

COVID-19, PCR, Respiratory Specimen (Lab Tests and Diagnostic Procedures)

Comment

The FDA has noted that unauthorized **fraudulent** COVID-19 test kits are being sold online. The FDA advises consumers and health professionals to be cautious of websites and stores selling products that claim to prevent, diagnose, treat, or cure COVID-19 ([FDA Fraudulent 2020](#)). Consumers and health care professionals can help by reporting suspected fraud to the [FDA's Health Fraud Program](#) or the [Office of Criminal Investigations](#).

The molecular tests for SARS-CoV-2 are designed to detect specific genetic sequences found in the genome of the virus. As the virus can mutate over time resulting in genetic variations, the FDA is alerting the health care community that false negative results may occur with any SARS-CoV-2 molecular test if a mutation occurs in the part of the virus' genome assessed by a particular test. Currently the FDA has identified six molecular tests that may be impacted by viral mutation and have reduced sensitivities ([FDA SARS-CoV-2 Mutations 2021](#)):

- **Accula SARS-CoV-2 Test** manufactured by Mesa Biotech Inc. Performance may be affected when patient sample has genetic variant at position 28881 to 28883 (GGG to AAC), and also 28877 to 28878 (AG to TC). This impact does not appear to be significant, however; the FDA is alerting health care providers.
- **TaqPath COVID-19 Combo Kit** (also labeled as TaqPath COVID-19 Combo Kit Advanced) manufactured by Thermo Fisher Scientific, Inc. One of three targets in this assay has significantly reduced sensitivity due to mutations including mutations in the B.1.1.7 variant. As this assay detects multiple genetic targets, the overall sensitivity should not be impacted. When certain mutations are present, the pattern of detection may help with early identification of new variants.
- **Linea COVID-19 Assay Kit** manufactured by Applied DNA Sciences, Inc. One of the two targets in this assay has significantly reduced sensitivity due to mutations, including a mutation in the B.1.1.7 variant. As this assay detects multiple genetic targets, the overall sensitivity should not be impacted. When certain mutations are present, the pattern of detection may help with early identification of new variants.
- **Xpert Xpress SARS-CoV-2, Xpert Xpress SARS-CoV-2 DoD, Xpert Omni SARS-CoV-2** manufactured by Cepheid. These tests are impacted by a single point mutation in the target area of the test. They look for both the N2 and E target regions within viral RNA. Two independent single point mutations reduce test sensitivity for detecting the N2 target. The E target is still detected when enough virus is present, and a *presumptive positive* result is reported with the **Xpert Xpress SARS-CoV-2** and **Xpert Xpress SARS-CoV-2 DoD** tests. With the **Xpert Omni SARS-CoV-2** test, detection of the E target without detecting the N2 target is reported as *positive*. As the testing is designed to detect multiple genetic targets, and the mutations do not lead to false negative results, the FDA states the impact on test performance does not appear to be significant.

- For more information see [FDA SARS-CoV-2 Mutations: Impact on COVID-19 Tests](#).

On September 17, 2021, the FDA issued an alert for clinical laboratory staff and health care providers of a potential for false positive results with the **Alinity m SARS-CoV-2 AMP Kit**, List number 09N78-095, and **Alinity m Resp-4-Plex AMP Kit**, List Number 09N79-096. Any positive results from either of these two assays should be considered presumptive and retesting with an alternative test is recommended on positive patient specimens performed within the last two weeks ([FDA Potential for False Positive 2021](#)).

With the development of the SARS-CoV-2 omicron variant (B.1.1.529), the FDA has currently identified assays that may be impacted ([FDA SARS-CoV-2 Mutations 2021](#)):

1. Tests with Reduced Ability to Detect the SARS-CoV-2 Omicron Variant:

- Meridian Bioscience, Inc. Revogene SARS-CoV-2: Single target test is expected to fail to detect the SARS-CoV-2 omicron variant, resulting in false negative results in patients with the omicron variant.
- Tide Laboratories DTPM COVID-19 RT-PCR test: Single-target test is expected to fail to detect the SARS-CoV-2 omicron variant, resulting in false negative results in patients with the omicron variant.

