of several hours	Higher throughput Relatively easy to perform depending on the assay	Longer result turnaround time More laboratory technologist training and expertise required depending on the assay
Testing Strategy	Definition	Advantages	Limitations
Individual testing	One specimen is tested individually with a given assay	Higher sensitivity compared with pooled testing (lower risk of false-negative results) Lower risk of laboratory error	Lower throughput compared with pooled testing Higher resource utilization per specimen tested
Pooled testing	Multiple specimens are combined and tested as one specimen with a given assay. If the pooled test is negative all	Higher throughput: increases the number of specimens that can be tested using the same amount of reagents and	Increased risk of false-negative results due to dilution of samples through the pooling process

	specimen samples are considered negative. If SARS-CoV-2 RNA is detected with the pooled test, each specimen within the pool is then individually tested to identify the positive sample	supplies Improved resource utilization when prevalence of SARS-CoV-2 is low and number of negative results is expected to be high Can estimate the positive rate in a given population	Requires high degree of laboratory organization to prevent errors
Testing Location	Definition	Advantages	Limitations
Laboratory-based	Tests performed in clinical laboratories that meet specific regulatory requirements for testing	Reference standard for molecular-based diagnosis given high sensitivity and specificity Higher throughput depending on the assay used	Longer result turnaround time Requires laboratory technologist training and expertise
Point of Care (POC)	Tests performed at or near specimen collection (eg, outpatient clinics, schools, congregate settings)	Low complexity testing requiring minimal operator experience and training Rapid result turnaround time	Variable sensitivity and specificity depending on the assay Lower throughput
Viral Testing Targets	Definition	Advantages	Limitations
Single viral target assay	Assay that detects the presence of only one viral target (SARS-CoV-2)	High sensitivity and specificity	Limited spectrum of pathogen identification
Small multiviral target assay	Assay that detects the presence of a few viral targets (Influenza A, Influenza B, SARS-CoV-2, ± respiratory syncytial virus)	High sensitivity and specificity Broader spectrum of pathogen identification compared with single viral target assays	Potential reduced sensitivity compared with single viral target assays Limited spectrum of pathogen identification compared with extended multiviral target assays
Extended multiviral target assay	Assay that detects the presence of many viral (and bacterial) targets	High sensitivity and specificity Broadest spectrum of pathogen identification	Potential reduced sensitivity compared with single viral target and small multiviral target assays

Data from Refs. [10,69–74](#)

Table 2

Test characteristics by specimen type

Specimen Type	Sensitivity (95% CI)	Specificity (95% CI)	Advantages	Limitations
NP	N/A (reference method)	N/A (reference method)	Reference method, reducing the potential for false-positive or false-negative results	Requires testing supplies Can only be collected by health-care professionals Higher potential for aerosol generation More invasive compared with other methods Uncomfortable procedure
Anterior nares swab (AN)	89% (83%–94%)	100% (99%–100%)	Potential for self-collection Less invasive compared with NP More comfortable procedure for patients Lower potential for aerosol generation	Reduced sensitivity compared with NP (higher risk of FN results) Requires testing supplies
Saliva (with or without coughing)	90%–99% (85%–100%)	96%–98% (83%–100%)	Potential for self-collection Less invasive compared with all other URT specimen types More comfortable procedure for patients Lower potential for aerosol generation (saliva without coughing only) Requires fewer testing supplies	Reduced sensitivity compared with NP (higher risk of FN results) Often requires formal specimen validation by clinical laboratories as specimen type is not frequently authorized for use on many commercially available NAAT platforms Higher risk of invalid results given complexity of specimen type which can lead to more repeat testing and delayed result turnaround time
MT swab	95% (83%–99%)	100% (89%–100%)	Potential for self-collection Less invasive compared with NP More comfortable procedure for	Reduced sensitivity compared with NP (higher risk of FN results) Requires testing supplies

