

Testing for COVID-19: PCR, Antigen, and Serology

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This document provides guidance for health care providers regarding diagnostic tests for SARS-CoV-2, the virus that causes COVID-19 disease. Polymerase chain reaction (PCR) tests remain the preferred tests for diagnosing acute COVID-19 infection. This guidance has been updated to reflect current guidance on when to use viral (nucleic acid or antigen) tests and serologic tests (antibody), including recommendations for use of viral tests on asymptomatic people.

Viral Tests

Viral tests detect nucleic acid or antigens of SARS-CoV-2 and are the only tests that are recommended to diagnose acute COVID-19 infection. The FDA maintains a [list of in vitro diagnostic tests](#) for COVID-19 granted Emergency Use Authorization (EUA). Only FDA EUA-approved viral diagnostic tests should be used.

What types of viral tests are commercially available?

- **Molecular tests:** These tests amplify and then detect specific fragments of viral RNA. Depending on the test, different sequences of RNA may be targeted and amplified. Examples of this method include *polymerase chain reaction (PCR)*, *loop-mediated isothermal amplification (LAMP)*, and *Nucleic Acid Amplification Test (NAAT)*.
- **Antigen tests:** These rapid tests identify viral protein fragments.
- The performance characteristics of EUA-approved assays are available in the “Instructions for Use” links on the FDA website (See <https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#covidinvitrodev>.)

Which tests are more reliable?

Not all viral tests have equivalent sensitivity and specificity. There is some variation in the sensitivity of different assays. Sensitivity is also highly dependent on the stage of the infection (higher early in infection), and negative results should always be interpreted in the context of the exposure history and clinical presentation.

Molecular tests

- When comparing molecular tests, tests that amplify two or more RNA gene targets are likely to have higher specificity (fewer false positives).
- The FDA has cautioned about false negative results with the Abbott ID NOW platform. If necessary for clinical management or infection control purposes, negative results obtained from this test should be confirmed with an alternative FDA authorized molecular assay.
- False-negative rate is lowest 3 days after onset of symptoms (approximately 8 days after exposure)¹.

¹ Kucirka et al. Variation in False-Negative Rate of Reverse Transcriptase Polymerase Chain Reaction–Based SARS-CoV-2 Tests by Time Since Exposure. *Annals of Internal Medicine*. May 13, 2020

- False positive results have been reported with the [BD Max SARS-CoV-2 RT-PCR reagents](#) on the BD Max system.

Antigen tests

- Antigen tests are used as rapid point-of-care tests and are not recommended for use on asymptomatic people, except in high prevalence settings.
- At this time, two antigen tests have received FDA EUA. The performance characteristics vary greatly between them. Neither have been specifically approved by the FDA for use on asymptomatic people.
 - The Quidel Sofia 2 reports a positive predictive agreement (PPA) of 96.7%.
 - The BD Veritor reports only 85% PPA; negative results with this test are considered presumptive and should be confirmed with an FDA authorized molecular assay, if necessary for clinical management or infection control.
- False positives have been reported when swabs are placed in viral transport medium prior to testing.
- For further information, see the Association of Public Health Laboratories (APHL) guidance document on the use of antigen tests, which is based on experience with rapid influenza diagnostics: <https://www.aphl.org/programs/preparedness/Crisis-Management/Documents/APHL-SARSCov2-Antigen-Testing-Considerations.pdf>

Can the cycle threshold (Ct) value be used in the interpretation of PCR results?

The FDA EUA PCR assays for SARS-CoV-2 are qualitative (detected vs. not detected) rather than quantitative assays that determine that amount of virus in a sample. Higher Ct values correlate with less SARS-CoV-2 RNA in the sample. Although attempts to culture virus from upper respiratory specimens have been largely unsuccessful when Ct values are high², SARS-CoV-2 PCR Ct values cannot be interpreted as a measure of viral burden, are not standardized by RT-PCR platform, and have not been approved by the FDA for use in clinical management. While the CDC does not recommend the use of Ct values for clinical management, they may be useful in the context of laboratory and clinical information available for a patient. Note that Ct values are not included routinely in lab reports and may be difficult to retrieve.