2. Tests with S-Gene Drop Out - SARS-CoV-2 Detection Should Not Be Impacted: Assays with multiple targets and one genetic target expected to have significantly reduced sensitivity due to a mutation in the SARS-CoV-2 omicron variant (specific deletion in the spike [S] gene [Δ 69-70]). As these tests are designed to detect multiple targets, overall test sensitivity should not be impacted. For a list of these assays and more information see [Omicron Variant: Molecular Tests That May Be Impacted](#).

On February 22, 2022 the FDA published a list of authorized at-home over the counter COVID-19 diagnostic tests. See <https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/home-otc-covid-19-diagnostic-tests>.

Prior receipt of a COVID-19 vaccine will **not** affect viral testing for SARS-CoV-2.

Related Information

- [COVID-19 Antibody \(IgG\), Quantitative, Serum or Plasma](#)
- [COVID-19 Antibody \(IgG\), Semi-quantitative, Serum or Plasma](#)
- [COVID-19 Antibody \(IgG\), Serum, Plasma, or Whole Blood](#)
- [COVID-19 Antibody \(IgM\), Serum or Plasma](#)
- [COVID-19 Antibody Total, Oral Fluid](#)
- [COVID-19 Antibody Total, Serum, Plasma, or Whole Blood](#)
- [COVID-19 Antibody, IgG/IgM Rapid Test, Serum, Plasma, or Whole Blood](#)
- [COVID-19 Antigen, Saliva](#)

- [COVID-19 Antigen, Upper Respiratory Specimen](#)
- [COVID-19 Neutralization Antibody, Serum or Plasma](#)
- [COVID-19, PCR, Saliva](#)
- [Influenza A/B, SARS-CoV-2, Respiratory Syncytial Virus, PCR](#)
- [Influenza and SARS-CoV-2 Antigen, Upper Respiratory Specimen](#)
- [Influenza SARS-CoV-2 Multiplex Assay, PCR, CDC](#)
- [Respiratory Panel, PCR, Nasopharyngeal](#)
- [T-Detect COVID Test, Whole Blood](#)

Overview

At the end of 2019, a novel coronavirus was identified as the cause of several cases of pneumonia in Wuhan City, Hubei Province, China. Initially linked to a large seafood and animal market suggesting animal-to-human spread, person-to-person transmission was quickly confirmed (Li Q 2020). The virus spread rapidly worldwide and in January 2020, the World Health Organization (WHO) declared the outbreak a "public health emergency of international concern." On March 11, 2020, the WHO publicly characterized COVID-19 as a pandemic. Currently, more than 400 million infections have been confirmed globally in over 200 countries and territories with over 6 million deaths ([WHO situation report 2022](#)).

The novel virus has been named **severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)** and the disease it causes has been named **coronavirus disease 2019, or COVID-19**. The virus spreads by contact with respiratory fluids (droplets or aerosol) produced when an infected person exhales (eg, breathes, speaks, coughs, sneezes, or sings). These droplet/aerosol particles can be

- inhaled directly into lungs
- directly deposited on exposed mucous membranes (eyes, mouth, nose)
- transferred to mucous membranes by hands contaminated with virus-containing respiratory fluids or by indirectly touching surfaces with virus on them ([CDC SARS-CoV-2 Transmission 2021](#))

COVID-19 symptoms typically appear within 2 to 14 days (median of 5 days) of exposure and include **fever, chills, fatigue, cough, shortness of breath, myalgia, recent loss of taste or smell, vomiting or diarrhea, and/or sore throat**. Sickness ranges from a mild respiratory illness to severe disease including respiratory failure, septic shock, or other organ failure. Most fatalities have occurred in patients with underlying comorbidities, with overall global fatalities around 2 percent ([WHO situation report 2022](#)). The CDC currently estimates that about 30% of COVID-19 infections are asymptomatic. ([CDC Pandemic Planning 2021](#)). The majority of SARS-CoV-2 transmission occurs early in the course of illness, generally in the 1 to 2 days prior to onset of symptoms and 2 to 3 days after symptom onset ([CDC Updates and Shortens 2021](#)).

