Summary Recommendations

- To diagnose acute infection of SARS-CoV-2, 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., a nasopharyngeal, nasal, or oropharyngeal specimen) (AIII).
- For intubated and mechanically ventilated adults who are suspected to have COVID-19 but who do not have a confirmed diagnosis:
  - 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 samples when collecting lower respiratory tract samples to establish a diagnosis of COVID-19 (BII).
- A NAAT should not be repeated in an asymptomatic person within 90 days of a previous SARS-CoV-2 infection, even if the person has had a significant exposure to SARS-CoV-2 (AIII).
- SARS-CoV-2 reinfection has been reported in people who have received an initial diagnosis of infection; therefore, a NAAT should be considered for persons 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).
- 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).
- The Panel recommends against the use of serologic (i.e., antibody) testing to determine whether a person is immune to SARS-CoV-2 infection (AIII).

Rating of Recommendations: A = Strong; B = Moderate; C = Optional

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, as well as 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. Ideally, diagnostic testing should also be performed for people who are likely to be at repeated risk of exposure to SARS-CoV-2, such as health care workers and first responders. Testing should also be considered for individuals who spend time in heavily populated environments (e.g., teachers, students, food industry workers) and for travelers. Testing requirements may vary by state, local, and employer policies. Travelers may need evidence of a recent negative test result to enter some states or countries; such documentation may be an acceptable alternative to quarantine upon arrival.

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), but no diagnostic test has been approved by the FDA.

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. Lower respiratory tract samples have a higher yield than upper tract samples, but they are often not obtained because of concerns about aerosolization of the virus during sample collection procedures. Some tests that have received EUAs can also be performed on saliva specimens. Studies are currently evaluating the use of other sample types, including stool samples.

Some tests that have received EUAs allow for self-collection of specimens at home, but these specimens...
must be sent to a laboratory for processing. In addition, some tests allow trained personnel to collect and test specimens in nonclinical settings, such as in the home or in nursing or assisted living facilities. This allows real-time antigen results to be obtained on site.

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

Reverse transcriptase 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 a variety of additional platforms (e.g., reverse transcriptase loop-mediated isothermal amplification [RT-LAMP]). 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 one 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 one 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 variant that has been associated with increased transmission carries 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 potentially discordant shedding of virus from the upper versus the 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 careful consideration of 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 (AIII). If there are concerns that an immunocompromised health care worker may still be infectious >20 days from the onset of SARS-CoV-2 infection, consultation with local employee health services regarding return-to-work testing policies is advised.

SARS-CoV-2 reinfection has been reported in people who have received an initial diagnosis of infection; therefore, a NAAT should be considered for persons 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). However, it should be noted that persons infected with SARS-CoV-2 may have a negative result on an initial NAAT and then have a positive result on a subsequent test due to intermittent detection of viral RNA and not due to reinfection. 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 RT-PCR-based tests, 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. Advantages of antigen-based tests are 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 where preventing transmission is critical. Antigen-based 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 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 additional NAATs 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 who is asymptomatic receives a positive result. 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)
• 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), the Panel does not recommend 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. 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 for SARS-CoV-2, 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). Serologic assays that have FDA EUAs should be used for public health and clinical use. 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 of tests detect neutralizing antibodies from recent or prior SARS-CoV-2 infection. It is unknown whether one 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 Panel recommends against the use of serologic testing to determine whether a person is immune to SARS-CoV-2 infection (AIII).

If SARS-CoV-2 antibodies are detected during a serologic test, the results should be interpreted with caution for the following reasons:

• It is unclear how long antibodies persist following infection; and
• It is unclear whether the presence of antibodies confers protective immunity against future 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, confirmatory testing using a distinct antibody assay, ideally one that uses a different antigenic target (e.g., the nucleocapsid phosphoprotein
if the first assay targeted the spike protein), can substantially improve the probability that persons with positive test results are antibody positive.

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

- Differentiate SARS-CoV-2 antibody responses to natural infection from vaccine-induced antibody responses to the SARS-CoV-2 spike protein antigen. Because nucleocapsid protein is not a constituent of vaccines that are currently available through EUAs or in late-stage clinical trials, serologic tests that detect antibodies by recognizing nucleocapsid protein can be used to distinguish antibody responses to natural infection from vaccine-induced antibody responses.
- Determine who may be eligible to donate convalescent plasma
- 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 (e.g., schools, dormitories, correctional facilities)
- Determine whether persons may return to the workplace
- Assess for prior infection solely to determine whether to vaccinate an individual
- Assess for immunity to SARS-CoV-2 following vaccination, except in clinical trials

References