What if I'm worried about false negatives?

If there is strong clinical suspicion for COVID-19 (compatible symptoms and/or exposure), and a patient has a negative viral test result, the patient should be isolated and treated as a presumed positive.

Due to lower sensitivities of antigen tests and the Abbott ID NOW test, any negative result with the Abbott ID NOW or BD Veritor *or* a negative Quidel Sofia 2 antigen result on a sample collected more than 5 days after symptom onset should be considered presumptively negative. Negative results from these tests should be confirmed with an FDA EUA-approved molecular assay, such as a PCR test, if necessary for clinical management or infection control.

What if I'm worried about false positives?

² Rao et al. A Narrative Systematic Review of the Clinical Utility of Cycle Threshold Values in the Context of COVID-19. Infect Dis Ther. July 2020.



False positive viral tests are rare but are known to occur when swabs are placed in an incorrect viral transport medium prior to testing. However, if a patient has a new positive viral test for COVID-19 and any of the following are true, treat as a case and isolate per CDC guidelines:

- The patient is symptomatic
- The patient is asymptomatic AND is a close contact with a known COVID-19 contact in the last two weeks
- The patient is asymptomatic AND tested for screening purposes AND has never tested positive in the past AND meets at least one of the following high-risk criteria
 - Lives or works in a community with ongoing high SARS-CoV-2 transmission
 - Has had potential exposures to COVID-19
 - Lives or works in a congregate setting (i.e., nursing home, prison)
 - Is going to be transferred to a congregate setting

What tests are appropriate to use on asymptomatic people?

Please refer to the FDA website for a current list of tests that have received EUA for use on asymptomatic people. There are situations where testing asymptomatic people may be appropriate, such as contact investigations, prior to surgical procedures, and prior to transfer to congregate living settings. Test results in asymptomatic people must be interpreted with caution given the unknown test characteristics in this population. Clinicians should consider the individual's pre-test probability of illness (i.e., overall prevalence of disease, risk factors) and be aware that the predictive value of a positive result is lower in asymptomatic people. When there is uncertainty regarding how to interpret a test result in an asymptomatic person, expert clinical consultation is advised.

What about pooled testing?

At this time, pooled testing can only be performed using a molecular assay that has received an FDA EUA for pooled testing, and that assay may only be used by the laboratory granted EUA status. Sample pooling, which is done in the laboratory, allows multiple specimens to be tested together in a "pool." If the pool tests positive, each sample from that pool must be tested individually to determine which sample(s) is positive from the pooled set. This testing strategy is most efficient in areas with low prevalence of infection.

Serologic Tests

Results from antibody testing should not be used to diagnose or exclude SARS-CoV-2 infections or to determine past infection status. None of the serologic assays that have been granted an FDA EUA are authorized for diagnosing acute COVID-19 infection. Negative results from antibody testing do not rule out SARS-CoV-2 infections, particularly in people who have been recently exposed to the virus and are still within the estimated incubation period. Similarly, a positive result may be due to past or present infection with a coronavirus other than SARS-CoV-2.

Serologic tests should NOT be used to diagnose acute COVID-19 infection or infer immunity from past infection because:

- There is a lag time to detection of antibodies. Studies suggest that fewer than 40% of patients will have detectable antibodies to SARS-CoV-2 in the first week after symptoms begin, and in some cases, it may be up to 21 days before antibodies are detectable. This includes IgM, which rises almost simultaneously with IgG. This delay means that serologic tests may have limited utility for diagnosing acute disease.

- Measurable antibody levels typically wane over time and may become undetectable in some people after infection.
- A positive serologic result does not necessarily indicate that a patient has protective immunity. Antibodies are produced to various viral antigens, and not all antibodies will protect against re-infection. At this time, there are no FDA EUA approved assays that can determine if a patient's antibodies will neutralize virus or protect from re-infection. It is not yet known whether or how long people who develop detectable antibodies are protected against re-infection with SARS-CoV-2. Until more information is available, patients with a positive serologic assay should not be assumed to be immune to SARS-CoV-2.