The Center of Disease Control and Prevention (CDC) developed a nucleic acid amplification test (NAAT) using a real time Reverse Transcription-Polymerase Chain Reaction (rRT-PCR) assay to detect SARS-CoV-2 in respiratory specimens. In the early stages of disease dissemination, the CDC was the only lab

conducting the SARS-CoV-2 testing; however, with the rapid spread of the viral infection, in February 2020 the FDA issued an Emergency Use Authorization (EUA) allowing qualified laboratories (as designated by CDC) to perform testing. Those facilities included US state and local public health laboratories, Department of Defense (DOD) laboratories, and select reference and international laboratories. Currently, under FDA guidance, there has been local, hospital-based SARS-CoV-2 test development. The developed tests may be based on the CDC, WHO, or manufacturer assays, or created de novo. The laboratories must perform an assay validation and pursue an FDA Emergency Use Authorization. See [Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency \(Revised\)](#).

As the pandemic has evolved, many FDA EUA nucleic acid amplification tests have entered the market for use in different settings,

- Laboratory-based testing (health care facilities and reference labs); useful for larger specimen volumes. Testing is performed by experienced laboratorians. Turnaround time can be up to 72 hours.
- At home self-collection devices to be sent into specific testing facilities for analysis
- Rapid, point-of-care tests which can provide results in 5 to 45 minutes
- Prescription tests that can be self-collected and performed at home or at point-of-care
- Over-the-counter (nonprescription), at-home, for use with a compatible mobile smart device
- Testing on pooled specimens. Tests process samples from multiple people at one time (can save time and money)
- Serial Screening. Repeated testing at different points in time, more likely to detect infection after known exposure. These qualitative NAATs can be used for individuals with or without symptoms. They provide better testing solutions for schools, workplaces, and communities when establishing SARS-CoV-2 detection protocols.
- Saliva testing. Saliva specimens can be collected at home or in a health care setting when determined to be appropriate by a health care provider. Such testing is becoming more prevalent as self collection reduces the need for personal protection equipment and the process is less invasive than other methods.
- Multianalyte panels. Molecular respiratory panels that include SARS-CoV-2 and several other well-known respiratory pathogens. These panels are intended for qualitative identification and differentiation of nucleic acid from multiple organisms (including influenza A and B) and assist health care providers in discerning various infectious agents that produce similar symptoms to COVID-19. Panel testing can also assist in diagnosing coinfections which have been noted in some COVID-19 patients (Ding 2020; Kim 2020).

As each of these assays has unique specificities regarding specimen, collection, transport and processing, it is important that health care providers understand testing options available and follow manufacturer instructions for proper specimen collection, processing, and transport. The level of sensitivity for the detection of SARS-CoV-2 RNA varies by the method used; laboratory-based NAATs

tend to have higher sensitivity than point-of-care or self-administered tests ([CDC NAATs 2021](#)). The information below covers the specimens and collection procedures required by the CDC.

Use/Indications

Aid in the diagnosis of coronavirus disease (COVID-19); detect current infection

Nucleic acid amplification testing was also originally recommended for determining resolution of infection, a **test-based** strategy which required serial tests and improvement of symptoms to determine when an individual with SARS-CoV-2 infection was no longer infectious and transmission-based precautions could be discontinued. However, the CDC has changed to a **symptom-based** strategy with recommendations based on severity of illness. It was found in the majority of cases, the test-based strategy actually prolonged the isolation of patients who continue to shed detectable SARS-CoV-2 RNA but are no longer infectious (virus is no longer replication-competent). A test-based strategy is still considered useful for some patients with severe illness or those who are severely immunocompromised. Such patients may produce replication-competent virus for longer than 10 to 20 days after symptom onset ([CDC Ending Isolation 2021](#); Baang 2021; Aydillo 2020) and molecular testing may be necessary to determine infection resolution.