patients
Lower potential for aerosol
generation

OP swab	76% (58%–88%)	98% (96%–99%)	Less invasive compared with NP More comfortable procedure for patients Lower potential for aerosol generation	Reduced sensitivity compared with all other URT specimen types (higher risk of FN results) Requires testing supplies Can only be collected by health-care professionals
Combined AN/OP swab	95% (69%–99%)	99% (92%–100%)	Potential for self-collection Less invasive compared with NP More comfortable procedure for patients Lower potential for aerosol generation	Reduced sensitivity compared with NP (higher risk of FN results) Requires testing supplies Requires formal specimen validation by clinical laboratories as combined specimen type is not authorized for use on commercially available NAAT platforms

Data from Refs. [15](#), [19](#), [75](#)

alone due to low sensitivity but instead to combine them with AN specimens. Studies evaluating the sensitivity of saliva in comparison with NP have yielded mixed results including decreased (compared with combination NP/OP specimens), equivalent, or greater sensitivity.¹⁶ Variability in reference tests, timing of specimen collection relative to symptom onset, specimen collection processes for saliva, patient demographics, comorbidities, and disease severity significantly limit the interpretation of testing performance across specimen types.^{15–19} Although perhaps slightly less sensitive than NP specimens, alternative URTS have the advantage of being less invasive and easier to collect which is especially important in frequent/routine testing schemes. Additionally, saliva specimens may be especially amenable to self-collection including in the home setting and reduce the need for swabs and other supplies that are prone to shortages, although specialized saliva collection kits may improve specimen adequacy. Of note, because many commercial NAAT platforms do not have FDA EUA for saliva, individual laboratories would be required to validate this sample type locally.²⁰

Testing timing. The sensitivity of SARS-CoV-2 NAAT is highly dependent on the quantity of viral RNA in a clinical specimen. Because the amount of viral RNA in a clinical specimen varies in relation to the time from infection and from symptom onset (in those who go on to develop symptoms), the timing of testing is an important factor when considering testing sensitivity. The SARS-CoV-2 virus becomes detectable in URTS at a median of 4 to 6 days after infection (median of 4 days for the Delta variant; incubation period 2–14 days; slightly shorter incubation period for Omicron) and 2 to 5 days before symptom onset. In symptomatic individuals, viral loads peak between 0.6 days prior and 5 days after symptom onset, then drop precipitously after 7 days of symptom onset but often remain at detectable levels for multiple weeks after infection.^{21–24} Therefore, testing done too early (before viral loads increase beyond the threshold of detection) or too late (after viral loads decrease below the threshold of detection) will lead to false-negative results.

Presence of symptoms and disease severity. Although viral loads have been demonstrated to peak early in the disease course and to progressively decline thereafter in most patients (apart from highly immunocompromised patients who may maintain prolonged high viral loads), the presence of symptoms and the severity of illness may also correlate with viral loads in clinical specimens and therefore the clinical sensitivity of molecular testing. Studies comparing viral loads in URTS of symptomatic versus asymptomatic or presymptomatic patients have yielded conflicting results, with most studies showing similar initial viral loads among mildly symptomatic, presymptomatic, and asymptomatic patients.^{24–32} The rate of viral clearance seems to be similar or slightly faster in asymptomatic patients compared with presymptomatic and symptomatic patients.^{33–35} Notably, assessing the rate of viral clearance is limited by the fact that the onset of viral replication in asymptomatic patients is often unknown leading to asynchronous viral dynamics with matched symptomatic patients. Additionally, multiple studies comparing mild and severe cases of COVID-19 have shown that viral loads tend to be higher at any point in time after symptom onset and persist longer in those with severe disease, suggesting that NAAT sensitivity is potentially higher in this population.^{21,36–42}

Cycle threshold values and their role in clinical decision making

Ct values are inversely proportional to the relative amount of viral RNA present in a sample, with lower Ct values representing high RNA titer (fewer PCR cycles needed to reach positive threshold) and higher Ct values representing lower RNA titer (more

