SARS-CoV-2 Testing Overview

Table of Contents

I. Background

The SARS-CoV-2 testing landscape is continually changing as new tests receive an emergency use authorization (EUA) from the Food and Drug Administration (FDA). Molecular, antigen, and serology tests are now available and additional technologies are anticipated. Evidence on the performance of these different tests and guidelines for their best use in different populations are currently limited.

This document provides an overview of SARS-CoV-2 tests with a focus on the use and interpretation of diagnostic (viral) tests. It will be updated as new tests and performance data become available, and as guidance from the Centers for Disease Control and Prevention (CDC), FDA, and New York City (NYC) Department of Health and Mental Hygiene and New York State (NYS) Department of Health is revised.

II. Testing Basics

Currently Available SARS-CoV-2 Test Types

- **Diagnostic (viral) tests** – molecular and antigen tests
  - **Molecular tests**, which directly detect and amplify specific fragments of viral RNA using nucleic acid amplification (NAA), are recommended for diagnosing current SARS-CoV-2 infections.
  - **Antigen tests**, which detect viral surface proteins, can also diagnose acute infection but are less sensitive and specific than molecular tests, especially when the likelihood of someone having a SARS-CoV-2 infection is low (for example, when asymptomatic or no recent exposure to someone with COVID-19) and the prevalence of COVID-19 in the community is low.
- **Serologic tests** – detect antibodies made by the immune system in response to SARS-CoV-2 infection, checking for previous infection.

Point-of-Care and At-Home COVID-19 Tests

Point-of-care (POC) tests are assays that can be conducted in a Clinical Laboratory Improvement Amendments (CLIA)-waived setting or facility outside of a clinical laboratory (such as an outpatient clinic, doctor's office, nursing home, worksite, school or mobile testing site). To use POC tests, facilities must have a New York State Limited Services Laboratory permit from the Clinical Laboratory Evaluation Program (CLEP) and a CLIA Certificate of Waiver. To date, the FDA has authorized NAA, antigen and serology tests for SARS-CoV-2 testing in CLIA-waived point-of-care settings.

It is critically important that testing sites observe best practices for handling patient specimens by safely following appropriate Universal Precautions guidelines. In addition, staff performing POC tests must be adequately trained to safely and accurately perform the test following the manufacturer's "Instructions for Use" to obtain accurate results. The NYS Wadsworth Center website provides guidance on the operation of COVID-19 testing. For additional information on POC testing, see CDC Guidance for SARS-CoV-2 POC Testing.

A handful of tests have also been given an EUA by the FDA for at-home collection of samples, which are then shipped to a laboratory for processing. Currently there are also EUAs from the FDA for a NAA-based test and two antigen-based tests that can be performed at home (without the need for sending samples to a laboratory).The NAA based Lucira test is prescription only (the test is also authorized for CLIA-waived settings for patients under 12 years old). Of the two antigen- based tests, one, Ellume, is available without a prescription and can be used for persons 2 years of age and older; the other, Abbott BinaxNOW requires a prescription and is authorized for use for persons 4 years of age and older .

Reporting SARS-CoV-2 Test Results

Results from SARS-CoV-2 tests performed in a clinical laboratory are reported electronically directly from the laboratory to the NYC and NYS Health Departments. However, SARS-CoV-2 POC test results and at-home test kit results (positive and negative) must be reported by providers and facilities via the Electronic Clinical Laboratory Reporting System (ECLRS) within 24 hours of receiving a result. Providers should instruct patients who test positive on an at-home test kits to appropriately isolate and encourage them to inform their household contacts and any other close contacts, so that they can quarantine and get tested.

Emergency Use Authorizations (EUAs)

Use only tests with emergency use authorizations (EUAs) from the FDA for patient care. A wide variety of SARS-CoV-2 NAA, antigen, and serologic tests now have EUAs and are listed on the FDA website. There are test-specific informational documents that must be provided to providers and patients for each test. For each test, it is important for clinicians to review the EUA documentation, particularly the "Instructions for Use" documents to understand the specific performance characteristics.