Special Instructions

With the rapid spread and newly acquired knowledge of COVID-19 along with the increased availability of tests, the CDC and IDSA testing recommendations are continuously changing, see

[Overview of Testing for SARS-CoV-2 \(COVID-19\)](#) and

[Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19](#) (IDSA [Hanson 2020]).

Note: Testing for other respiratory pathogens by the provider should be done as part of the initial work-up (as indicated) and should not delay specimen processing or shipping to qualified testing laboratory.

Test Includes

Qualitative detection of nucleic acid (one or more RNA gene targets) specific for SARS-CoV-2

Turnaround Time

Result turnaround varies with the type of molecular test performed.

- Standard hospital or large testing facility molecular test: 1 to 3 days
- Rapid point-of-care test: 15-45 minutes
- At home NAAT: 20-60 minutes
- At home mail-in test: Up to 1 week

Specimen

Upper Respiratory Tract Specimen

For initial COVID-19 testing, the CDC recommends collecting an upper respiratory specimen. This includes ([CDC Interim Guidelines for Collecting 2021](#)):

- Nasopharyngeal (NP) specimen collected by a trained health care professional. **Or**
- Oropharyngeal (OP) specimen collected by a trained health care professional. **Or**
- Nasal mid-turbinate (NMT) specimen collected by a trained health care professional or by a supervised or unsupervised onsite self-collection (using flocked tapered swab), or self-collected at home following kit instructions. Both nares should be swabbed. **Or**
- Anterior nares (nasal swab; NS) specimen collected by a trained health care professional, or by a supervised or unsupervised onsite self-collection (using a flocked or spun polyester swab), or self-collected at home following kit instructions. Both nares should be swabbed using the same swab. **Or**
- Nasopharyngeal wash/aspirate or nasal wash/aspirate (NW) specimen collected by health care professional. **Or**
- Saliva, self-collection at home or at testing site
- **Note:** The IDSA recommends collecting an NP swab, NMT swab, anterior nasal swab, saliva, or a combined anterior nasal/oropharyngeal swab rather than an oropharyngeal swab alone for nucleic acid testing in symptomatic patients ([IDSA \[Hanson 2020\]](#)).

Lower Respiratory Tract Specimen

- Bronchoalveolar lavage (BAL), tracheal aspirate, pleural fluid, lung biopsy
- Sputum - for patients who develop a productive cough; induction of sputum is **not** recommended.

Note: SARS-CoV-2 RNA has also been detected in stool, blood, and tissue, and semen (Li D 2020).

Container(s)

- Confer with testing laboratory for proper specimen container and collection.
- Lower respiratory specimen: Sterile, screw-cap plastic container
- Nasal mid-turbinate (NMT) specimen: Flocked tapered swab
- Nasal swab or anterior nares (NS) specimen: Flocked or spun polyester swab
- Nasopharyngeal or oropharyngeal specimen: Use only synthetic fiber swab with plastic or wire shaft. Do not use calcium alginate swab or cotton swab with wooden shaft.
- Nasopharyngeal wash/aspirate or nasal wash/aspirate specimen: Sterile viral transport media tube
- Saliva: Sterile, screw-cap plastic container

