Testing for SARS-CoV-2 Infection

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<table>
<thead>
<tr>
<th>Summary Recommendations</th>
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<tr>
<td>• The COVID-19 Treatment Guidelines Panel (the Panel) recommends using a nucleic acid amplification test (NAAT) with a sample collected from the upper respiratory tract (i.e., nasopharyngeal, nasal mid-turbinate, anterior nasal, or oropharyngeal) to diagnose acute infection of SARS-CoV-2; if it is not practical to use a NAAT or if NAATs are not available, an antigen test may be used (AIII).</td>
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<td>• For intubated and mechanically ventilated adults who are suspected to have COVID-19 but who do not have a confirmed diagnosis:</td>
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<td>• The Panel recommends obtaining lower respiratory tract samples to establish a diagnosis of COVID-19 if an initial upper respiratory tract sample is negative (BII).</td>
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<td>• The Panel recommends obtaining endotracheal aspirates over bronchial wash or bronchoalveolar lavage samples when collecting lower respiratory tract samples to establish a diagnosis of COVID-19 (BII).</td>
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<td>• A NAAT should not be repeated in an asymptomatic person (with the exception of health care workers) within 90 days of a previous SARS-CoV-2 infection, even if the person has had a significant exposure to SARS-CoV-2 (AIII).</td>
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<td>• SARS-CoV-2 reinfection has been reported in people after an initial diagnosis of the infection; therefore, clinicians should consider using a NAAT for those who have recovered from a previous infection and who present with symptoms that are compatible with SARS-CoV-2 infection if there is no alternative diagnosis (BIII).</td>
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<td>• The Panel recommends against the use of serologic (i.e., antibody) testing as the sole basis for diagnosis of acute SARS-CoV-2 infection (AIII).</td>
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<td>• There is insufficient evidence for the Panel to recommend either for or against the use of SARS-CoV-2 serologic testing to assess for immunity or to guide clinical decisions about using COVID-19 vaccines or anti-SARS-CoV-2 monoclonal antibodies in certain people.</td>
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Rating of Recommendations: A = Strong; B = Moderate; C = Weak
Rating of Evidence: I = One or more randomized trials without major limitations; IIa = Other randomized trials or subgroup analyses of randomized trials; IIb = Nonrandomized trials or observational cohort studies; III = Expert opinion

Diagnostic Testing for SARS-CoV-2 Infection

Everyone who has symptoms that are consistent with COVID-19 and people with known high-risk exposures to SARS-CoV-2 should be tested for SARS-CoV-2 infection. Such testing should employ either a nucleic acid amplification test (NAAT) or an antigen test to detect SARS-CoV-2. Testing may also be used for screening, determining the length of a patient’s isolation period, and other nondiagnostic purposes.¹

A number of diagnostic tests for SARS-CoV-2 infection (e.g., NAATs, antigen tests) have received Emergency Use Authorizations (EUAs) from the Food and Drug Administration (FDA),² but no diagnostic test has been approved by the FDA. Diagnostic tests have been authorized for use by trained personnel in several settings, including lab facilities. They can also be used in point-of-care settings, where the test is performed by trained personnel at or near the place where the specimen was collected. Point-of-care settings include physician offices, pharmacies, long-term care facilities, and school clinics.

Antigen tests can be self-administered, and most can be used in point-of-care settings, allowing results to be available within minutes. Some NAATs can also be self-administered at home or in other non-health care locations and shipped to a laboratory for testing.

Although nasopharyngeal specimens remain the recommended samples for SARS-CoV-2 diagnostic testing, nasal (anterior nares or mid-turbinate) or oropharyngeal swabs are acceptable alternatives.³ Lower respiratory tract samples have a higher yield than upper respiratory tract samples, but they
are often not obtained because of concerns about aerosolization of the virus during sample collection procedures. Some of the tests that have received EUAs can also be performed on saliva specimens, but the quality of saliva specimens can be highly variable. Studies are currently evaluating the use of other sample types, including stool samples.