III. More Detail on SARS-COV-2 Test Types

Molecular Tests

Molecular tests detect the unique genetic sequence of the SARS-CoV-2 virus using nucleic acid amplification (NAA) technology, such as real-time reverse transcription polymerase chain reaction (rRT-PCR) or other amplification methods (e.g., transcription-mediated amplification (TMA) or loop-mediated isothermal amplification (LAMP)). Most NAA tests are of moderate or high complexity and must be run in a laboratory. However, some are authorized to be conducted in POC settings.

Laboratory-based NAA tests are the most accurate tests for diagnosing current SARS-CoV-2 infection. They are considered very sensitive and specific. However, their sensitivity is affected by the timing of testing, sampling technique and sample type. Furthermore, not all molecular tests have equivalent performance, so it is important for providers to be familiar with the characteristics of the specific test that is being used. NAA tests currently available for use in the POC setting may be less sensitive than laboratory-based NAA tests.

In addition to the limited test performance data provided by the manufacturer in the EUA documentation, the FDA has published comparative performance data of NAA against a standard reference panel.

Interpretation of NAA test results:

- Positive NAA test result generally confirms a SARS-CoV-2 infection. Note: NAA tests may remain persistently positive for prolonged periods (up to 12 weeks or longer) after a patient has recovered, due to prolonged presence of non-viable RNA (see CDC Decision Memo).
- Negative NAA tests must be interpreted in the context of the exposure history and clinical presentation. False-negative tests have been documented, especially early in the clinical course (see Variation in False-Negative Rate of rRT-PCR–Based SARS-CoV-2 Tests by Time Since Exposure).

Laboratory turn-around time (TAT) for NAA test results should be less than 48 hours. However, results can be delayed if laboratories are experiencing high volumes of specimens or shortages of reagents. Providers should be aware of current TAT and make sure patients with symptoms isolate while waiting for their test result. Currently available POC NAA tests results have a TAT as short as 15 to 45 minutes. See FDA list of NAA tests with EUAs for more information on the performance and use of specific authorized tests.

Antigen Tests

Antigen tests detect the presence of SARS-CoV-2 viral surface proteins, which indicate current viral infection. The main advantages of POC antigen tests are that their results are available within 15 to 45 minutes, they are relatively simple to perform and they are less expensive than NAA tests. There are a number of available SARS-CoV-2 antigen tests with an EUA and designed

for use in a CLIA-waived setting. **They are intended for use on symptomatic persons within 5 to 12 days of symptom onset** (the number of days varies for each test).

Antigen tests are considered specific for the virus when used as designated in the "Instructions for Use" (i.e., on symptomatic patients only) but are less sensitive than most NAA tests. See FDA's list of EUAs for Antigen Tests for more information about the performance and use of specific authorized tests.

The best use of antigen tests is still being evaluated. They can supplement other testing methodologies, especially in settings where NAA testing capacity is limited or testing results are delayed, and can add both clinical and infection control value by enabling the prompt identification of SARS-CoV-2 infection to expedite response in outbreak settings (for example, in congregate settings). However, antigen tests were developed for and evaluated on symptomatic persons early in the clinical course, and there is limited data to guide their use for screening asymptomatic persons with no known exposure to COVID-19.

Nonetheless, antigen tests are increasingly being used for asymptomatic persons. With this more widespread use, a growing number of false-positive and false-negative results have been reported. For this reason, confirmatory NAA testing at a clinical laboratory is needed when:

- An individual with a high pre-test probability (such as a person with symptoms of COVID-19 or recent exposure to SARS-CoV-2, especially in a setting with widespread community transmission or an outbreak setting) has a negative rapid antigen test result
- An individual with a low pre-test probability (such as a person who is asymptomatic with no recent exposure to SARS-CoV-2, especially when there is limited-to-no community transmission) has a positive rapid antigen test result

Confirmatory NAA testing should be done within 48 hours of initial specimen collection, and the individual should be directed to isolate at home while awaiting NAA results. Ideally, two specimens should be collected (nasal swab for the antigen test and a nasopharyngeal swab for laboratory-based NAA test) at the same patient encounter when antigen tests are being used in the outpatient setting.