Volume / Minimum Volume

- 1 NP swab or 1 OP swab or 1 NS swab or 1 NMT swab
- Fluids: 2-3 mL

- Saliva: 1-5 mL

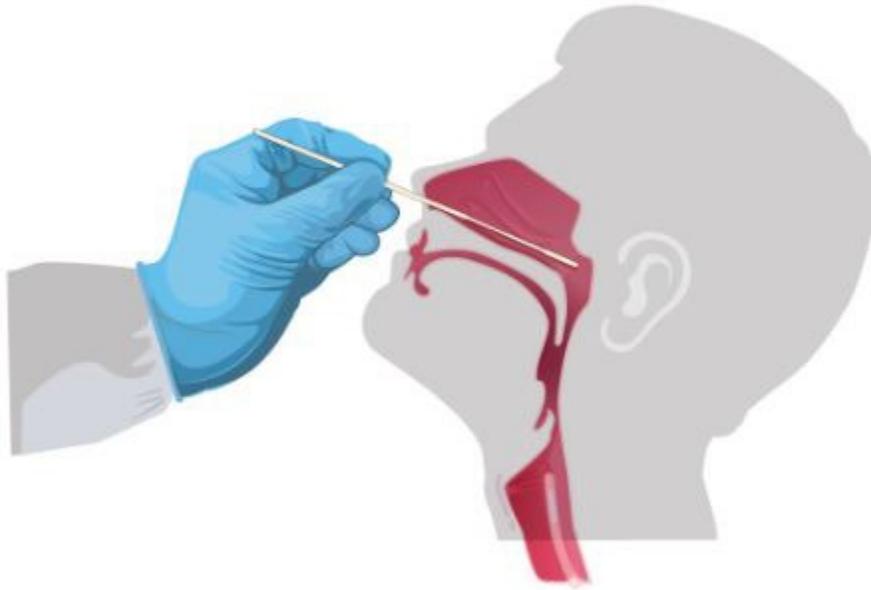
Collection

Prior to specimen collection, patient identity should be confirmed using two independent identifiers; use of a patient identification arm band or similar system is recommended. Specimen label(s) should include the two independent identifiers and the date of collection. There should be a method to identify the individual collecting the specimen. The specimen container(s) should be labeled in the presence of the patient after specimen is collected. Container(s) should **not** be pre-labeled. Use computer-generated label(s), if available, to avoid transcription errors.

Collect specimen from patient as soon as possible, regardless of time of symptom onset. Health care professionals collecting specimens should use proper infection control techniques and wear personal protective equipment, including an N95 respirator (or facemask if respirator is not available), eye protection, gloves, and a gown.

Upper Respiratory Tract Specimen

- **NP swab:** Tilt patient's head back 70 degrees. Insert a minitip swab with flexible (wire or plastic) shaft through the nostril parallel to the palate (not upwards) until resistance is detected or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. (ie, swab should reach depth equal to distance from nostrils to outer opening of the ear). Gently rub and roll swab. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it. Specimens can be collected from both sides using the same swab, but it is not necessary to collect from both sides if the minitip is saturated with fluid from the first collection. If a deviated septum or blockage create difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril. Place swab immediately into tube provided or sterile tube containing 2 to 3 mL of viral transport media (VTM), Amies transport medium, phosphate-buffered saline, or sterile saline. If both are collected, NP and OP specimens should be placed in the same vial at collection site. **Note:** Some point of care assays are designed to analyze the specimen **directly (without medium)**; use swabs provided and follow manufacturers instructions for swab storage and processing. See [CDC Nasopharyngeal Specimen Collection Steps](#).