**Nucleic Acid Amplification Testing for SARS-CoV-2 Infection**

Reverse transcription polymerase chain reaction (RT-PCR)-based diagnostic tests (which detect viral nucleic acids) are considered the gold standard for detecting current SARS-CoV-2 infection. More recently, NAATs have included isothermal amplification platforms (e.g., nicking endonuclease amplification reaction [NEAR], loop-mediated isothermal amplification [LAMP], transcription-mediated amplification [TMA]). Some NAATs have also received EUAs for use in different settings, such as in laboratory facilities and point-of-care settings. Laboratory-based NAATs generally have higher sensitivity than point-of-care tests.

Clinically, there may be a window period of up to 5 days after exposure before viral nucleic acids can be detected. Diagnostically, some NAATs may produce false negative results if a mutation occurs in the part of the virus’ genome that is assessed by that test. The FDA monitors the potential effects of SARS-CoV-2 genetic variations on NAAT results and issues updates when specific variations could affect the performance of NAATs that have received EUAs. Generally, false negative results are more likely to occur when using NAATs that rely on only 1 genetic target. Therefore, a single negative test result does not exclude the possibility of SARS-CoV-2 infection in people who have a high likelihood of infection based on their exposure history and/or their clinical presentation.

Many commercial NAATs that use RT-PCR rely on multiple targets to detect the virus, such that even if a mutation impacts 1 of the targets, the other RT-PCR targets will still work. NAATs that use multiple targets are less likely to be impacted by an increased prevalence of genetic variants. In fact, because each of these tests target multiple locations on the virus’ genome, they can be helpful in identifying new genetic variants before they become widespread in the population. For example, the B.1.1.7 (Alpha) variant and the BA.1 subvariant of the B.1.1.529 (Omicron) variant, both of which have been associated with increased transmission, carry many mutations, including a double deletion at positions 69 and 70 on the spike protein gene (S-gene). This mutation appears to impact the detection of the S-gene but does not impact other genetic targets in certain NAATs. If COVID-19 is still suspected after a patient receives a negative test result, clinicians should consider repeating testing; ideally, they should use a NAAT with different genetic targets.

SARS-CoV-2 poses several diagnostic challenges, including the potential for discordant viral shedding between the upper and lower respiratory tract. However, due to the high specificity of NAATs, a positive result on a NAAT of an upper respiratory tract sample from a patient with recent onset of SARS-CoV-2-compatible symptoms is sufficient to diagnose COVID-19. In patients with COVID-19, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS), lower respiratory tract specimens have a higher viral load and thus a higher yield than upper respiratory tract specimens. For intubated or mechanically ventilated patients with clinical signs and symptoms that are consistent with COVID-19 pneumonia, the COVID-19 Treatment Guidelines Panel (the Panel) recommends obtaining lower respiratory tract samples to establish a diagnosis of COVID-19 if an initial upper respiratory tract sample is negative (BII). The Panel recommends obtaining endotracheal aspirates over bronchial wash or bronchoalveolar lavage (BAL) samples when collecting lower respiratory tract samples to establish a diagnosis of COVID-19 (BII).

BAL and sputum induction are aerosol-generating procedures that should be performed only after carefully considering the risk of exposing staff to infectious aerosols. Endotracheal aspiration appears to
carry a lower risk of aerosol generation than BAL, and some experts consider the sensitivity and specificity of endotracheal aspirates and BAL specimens comparable in detecting SARS-CoV-2.

**Nucleic Acid Amplification Testing for Individuals With a Previous Positive SARS-CoV-2 Test Result**

NAATs can detect SARS-CoV-2 RNA in specimens obtained weeks to months after the onset of COVID-19 symptoms. However, the likelihood of recovering replication-competent virus >10 days from the onset of symptoms in those with mild disease and >20 days in those with severe disease is very low. Furthermore, both virologic studies and contact tracing of high-risk contacts show a low risk for SARS-CoV-2 transmission after these intervals. Based on these results, the Centers for Disease Control and Prevention (CDC) recommends that NAATs should not be repeated in asymptomatic persons within 90 days of a previous SARS-CoV-2 infection, even if the person has had a significant exposure to SARS-CoV-2. An exception to this is for health care workers who meet the specific criteria found in CDC guidance. If there are concerns that an immunocompromised health care worker may still be infectious >20 days from the onset of SARS-CoV-2 infection, consulting local employee health services about return-to-work testing policies is advised.