PCR cycles needed to reach the positive threshold). Given this relationship, there has been significant interest in using Ct values as surrogate markers of genomic load in the clinical setting. In particular, Ct values have been used as a proxy for transmissibility, to predict illness severity, and to help distinguish active infection, which would require potential treatment and isolation, from persistent RNA detection.^{43–45} However, although Ct values do generally correlate well with RNA concentrations, limited data exist to support the use of Ct values in the above-described settings. Additionally, as RT-PCR tests are designed to be qualitative in nature, these assays have not undergone extensive evaluation to optimize the relationship between Ct values and RNA levels, which can lead to lower correlation at low and high RNA concentrations.⁴⁶ Finally, significant variability in Ct values has been shown both within and across RT-PCR platforms; Rhoads and colleagues reported intra-assay variability of up to 3 cycles (10-fold viral load difference) and interassay variability of up to 14 cycles (4000-fold viral load difference) for Ct values when reviewing proficiency testing across 700 laboratories in the United States.⁴⁷ Due to all of these factors, IDSA does not recommend the use of Ct values for clinical decision making and cautions providers about using these data to predict disease severity or to determine infection status and infectivity.⁴⁶ If Ct values are to be used for clinical decision making, consultation with infectious diseases experts should be considered.

Antigen-Based Testing

Overview

Antigen tests are immunoassays that use tagged antibodies to detect specific SARS-CoV-2 antigens within a primary specimen. Many assays are currently authorized by the FDA for the diagnosis of COVID-19, most of which target the N and S proteins (see [Fig. 3](#)) and can be performed on NP or nasal swab specimens.^{7,48,49}

Antigen test characteristics

Overall, antigen tests are sensitive and specific tools for SARS-CoV-2 detection with reported 81% sensitivity (95% CI 72%–88%) and 99% specificity (95% CI: 99–100) compared with NAAT.⁵⁰ Sensitivity is improved when used within 7 days of symptom onset, when viral burden in the URT is highest.⁵⁰ Additionally, studies comparing antigen testing and NAAT to viral culture have shown higher correlation between positive antigen tests and culture positivity when compared with NAAT, suggesting that antigen tests may be a better marker of infectiousness and transmissibility in certain clinical situations.⁵¹ However, more data are needed to better understand the association between antigen tests and transmissibility; until then, antigen tests should only be used to approximate infectiousness in individuals with COVID-19.⁵⁰

As with other respiratory antigen detection tests and compared with most commercially available NAATs, SARS-CoV-2 antigen tests are relatively inexpensive and easy to perform, require little operator training and experience, and provide rapid results (approximately 15–30 minutes).^{10,11,50,52} Given these characteristics, antigen tests are commonly used at the POC where results can be communicated to providers and patients in real time to allow for more informed clinical decision making.

Clinical utility of antigen testing compared with nucleic acid amplification testing

Although NAAT remains the diagnostic test standard for acute COVID-19 infection, antigen tests can be used to diagnose acute infection in both asymptomatic and symptomatic individuals when NAAT is unavailable or results are delayed; testing should be performed within 7 days of symptom onset to maximize sensitivity.⁵⁰ Given the reduced sensitivity of antigen detection tests compared with NAAT, negative results must be interpreted in the appropriate clinical context, which includes a

comprehensive evaluation of an individual's pretest probability for COVID-19 infection. Negative antigen tests in symptomatic individuals with high clinical suspicion for COVID-19 should have results confirmed with NAAT given the potential for false-negative antigen test results.^{7,11,48,50} In contrast, given their high specificity, positive antigen test results in symptomatic individuals generally do not require confirmatory NAAT and individuals with positive results should be considered infected with COVID-19.^{48,50} Confirmatory NAAT can be considered in select situations in antigen-positive, symptomatic individuals with low likelihood of SARS-CoV-2 infection because, although rare, false-positive results have been reported. CDC-designed algorithms using antigen based testing in these population are available at <https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html> and provide specific guidance for test interpretation, including indications for additional testing, depending on the antigen test results.^{48,53}