CDC Interim Guidelines for Collecting 2021

- **OP (throat) swab:** Avoiding the tongue, teeth, and gums, insert swab into the posterior of pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx. Place swab immediately into tube provided or sterile tube containing 2 to 3 mL of viral transport media (VTM), Amies transport medium, phosphate-buffered saline, or sterile saline. If both are collected, NP and OP specimens should be placed in the same vial at collection site. **Note:** Some point of care assays are designed to analyze the specimen **directly (without medium)**; use swabs provided and follow manufacturers instructions for swab storage and processing.
- **NMT swab (deep nasal swab):** Use a flocked tapered swab. Tilt patient's head back 70 degrees. While gently rotating, insert swab less than one inch (about 2 cm) into nostril, parallel to the palate (not upwards), until resistance is met at turbinates. Rotate the swab several times against nasal wall and repeat in other nostril using the same swab. Place swab immediately into tube provided or sterile tube containing 2 to 3 mL of viral transport media (VTM), Amies transport medium, phosphate-buffered saline, or sterile saline. **Note:** Some point of care assays are designed to analyze the specimen **directly (without medium)**; use swabs provided and follow manufacturers instructions for swab storage and processing. See [CDC Nasal Mid-turbinate Specimen Collection Steps](#).
- **NS (anterior nares):** Using a flocked or spun polyester swab, insert the entire tip of swab 0.5 to 0.75 inch (1 to 1.5 cm) inside the nostril (naris) and firmly sample the nasal membrane by rotating the swab in a circular path against the nasal wall at least 4 times. Take approximately 15 seconds to collect the sample. Be sure to collect any nasal drainage that may be present on the swab. Sample both nostrils with same swab. Place swab immediately into tube provided or

sterile tube containing 2 to 3 mL of viral transport media (VTM), Amies transport medium, phosphate-buffered saline, or sterile saline. **Note:** Some point of care assays are designed to analyze the specimen **directly (without medium)**; use swabs provided and follow manufacturers instructions for swab storage and processing. See [CDC How to collect Your Anterior Nasal Swab Sample for COVID-19 Testing](#).

- **NP wash/aspirate or nasal wash/aspirate (NW):** Sterile collection by health care provider. Attach catheter to suction apparatus. Tilt patient's head back 70 degrees. Instill 1 to 1.5 mL of non-bacteriostatic saline (pH 7.0) into one nostril. Insert the tubing into the nostril parallel to the palate (not upwards). Catheter should reach depth equal to distance from nostrils to outer opening of ear. Begin gentle suction/aspiration and remove catheter while rotating it gently. Place specimen in a sterile viral transport media tube.
- **Saliva:** Supervised, patient self-collection of 1 to 5 mL saliva into sterile, leakproof container; without preservative

Lower Respiratory Tract Specimen

- **BAL, tracheal aspirate, pleural fluid, lung biopsy:** Sterile collection by health care provider (in the hospital setting). Collect 2 to 3 mL into sterile, leakproof, screw-cap container. Collection of lower respiratory specimens may be limited to hospitalized patients with severe disease. A lower respiratory specimen is advised if collected later in the course of disease or in patients with a negative upper respiratory test and a strong clinical suspicion of COVID-19 infection (WHO Diagnostic testing 2020).
- **Sputum:** Patient should be educated on difference between sputum and saliva. Patient should rinse mouth with water then expectorate deep cough sputum directly into sterile, leakproof screw-cap container. Health care personnel should apply standard precautions and wear an N95 or equivalent or higher respirator when collecting specimens. **Note:** Sputum induction is **not** recommended.

For more information see: [CDC Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing](#).

See [Guidelines for submitting specimens to CDC](#) for laboratory testing for SARS-CoV-2.

Processing and Storage

- For all specimens: Refrigerate at 2°C to 8°C and ship overnight on ice pack to CDC (or qualified laboratory). Specimens are stable 72 hours.
- For longer storage, freeze specimen(s) at -70°C.
- For state, local, or reference laboratories, follow specific shipping guidelines.