SARS-CoV-2 reinfection has been reported in people after an initial diagnosis of infection; therefore, clinicians should consider using a NAAT for those who have recovered from a previous infection and who present with symptoms that are compatible with SARS-CoV-2 infection if there is no alternative diagnosis. However, a negative result on an initial NAAT followed by a positive result on a subsequent test does not necessarily mean a person has been reinfeected; this can occur due to intermittent detection of viral RNA. When the results for an initial and a subsequent test are positive, comparative viral sequence data from both tests are needed to distinguish between the persistent presence of viral fragments and reinfection. In the absence of viral sequence data, the cycle threshold (Ct) value from a positive NAAT result may provide information about whether a newly detected infection is related to the persistence of viral fragments or to reinfection. The Ct value is the number of PCR cycles at which the nucleic acid target in the sample becomes detectable. In general, the Ct value is inversely related to the SARS-CoV-2 viral load. Because the clinical utility of Ct values is an area of active investigation, an expert should be consulted if these values are used to guide clinical decisions.

**Antigen Testing for SARS-CoV-2 Infection**

Antigen-based diagnostic tests (which detect viral antigens) are less sensitive than laboratory-based NAATs, but they have similarly high specificity. Antigen tests perform best early in the course of symptomatic SARS-CoV-2 infection, when the viral load is thought to be highest. Early data suggest that antigen tests can detect the Omicron variant, but they may have lower sensitivity to this variant compared to earlier variants. Advantages of antigen tests include their low cost and rapid turnaround time. The availability of immediate results makes them an attractive option for point-of-care testing in high-risk congregate settings (e.g., long-term care facilities, schools, dormitories, correctional facilities) and community settings where preventing transmission is critical. These tests can also be used to inform decisions about the use of post-exposure prophylaxis (PEP). Antigen tests also allow for repeat testing to quickly identify persons with SARS-CoV-2 infection.

Increasingly, data are available to guide the use of antigen tests as screening tests to detect or exclude SARS-CoV-2 infection in asymptomatic persons, or to determine whether a person who was previously confirmed to have SARS-CoV-2 infection is still infectious. The CDC has developed an antigen testing algorithm for persons in congregate living settings and community settings who have symptoms of COVID-19, those who are asymptomatic and have a close contact with COVID-19, and those who are asymptomatic and have no known exposure to a person with COVID-19. The CDC testing algorithm
recommends performing additional confirmatory testing with a laboratory-based NAAT when a person who is strongly suspected of having SARS-CoV-2 infection (i.e., a person who is symptomatic) receives a negative result and when a person in a congregate living setting is asymptomatic but receives a positive result. People in congregate living settings who test positive for SARS-CoV-2 infection may need to be isolated as a group; therefore, correct identification of these individuals is especially important in this setting.23

Antigen tests can yield false positive results for a variety of reasons, including:

- Incomplete adherence to the instructions for antigen test performance (e.g., reading the results outside the specified time interval or storing test cartridges/cards inappropriately);
- Test interference due to human antibodies (e.g., rheumatoid factor or other nonspecific antibodies); and
- Use in communities that have a low prevalence of SARS-CoV-2 infection.

### Serologic or Antibody Testing for Diagnosis of SARS-CoV-2 Infection

Unlike NAATs and antigen tests for SARS-CoV-2 that detect the presence of the virus, serologic or antibody tests can detect recent or prior SARS-CoV-2 infection. Because it may take 21 days or longer after symptom onset for seroconversion to occur (i.e., the development of detectable immunoglobulin [Ig] M and/or IgG antibodies to SARS-CoV-2),24-29 the Panel does not recommend using serologic testing as the sole basis for diagnosing acute SARS-CoV-2 infection (AIII). Because NAATs and antigen tests for SARS-CoV-2 occasionally yield false negative results, serologic tests have been used in some settings as an additional diagnostic test for patients who are strongly suspected to have SARS-CoV-2 infection. Using a serologic test in combination with a NAAT to detect IgG or total antibodies 3 to 4 weeks after symptom onset maximizes the sensitivity and specificity to detect past infection.