Asymptomatic, exposed individuals may also benefit from antigen testing when NAAT is unavailable because positive tests do reliably indicate SARS-CoV-2 infection. As with NAAT, negative antigen results do not preclude COVID-19 infection in asymptomatic individuals with high risk for infection (close contact or suspected exposure) and do not obviate the need for quarantine. Per CDC guidance, both NAAT and antigen tests can be used to shorten the duration of quarantine in exposed unvaccinated asymptomatic individuals if performed at least 5 days after last exposure.⁵⁴

Finally, the rapid turnaround time and relatively low cost of antigen detection tests compared with NAATs make these tests appealing serial screening tools for SARS-CoV-2 detection in high-risk congregate and community settings, where identifying infected individuals quickly is paramount to preventing transmission.⁴⁸

Serologic Testing

Overview

Serologic testing detects the presence of SARS-CoV-2 specific antibodies in clinical specimens from individuals with prior SARS-CoV-2 infection, vaccination, and/or recent receipt of anti-SARS-CoV-2 monoclonal antibody therapy. Currently, nearly 100 assays have received FDA EUA for clinical use; common antibody targets include the nucleocapsid (anti-N) protein, which is produced in response to natural infection and the spike (anti-S) protein, which is produced in response to either natural infection or vaccination (**Table 3** for guidance on the interpretation of SARS-CoV-2 serologic assays by vaccination status). Common specimen types include serum and/or plasma. Depending on the assay, different classes of antibodies may be detected, ranging

Vaccination Status	Anti-N Antibody	Anti-S Antibody	Interpretation
Unvaccinated	Positive	Positive	Previously infected
Unvaccinated	Negative	Negative	Not previously vaccinated or infected
Vaccinated	Positive	Positive	Vaccinated and previously infected
Vaccinated	Negative	Positive	Vaccinated and not previously infected ^a

^aImmunocompromised patients may have negative serology postvaccination.

Adapted from Centers for Disease Control and Prevention. Interim Guidelines for COVID-19 Antibody Testing. *Cent Dis Control Prev.* 2020:1-8. <https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html>. Accessed October 20, 2021.

from IgM, IgG, IgA, or total antibody, the latter of which does not discern between specific class types. Assays are qualitative or semiquantitative in nature and, generally, do not distinguish between neutralizing (protective) and nonneutralizing antibodies.

Serologic testing characteristics

In general, SARS-CoV-2 serologic assays are reasonably sensitive and specific; however, several factors, including (1) test method, (2) test timing in relation to symptom onset, (3) type of antibodies measured, and (4) host type, can affect test performance.⁵⁵ These factors are discussed below:

Test method. There are several available SARS-CoV-2 serologic testing methods including lateral flow assays (LFA), enzyme-linked immunosorbent assay (ELISA), and chemiluminescent immunoassays (CIA).^{56,57} LFA are generally low complexity tests that can be performed at the POC, whereas ELISA and CIA are laboratory-based tests with the potential for high-throughput testing.⁵⁷ In general, LFA are less sensitive than ELISA and CIA, with a recent-meta-analysis showing a pooled sensitivity of only 66% for LFA compared with 84.3% and 97.8% for ELISA and CIA, respectively.^{9,10,55} Pooled specificity ranged from 99.6% to 99.7%.⁵⁵ Given this observed variability across assay methodologies, clinicians should be aware of the test characteristics of the specific platforms used by their institutions so as to interpret negative test results in the appropriate context.