Stability

- Refrigerated: 72 hours
- Frozen (-70°C or lower): Not established

Causes for Rejection

Specimen stored at 2°C to 8°C over 72 hours

Methodology

- Nucleic acid amplification testing (NAAT), with real time Reverse transcription polymerase chain reaction (RT-PCR). Gold standard method for detection of SARS-CoV-2. This method amplifies a small segment of virus genetic material. Extracted viral RNA is converted to (complementary) cDNA which subsequently undergoes amplification using specific primers for the SARS-CoV-2 genes of interest. DNA amplification is monitored in real time using fluorescence. Cycling of temperatures is required [Zhao 2015]. This method has high sensitivity and specificity but is costly, requires professional skills, is time-consuming, and must be performed in a laboratory.
- Nucleic acid amplification testing (NAAT) with real time Reverse Transcription polymerase chain reaction (RT-PCR) with MALDI-TOF mass spectrometer. Ionized PCR products are separated and identified based on their mass-to-charge ratio (FDA 2020 MassArray Test). This is a newer, less utilized technique.
- Isothermal amplification. This method amplifies the target sequence at a constant temperature. [Zhao 2015])
 - Loop-mediated isothermal amplification (LAMP)
 - LAMP and Clustered regularly interspaced short palindromic repeats (CRISPR)
 - Transcription Mediated Amplification (TMA)
 - Helicase-dependent Amplification (HAD)
 - Nicking endonuclease amplification reaction (NEAR)
 - Strand displacement amplification (SDA) ([CDC NAATs 2021](#))

Note: The IDSA currently recommends rapid RT-PCR assays or standard laboratory-based NAATs be used over Rapid isothermal NAAT in symptomatic individuals due to decreased sensitivity in the rapid isothermal molecular tests (IDSA [Hanson 2020]).

Note: The above list is not comprehensive.

Normal Values/Findings

Not detected

Note: If a negative result is obtained from a patient with a high index of suspicion, the World Health Organization (WHO) recommends resampling with repeat nucleic acid testing; the collection of additional specimens, including the lower respiratory tract may be necessary (WHO Diagnostic testing 2020).

All positives must be reported to local/state health departments.

Interpretative Information

- A positive result indicates the presence SARS-CoV-2 RNA; results must be correlated with patient history and clinical symptoms. Positive results do not rule out infection with other pathogens (bacteria or coinfection with other viruses).
- A Not Detected test result means that SARS-CoV-2 RNA was not present in the specimen above the limit of detection, thus a negative result does not rule out the possibility of COVID-19 and should not be used as the sole basis for patient management decisions.

Limitations

Factors that can lead to a false-negative result include:

- Poor specimen quality; small amount of patient specimen
- Specimen collected early or late in the infection process. A study of PCR-based SARS-CoV-2 tests, funded by the National Institute of Allergy and Infectious Diseases (NIAID) and the CDC, found the median false-negative rate on the first day of symptom onset was 38%, and decreased to 20% three days after symptom onset. This percentage began to increase on day 4, and 16 days after symptom onset the false-negative rate was up to 66% (Kucirka 2020).
- Genetic variations of SARS-CoV-2 can cause false-negative results.
- Inadequate processing, storage, or shipping of specimen
- Technical reasons inherent in the test (eg, virus mutation, PCR inhibition) (FDA novel coronavirus EUA 2020)

False positive results are rare but can occur due to technical error or reagent contamination

Diagnostic Role

Gold standard confirmation of acute SARS-CoV-2 infection is based on the detection of unique viral sequences by nucleic acid amplification tests (NAATs). These assays' targets include regions on the nucleocapsid (N), envelope (E), spike (S), and RNA-dependent RNA polymerase (RdRp) genes (WHO Diagnostic testing 2020). Although this testing is both sensitive and specific, an accurate result is dependent on the type and quality of the specimen and the duration of illness at the time of testing.

The viral load tends to be higher in lower respiratory tract (LRT) specimens; Wang et al found highest (RT-PCR) positive rates in bronchoalveolar lavage (BAL) specimens (93%), followed by sputum (72%), nasal swab (63%), and pharyngeal swab (32%) (Wang 2020b). As such an LRT specimen may be recommended when an upper respiratory tract (URT) specimen is negative for SARS-CoV-2.

SARS-CoV-2 may be detected in the URT 1 to 3 days prior to development of symptoms (Furukawa 2020; WHO Diagnostic testing 2020); the viral concentration is highest around the time of symptom onset and then gradually declines (He 2020; Zou 2020). Although it is important to test the suspected positive patient as soon as possible, the duration of the illness at specimen collection must be considered when interpreting results.