See HAN#39 titled, Proper Use and Interpretation of SARS-CoV-2 Antigen Tests, for more information.

Guidelines released by the CDC outline scenarios where antigen tests may be considered for screening asymptomatic persons. To learn more, see:

- Association of Public Health Laboratories (APHL) - Considerations for Implementation of SARS-CoV-2 Rapid Antigen Testing
- CDC - Interim Guidance for Rapid Antigen Testing for SARS-CoV-2
- CDC - Considerations for Use of SARS-CoV-2 Antigen Testing in Nursing Homes

Serology Tests

Serology tests detect waning or past SARS-CoV-2 virus infection indirectly, by measuring the antibody response to the virus. Serologic tests should not replace diagnostic viral tests for diagnosing active (current) SARS-CoV-2 infection. Currently available serology tests are also insufficient to determine immune status (whether the patient is protected from future infection). See FDA list of EUA authorized serology tests and performance information. Some COVID-19 vaccines may result in a positive serologic test, refer to Section VI. Interpretation of SARS-CoV-2 test results in vaccinated persons**,** for more information.

CDC Recommendations for Use of Serologic Tests include:

- To help establish a diagnosis when a patient presents with late complications of COVID-19 illness, such as multisystem inflammatory syndrome in children (MIS-C) or adults, or signs or symptoms of late sequelae of COVID-19.
- To support diagnosis of acute COVID-19 illness for persons who present late. For persons who present 9 to 14 days after illness onset, serologic testing can be offered in addition to recommended testing for viral detection. This will improve diagnostic accuracy at a time in the clinical course when the sensitivity of viral detection is decreasing and the antibody response is increasing.

For more information, see:

- CDC Interim Guidelines for COVID-19 Antibody Testing
- Infectious Diseases Society of America (IDSA) Guidelines on the Diagnosis of COVID-19: Serologic Testing
- FDA Serology/Antibody Test FAQs

IV. Diagnostic Test Performance and Characteristics and Pre-Test Probability

Decisions regarding which SARS-CoV-2 diagnostic test to use and how to interpret test results should take the accuracy of the test and pre-test probability into account.

Interpretation of SARS-CoV-2 Diagnostic (Viral) Test Results – Key Points

- **Accuracy of test results** is affected by:
  - Sensitivity and specificity of the test
  - Whether the test is used as directed in the EUA
  - Proper administration of the test, including specimen collection methods
  - Timing in relation to onset of symptoms, if present
- The **pre-test probability** is the probability that a patient is infected with SARS-CoV-2. Factors that increase the pre-test probability of infection include:
  - Symptoms of COVID-19
  - History of exposure to someone with COVID-19 in the past 14 days
  - Residence or work in a setting with an ongoing outbreak or high incidence of COVID-19
- When the **pre-test probability** is **high**:
  - A positive viral test result is likely to indicate current infection with SARS-CoV-2.
  - A negative viral test result should be interpreted in the context of the exposure history and clinical presentation. If result was from an antigen test or POC NAA, conduct confirmatory NAA testing at a clinical laboratory within 48 hours of the initial specimen.
- When the **pre-test probability** is **low**:
  - A negative viral test result is likely to be a true result.
  - A positive viral test result should be interpreted in the context of the exposure history and clinical presentation. If result was from an antigen test, conduct confirmatory NAA testing at a clinical laboratory within 48 hours of the initial specimen collection.
  - Note that if more than 48 hours separate the two specimen collections, or if there have been opportunities for new exposures, a NAA should be considered a separate test rather than a confirmatory test.