When might serologic testing be appropriate?

- Serologic tests are most appropriate as a surveillance tool (i.e., providing population-level estimates of exposure to SARS-CoV-2), rather than as a diagnostic tool for individual patients. Serologic tests may be used to document seropositivity for plasma donation or vaccine studies.
- Clinically, serologic tests may be useful in individual patients with negative PCR testing results but a high clinical suspicion for COVID-19 disease. An example might include:
 - A hospitalized patient, 12 days after symptom onset of fever and non-productive cough, found to have ground glass opacities on chest imaging and progressive respiratory failure, whose initial SARS-CoV-2 PCR testing from pooled oropharyngeal + nasopharyngeal swabs was negative. Respiratory viral panel testing was also negative, and no other cause of respiratory failure has been identified. Patient is currently on high-flow oxygen therapy, and a lower respiratory tract specimen for repeat SARS-CoV-2 PCR test is difficult to obtain.

A positive COVID-19 serologic test may be consistent with any of the following:

- Recent SARS-CoV-2 infection, whether symptomatic (>1 week after onset of symptoms) or asymptomatic
- Past infection, whether symptomatic or asymptomatic
- False positive results and no SARS-CoV-2 infection

A negative COVID-19 serologic test may be consistent with any of the following:

- No recent or prior SARS-CoV-2 infection
- Early SARS-CoV-2 infection, after exposure but prior to the development of antibodies
- Undetectable antibody levels after SARS-CoV-2 infection
- False negative results after true SARS-CoV-2 infection

What types of serologic tests are commercially available?

The FDA maintains a [list of in vitro diagnostic tests](#) for COVID-19 that have been granted an EUA. Only FDA EUA-approved serologic tests should be used.

Serologic tests vary in the following ways:

- Methodology: Rapid tests or point-of-care tests often use a lateral flow methodology and provide qualitative (positive or negative) results. Note that rapid serologic tests typically are validated with serum (from a venous blood draw) and are often less sensitive and specific when performed on capillary blood (i.e., from a finger-prick). Other serologic tests, typically - Enzyme immunoassay (EIA)



or Enzyme-linked immunosorbent assay (ELISA), can be qualitative or quantitative and are performed in high complexity laboratories.

- Classes of antibody detected: Serologic tests may measure levels of IgM, IgG, IgA, or a combination of these. Antibody dynamics and correlates of immunity for COVID-19 are not well understood at this time.
- Target viral antigens: Assays vary in the viral antigen(s) they use to detect antibody. Most SARS-CoV-2 serology assays target antibodies to the spike protein (S protein) or to a specific region of the S protein (e.g., the receptor-binding domain). The clinical significance of each target and correlates of immunity are not fully understood. Assays that target antibodies to the S protein tend to be more specific for SARS-CoV-2 than assays that target the nucleocapsid (N) protein that is more conserved across other coronaviruses.

Which serologic tests are more reliable?

- There is a high degree of variability in sensitivity and specificity between different serologic assays.
- Serologic tests are generally less specific than PCR tests and have a greater potential to cross-react with coronaviruses other than SARS-CoV-2.
- The positive predictive value of a test depends not only on the sensitivity and specificity of a test, but also on the pre-test probability of disease, as measured by the population prevalence of disease. For example, in a population with an expected 5% prevalence of SARS-CoV-2 infection, a serologic test with 95% sensitivity and 95% specificity will have 50% positive predictive value.
- Even in heavily impacted settings, the prevalence of SARS-CoV-2 can be low, and serologic testing may result in false positive results.
- The FDA has provided guidance for how to evaluate the approved serologic assays at <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance>.

Are serology results reported to public health departments?