Test timing. Due to the kinetics of antibody production in response to infection, sensitivity for all serologic assays is low within the first 2 weeks following infection, with reported pooled sensitivities of 23% to 63% at week 1 and 68% to 96% at week 2.⁵⁷ Sensitivity significantly improves by 3 weeks after infection with pooled sensitivity of 84% to 95%.⁵⁷ Due to this reduced sensitivity early in infection, serologic testing should be avoided during this time frame to prevent potential false-negative results. Levels of S-specific, RBD-specific, and N-specific IgM and IgG antibodies seem to develop concurrently after infection and follow similar dynamic changes during convalescence.⁵⁸

Types of antibodies measured. Because small differences in specificity can have disproportionate effects on false-positive rates when SARS-CoV-2 prevalence is low, IDSA recommends using serologic tests with high specificity ($\geq 99.5\%$).⁵⁷ Generally, serologic assays that detect IgG or total antibodies (IgM, IgA, and IgG) have higher specificities ($\geq 99\%$) compared with assays that detect IgM or IgA only or combined IgM and IgG; these assays should be prioritized for use if serologic testing is indicated.⁵⁷ When a false-positive test is suspected based on low pretest probability and/or low prevalence, orthogonal testing using two sequential serologic assays that target different protein targets can be done to increase the positive predictive value of an initial positive result.

Host type. Patients with immune deficiencies limiting their host immune response to SARS-CoV-2 antigens, especially those on lymphocyte depleting or suppressing agents, may fail to seroconvert after exposure to SARS-CoV-2. As a result, serologic methods may not reliably identify immunosuppressed individuals with prior infection.

Clinical utility of serologic testing

A detailed description of “best use” scenarios for SARS-CoV-2 serologic testing can be found in [Table 4](#). In general, serologic testing plays a limited role in the diagnosis of acute infection and is more commonly used for surveillance purposes. Currently authorized antibody tests provide a qualitative result (positive/negative). Although

NAAT¹⁵	Antigen-Based⁵⁰	Serology⁵⁷
Symptomatic individuals suspected of having COVID-19	Screening in high-risk congregate or community settings	Confirmation of past SARS-CoV-2 infection, 3–4 wk after symptom onset
Asymptomatic individuals with possible or known exposure to COVID-19	Symptomatic individuals suspected of having COVID-19, when NAAT is not easily available	Diagnose COVID-19 infection in symptomatic patients with high clinical suspicion and negative NAAT testing
Asymptomatic individuals being admitted to the hospital, regardless of exposure history to COVID-19	Prior to an event or travel to identify asymptomatic or presymptomatic COVID-19 infection	Diagnose current or past COVID-19 infection in patients with MIS
Asymptomatic patients undergoing procedures when PPE or other resources are limited	Congregate settings experiencing an outbreak in need of rapid turnaround	

some tests additionally provide a numerical result, these tests are classified as semi-quantitative rather than quantitative by the FDA; hence, the utility of numerical values is not known at this time. One exception is their use to support the diagnosis of multi-system inflammatory syndrome (MIS) in children and adults as NAAT can be negative because most cases present 2 to 6 weeks after initial infection. Additionally, recent data have suggested that knowing SARS-CoV-2 serostatus in hospitalized individuals suffering from COVID-19 may be helpful for making treatment decisions related to anti-SARS CoV-2 monoclonal antibody therapy, particularly in immunosuppressed patients unlikely to mount any immune response; however, more data are needed to support the routine use of these assays in this setting.⁵⁹ Importantly, although good correlation between some serologic assays (anti-S) and neutralizing antibodies has been reported, full correlates of immunity have not been established and may differ across viral variants, antibody types, assays, and patient populations. Thus, serologic testing should not routinely be used to determine immunity in previously infected or vaccinated individuals.^{57,60,61}