V. Considerations for Testing Persons Without Symptoms or a Known Exposure to SARS-CoV-2

Due to uncertainties in test performance in asymptomatic individuals and those who have a lower likelihood of having been exposed to SARS-CoV-2, clinical judgment should be used to determine if a person with a questionable test result may warrant additional NAA testing at a clinical laboratory. There are some situations where testing people without symptoms or a known exposure (i.e., screening) can play an important role in viral transmission reduction.

Screening for SARS-CoV-2 is intended to identify infections among people who have no symptoms and no known recent exposure to SARS-CoV-2 to reduce community transmission. Screening is currently being conducted in several NYC settings in accordance with NYS Department of Health guidance, including:

- Nursing homes and other congregate residential settings that serve people with an increased risk for severe COVID-19, or where spread could occur rapidly due to

proximity of residents, and test results can inform immediate decisions regarding the need to isolate residents to prevent ongoing spread
- Schools and other educational settings
- Newly admitted patients in health care facilities (to prevent persons who are entering a facility from spreading the infection to staff or other patients)

Periodic screening of other persons who have an increased risk of being exposed to or spreading SARS-CoV-2 due to their occupation or place of residence is also being conducted (see NYC Health Alert #38).

Given emerging evidence that SARS-CoV-2 antigen tests produce both false-positive and false-negative results, and the fact that they have not been authorized by the FDA for testing of asymptomatic individuals, providers and facilities should consider using NAA-based tests for screening. Nonetheless, SARS-CoV-2 antigen tests may be the most feasible option for some facilities or screening programs. Serial antigen testing of persons without within a closed congregate setting, such as a long-term care facility or a correctional facility, has been suggested by the CDC as a potential strategy to rapidly identify cases of SARS-CoV-2 infection to prevent further transmission in the facility. CDC cites modeling evidence showing that outbreak control depends largely on the frequency of testing and the speed of reporting and is only marginally improved by high test sensitivity.

The Centers for Medicare and Medicaid Services (CMS) has stated that, for the duration of the COVID-19 public health emergency, they will exercise enforcement discretion and not cite facilities with a CLIA certificate of waiver when SARS-CoV-2 POC antigen tests are performed on asymptomatic individuals. Providers who screen individuals with no COVID-19 symptoms and no known recent exposure are encouraged to confirm positive antigen results with an NAA-based test, as outlined above.

When Testing Asymptomatic Persons Without a Known Exposure – Interpretation and Patient Management

- It is important to emphasize that negative test results only indicate that the test did not detect the virus at the time the test was taken. When there is widespread community transmission, any person who interacts with other people, especially among infected household members, runs a daily risk of acquiring COVID-19. This daily risk increases in crowded places, in confined spaces (especially indoors), with close contact, and when protective actions (such as maintaining physical distancing, correctly using face coverings when around others, frequent hand hygiene, and other protective measures) are not followed. A negative test does NOT mean the person can safely ignore physical distancing and face covering requirements.
- **A false negative result** in a person with a high pre-test probability can happen if an infected individual is still incubating the infection or as detectable levels of antigen begin to decline (i.e., if the test was done too early or late in the course of the infection), or if the test simply fails to detect the SARS-CoV-2 virus.
- **A false positive test result**, as has been seen most often in asymptomatic individuals who have no recent exposure to SARS-CoV-2 (low pre-test probability), can occur as a result of test interference from patient-specific factors, such as the presence of non-specific antibodies (such as rheumatoid factor) or a highly viscous specimen.