No serologic tests for SARS-CoV-2 are approved by the FDA; some, but not all, commercially available serologic tests for SARS-CoV-2 have received EUAs from the FDA.30 Several professional societies and federal agencies, including the Infectious Diseases Society of America, the CDC, and the FDA, provide guidance on the use of serologic testing for SARS-CoV-2.

Several factors should be considered when using serologic tests, including:

- Important performance characteristics of many of the commercially available serologic tests have not been fully characterized, including the sensitivity and specificity of these tests (i.e., the rates of true positive and true negative results). Only serologic assays that have FDA EUAs should be used in public health or clinical settings. Formal comparisons of serologic tests are in progress.
- Two types of serologic tests have received EUAs from the FDA. The first type are antibody tests that detect the presence of binding antibodies, which bind to a pathogen (e.g., a virus). The second type detects neutralizing antibodies from recent or prior SARS-CoV-2 infection. It is unknown whether 1 type of test is more clinically meaningful than the other.
- Serologic assays may detect IgM, IgG, IgA, and/or total antibodies, or a combination of IgM and IgG antibodies. Serologic assays that detect IgG and total antibodies have higher specificity to detect past infection than assays that detect IgM and/or IgA antibodies or a combination of IgM and IgG antibodies.
- False positive test results may occur due to cross-reactivity from pre-existing antibodies to other coronaviruses.
Serologic Testing and Immunity to SARS-CoV-2 Infection

The FDA has issued EUAs for more than 80 SARS-CoV-2 serologic tests since the start of the pandemic. However, these tests are not currently authorized for routine use in making individual medical decisions. SARS-CoV-2 serologic tests are authorized for detecting antibodies, but their ability to predict protective immunity has not been validated. The majority of these tests are not standardized. Furthermore, as SARS-CoV-2 is not a well-conserved virus, mutations in the receptor binding domain of the virus could lead to decreased binding affinity between antibodies and SARS-CoV-2-specific antigens.

Given the available information, there is insufficient evidence for the Panel to recommend either for or against the use of SARS-CoV-2 serologic testing to assess for immunity or to guide clinical decisions about using COVID-19 vaccines or anti-SARS-CoV-2 monoclonal antibodies in certain people.

If a serologic test is performed, the result should be interpreted with caution. It remains unclear how long SARS-CoV-2 antibodies persist following either infection or vaccination. A negative serologic test result also does not preclude prior SARS-CoV-2 infection or vaccination against COVID-19. Some people who are infected with SARS-CoV-2 or who are vaccinated against COVID-19 may not develop measurable antibodies (e.g., those who are immunocompromised). It is presumed that those who do not have measurable antibodies after vaccination are at higher risk of SARS-CoV-2 infection.

In communities that have a low prevalence of SARS-CoV-2 infection, the proportion of positive test results that are false positives may be quite high. In these situations, performing confirmatory testing with a distinct antibody assay, ideally an assay that uses a different antigenic target (e.g., the nucleocapsid phosphoprotein if the first assay targeted the spike protein), can substantially reduce false positives.

Assuming that the test is reliable, serologic tests that identify recent or prior SARS-CoV-2 infection may be used to:

- Differentiate between SARS-CoV-2 antibody responses to natural infection and vaccine-induced antibody responses to the SARS-CoV-2 spike protein antigen. Because nucleocapsid protein is not a constituent of the vaccines that are currently approved by the FDA, available through EUAs, or in late-stage clinical trials, serologic tests that detect antibodies by recognizing nucleocapsid proteins can be used to distinguish between antibody responses to natural infection and vaccine-induced antibody responses.

- Determine who may be eligible to donate convalescent plasma

- Define multisystem inflammatory syndrome in children (MIS-C) and multisystem inflammatory syndrome in adults (MIS-A)

- Estimate the proportion of the population that has been exposed to SARS-CoV-2

Based on current knowledge, serologic tests should not be used to (AIII):

- Make decisions about how to group persons in congregate settings;

- Determine whether someone may return to the workplace; or

- Assess for immunity to SARS-CoV-2 following vaccination in immunocompetent individuals, except in clinical trials.

References


2. Food and Drug Administration. Coronavirus disease 2019 (COVID-19) emergency use authorizations for