It is important to note that the various NAATs available have different limits of detection (LOD), thus varying sensitivities. Specimens tested on different platforms could have different results. For more information see [SARS-CoV-2 Reference Panel Comparative Data](#).

Laboratory/Diagnostic Pearls

- For biosafety reasons it is not recommended to perform viral isolation in cell culture for SARS-CoV-2 or perform initial characterization of viral agents recovered in cultures of specimens from patients.
- Studies indicate SARS-CoV-2 may persist on surfaces for a few hours up to days dependent on conditions (eg, type of surface, temperature or humidity of the environment). Warmer temperatures and exposure to sunlight reduces viral survival time ([CDC EPA 2020](#)).
- Concentrations of SARS-CoV-2 RNA in upper respiratory specimens declines after onset of symptoms as does the recovery of replication-competent virus. In patients with mild to moderate disease, replication-competent virus has not been recovered after 10 days following symptom onset. In patients with severe COVID-19 disease or some immunocompromised patients replication-competent virus has been recovered 10 to 20 days post symptom onset. Some severely immunocompromised have had replication-competent virus recovered at much longer than 20 days post symptom onset (Baang 2021; Aydillo 2020), thus making a case for test-based strategy for determining resolution of infection in these patients.
- Current data indicate that low levels of SARS-CoV-2 may persist for up to 3 months in the respiratory tracts of persons recovered from COVID-19 infection. This latent shedding has **not** been found to be replication competent. If tested within 3 months of initial infection, individuals may continue to have a positive test result, however; infectiousness is unlikely ([CDC Ending Isolation 2021](#)).
- The COVID-19 molecular assays are designed to target specific RNA sequences and the persistent genetic variation of the SARS-CoV-2 virus can affect test performance if a mutation occurs in the region of the genome targeted by the test. Assays that rely on detection of multiple genome regions may be less impacted by genetic variations. The FDA has updated [In Vitro Diagnostics EUAs - Molecular Diagnostic Tests for SARS-CoV-2](#) to include the labeling of single- and multiple-target assays.

Additional Information

FDA recommendations for clinical laboratory and health care providers who use molecular testing to detect SARS-CoV-2 (FDA SARS-CoV-2 Viral Mutations 2021):

- Be aware that genetic variants of SARS-CoV-2 arise regularly and false negative test results can occur.
- Consider negative results in combination with clinical observations, patient history, and epidemiological information.
- Consider repeat testing with a different EUA-authorized or FDA-cleared Molecular diagnostic test (with different genetic targets) if COVID-19 is still suspected after receiving a negative test result.
- Test performance may be impacted by certain variants

- Tests with single targets are more susceptible to changes in performance due to viral mutations and more likely fail in detecting new variants.
- Be aware that tests that use multiple genetic targets to determine a final result are less likely to be impacted by increased prevalence of genetic variants.

The SARS-CoV-2 omicron variant, B.1.1.529, has markedly more mutations than previous variants, particularly in the S-gene.

For more information, interim guidance has been issued by the United States [CDC](#) and the [World Health Organization](#).

See: [Guidance for Healthcare Workers about COVID-19 \(SARS-CoV-2\) Testing](#)

See: [Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing](#)

See: [Information for Laboratories about Coronavirus \(COVID-19\)](#)

See: [Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 \(COVID-19\)](#)

See: FDA [Emergency Use Authorizations](#)

Index Terms

2019 Novel Coronavirus; 2019-nCoV; COVID-19 PCR; COVID-19, Qualitative, PCR; Covid19; nCoV, 2019; Novel Coronavirus, 2019; Qualitative COVID-19; SARS-CoV-2; Severe Acute Respiratory Syndrome Coronavirus 2; Wuhan

Applies to

Pandemic; Pneumonia of Unknown Etiology

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