Serology results are currently reportable to local and state health departments via electronic lab reporting (ELR), but patients with positive serology results who do not also have documented positive PCR are not counted as confirmed COVID-19 cases, and serology results are not currently included in the Council of State and Territorial Epidemiologists (CSTE) laboratory criteria for [confirmed cases of COVID-19](#). Local health departments may use serology results as needed for surveillance and tracking probable cases. However, there are no requirements to take any actions based on serology results.

At Home Testing

Several home collection kits, where patients collect their own nasal swabs or saliva samples and send it back to a laboratory for analysis, have FDA EUA and can be useful in a number of clinical and public health situations.

Fraudulent Test Kits

The FDA has reported on the sale of unauthorized fraudulent test kits for COVID-19. At this time, there are no kits approved for at-home testing of COVID-19 where patients collect their own nasal swabs or saliva samples and process the tests in their own home. Please counsel patients to avoid home testing where the patient is analyzing the specimen at home for a result.

Where can I find more information?

- IDSA Guidelines on the Diagnosis of COVID-19
<https://www.idsociety.org/practice-guideline/covid-19-guideline-diagnostics/>
- IDSA COVID-19 Antibody Testing Primer
<https://www.idsociety.org/globalassets/idsa/public-health/covid-19/idsa-covid-19-antibody-testing-primer.pdf>
- CDC Interim Guidelines for COVID-19 Serology
<https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html>
- UpToDate: Coronavirus disease 2019 (COVID-19): Diagnosis (free access)
<https://www.uptodate.com/contents/coronavirus-disease-2019-covid-19-diagnosis>
- BMJ: Interpreting a COVID-19 test result
<https://www.bmj.com/content/bmj/369/bmj.m1808.full.pdf>
- Johns Hopkins Center for Health Security – Serology testing for COVID-19
<https://www.centerforhealthsecurity.org/resources/COVID-19/COVID-19-fact-sheets/200228-Serology-testing-COVID.pdf>
- Johns Hopkins Center for Health Security – Developing a National Strategy for Serology (Antibody Testing) in the United States
https://www.centerforhealthsecurity.org/our-work/pubs_archive/pubs-pdfs/2020/200422-national-strategy-serology.pdf
- FDA maintains a list of in vitro diagnostic tests for COVID-19 granted EUA at:
<https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas>

Definitions

- Sensitivity: The proportion of patients with COVID-19 who have a positive test, i.e., “true positives”
- Specificity: The proportion of patients without COVID-19 who have a negative test, i.e., “true negatives”
- Positive percent agreement: The proportion of samples that test positive compared with the reference test.
- Positive predictive value: The probability that patients with a positive test truly have COVID-19.
- Pre-test probability: The likelihood a person has COVID-19 based on their characteristics.

COVID-19 testing overview

	Viral Test		Serology (Antibody)
	Molecular	Antigen	
Method	Amplifies specific fragment of viral RNA using RT-PCR, LAMP, or NAAT	Detects fragments of viral proteins	Detects antibodies to specific viral protein targets
Sample type*	Nasopharyngeal (NP), oropharyngeal, or nasal swab; saliva; lower respiratory tract specimens	NP or nasal swab	Blood-based test
Timing	Most likely to be positive 1-2 days before symptom onset and in early days of symptomatic	In symptomatic infection, more likely to be positive as symptoms increase,	Takes at least 7-14 days after symptom onset to develop antibodies, and



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	infection; positivity wanes over time but can persist. The false negative rate is lowest 3 days after onset of symptoms, or approximately 8 days after exposure. ¹	sensitivity declines over time.	varies depending on antibody class measured
Performance	Sensitivity depends on timing of sample collection, sampling technique and sample type	Performance is variable and may not be appropriate for use in asymptomatic persons.	Sensitivity and specificity are highly variable depending on the test
Examples	CDC SARS-CoV-2 Assay; Panther Fusion (real-time RT-PCR) Abbott ID Now (LAMP)	Quidel Sofia 2; BD Veritor	Abbott Architect; Diasorin Liaison; Bio-Rad Platelia

* Refer to FDA EUA information for sample types approved for specific tests