Effects of SARS-CoV-2 Variants on Diagnostic Testing Performance

Because the SARS-CoV-2 pandemic has continued, many SARS-CoV-2 variants, with various mutations in the viral genome, have emerged. In addition to affecting the overall clinical characteristics of this virus, the presence of these mutations has also affected the test performance of certain diagnostics tests, leading to false-negative results.⁶² For example, specific spike protein mutations identified in Alpha (B.1.1.7) and Omicron (B.1.1.529) variants can cause false-negative NAAT results (“S gene target failure” [SGTF]) if the S gene is the predominant gene target for a given NAAT. Importantly, most NAATs detect more than one viral target, minimizing the likelihood of a false-negative result even if one target is affected by a specific mutation present in a circulating variant. In fact, in some instances, failure of a specific gene target to amplify in the setting of other amplified SARS-CoV-2 gene targets (such as SGTF) can even be advantageous because these patterns can serve as proxies for detection

of certain variants. For example, the identification of the SGTF pattern has been used as a proxy for detection of Alpha and Omicron variants both in the United States and abroad (ECDC).^{7,63,64} In contrast, S gene mutations in the Delta variant (B.1.617.2) typically do not lead to S gene drop out. Currently, the FDA monitors the potential impact of novel mutations on test performance; up-to-date information can be found at: <https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-viral-mutations-impact-covid-19-tests>. Additionally, in February of 2021, the FDA published guidance for test developers and manufacturers for the evaluation of novel viral mutations on COVID-19 test performance in an effort to identify issues with commercial testing in real-time.⁶⁵

Compared with NAAT, most antigen tests detect the viral nucleocapsid protein and would be unaffected by mutations in the spike protein, although rare mutations in the N gene that may affect the sensitivity of diagnostic tests have been reported.⁴⁹ More recently, reports of reduced antigen-testing sensitivity have been reported for the rapidly emerging Omicron variant.⁶² Reasons for this reduced sensitivity are as of yet unclear but include mutations in target antigens, variations in viral burden, or tropism to an anatomic site/specimen other than the one being tested (such as for saliva when nasal swab being performed). Evaluation of these reports related to Omicron is an area of ongoing investigation.

Testing in Specific Patient Populations/Clinical Settings

Reinfected individuals

Genotypically confirmed reinfection with SARS-CoV-2 has been documented in both immunocompetent and immunocompromised individuals with most cases occurring ≥ 90 days after the primary infection, presumably because of the development of short-term protective immunity. Considering this and the potential for prolonged shedding of viral particles without true infectiousness for weeks to months after initial infection, retesting should generally be avoided within 90 days of primary infection. In symptomatic or asymptomatic individuals presenting ≥ 90 days after initial infection/illness, standard testing criteria should be applied. For individuals presenting 45 to 89 days after initial infection and with high suspicion for reinfection based on clinical criteria without or without additional predisposing host factors and no alternate cause of symptoms, testing should be considered because some cases of reinfection have occurred as early as 48 days after initial infection.^{66,67} In these circumstances, a repeat positive NAAT test with a low Ct value (lower than 33 per CDC investigative reinfection criteria) would be suggestive of reinfection.⁶⁸ Ultimately, however, reinfection can only be confirmed with genomic sequencing of both the initial and subsequent infecting viruses to determine if the patient was reinfected with a different virus.

SUMMARY

The diagnosis of SARS-CoV-2 infection relies on several considerations including the presence and duration of COVID-19 symptoms and the diagnostic testing methods used. Molecular testing, most often RT-PCR, offers the highest sensitivity and specificity during acute infection, with URTS being the preferred initial specimen type for testing. Antigen testing can also be used for acute diagnosis when RT-PCR is unavailable or not easily accessible and is of particular use for serial screening in high-risk asymptomatic populations and congregate settings due to its low cost and ease of performance. Finally, antigen testing may have the potential to approximate infectiousness in individuals with COVID-19 infection but the current data are inconclusive and more studies are needed to establish their use in this setting. In contrast, serologic

testing is more useful for diagnosing recent or past infection and MIS, with improved sensitivity when performed after more than 14 days of symptoms. In keeping with current guidelines, COVID-19 serologic assays should not be used during the acute phase of infection.

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