Table 1. SARS-CoV-2 Diagnostic (Viral) Test Comparison Summary Grid

Molecular Tests	Antigen Tests
Test Methodology	
Amplify specific fragment of viral RNA using nucleic acid amplification (NAA). Examples include: ▪ Reverse-transcription real time polymerase chain reaction (rRT-PCR) ▪ Transcription mediated amplification (TMA) ▪ Isothermal amplification method including LAMP, NEAR, etc., which are ultrafast NAA	Lateral flow immunoassay to detect viral surface proteins
Specimen Types Refer to the manufacturer for acceptable sample type(s) for each test	
Nasopharyngeal, oropharyngeal, or nasal swab; saliva; lower respiratory tract specimens	Nasopharyngeal or nasal swab
Authorized for Point-of-Care (POC) or At-Home Test Kit?	
Most are not, but some are. To use POC tests, facilities must have a NYS Limited Services Laboratory permit from the Clinical Laboratory Evaluation Program (CLEP) and a CLIA Certificate of Waiver. Home test kits include home collection tests kits for which specimens are sent to a laboratory and those that are performed at home.	Yes. To use POC tests, facilities must have a NYS Limited Services Laboratory permit from the Clinical Laboratory Evaluation Program (CLEP) and CLIA Certificate of Waiver. Home test kits include those that are performed at home.
Turn-Around Time for Results	
Laboratory-based NAA: less than 48 hours (but may be longer if the laboratory is experiencing a backlog or reagents are in short supply) POC tests and home test kits performed at home range from 15 to 45 minutes.	POC tests range from 15 to 30 minutes. Home test kits performed at home range from 15 to 45 minutes.

Performance Characteristics Test results should always be interpreted in the context of the pre-test probability, which is informed by the clinical presentation, exposure history of the person being tested, and the prevalence of COVID-19 in their community	
Laboratory-based NAA tests have high sensitivities and specificities. They are the most accurate tests available for clinical diagnostic detection of SARS-CoV-2. Current NAA POC tests and home test kits performed at home use a methodology that is different from rRT-PCR and may be less sensitive.	Antigen tests are less sensitive than NAA tests. Antigen levels in specimens collected more than 5 to 7 days after the onset of symptoms may drop below the limit of detection of the test leading to a negative test result, while NAA-based testing may still detect viral RNA. It may be necessary to confirm an antigen test result with a NAA test if the result is inconsistent with the clinical context. See CDC rapid antigen testing guidelines for more discussion.
Positive Test Result Interpretation	
High pre-test probability: A **positive** result indicates SARS-CoV-2 RNA was detected. The patient is considered infected and contagious and should be managed appropriately. Low pre-test probability: While uncommon, **false positive** results have been reported with NAA tests. If a false positive is suspected, repeat NAA testing and direct patient to isolate while awaiting NAA result.	High pre-test probability: In most cases a **positive** result indicates SARS-CoV-2 antigens were detected. The patient is considered infected and contagious and managed appropriately. Low pre-test probability: May be a **false positive** result and confirmatory testing by NAA is needed. Direct the patient to isolate while awaiting confirmatory test results.
Negative Test Result Interpretation	
High pre-test probability: A **negative** result is likely to be a true negative; however, if based on clinical judgment there is reason to suspect SARS-CoV-2 infection, repeat NAA testing and direct patient to isolate while awaiting NAA result. Low pre-test probability: A **negative** result is likely a true negative.	High pre-test probability: May be a **false negative** and should be confirmed with a laboratory-based NAA test within 48 hours of the initial specimen collection and direct patient to isolate while awaiting NAA result. Low pre-test probability: A **negative** is likely to be a true negative and confirmatory testing not needed unless clinical judgment deems it important for patient management or infection control.

Considerations for Use	
NAA-based testing is recommended for the following: ▪ Persons with symptoms of COVID-19 ▪ Close contacts of someone with COVID-19 ▪ For people without symptoms living or working in a high-risk setting (such as a skilled nursing facility) or who are identified as part of outbreak response	Antigen-based testing is recommended for the following: ▪ Persons with symptoms of COVID-19, within 5 to 12 days of onset (varies by test) ▪ Close contacts of someone with COVID-19 These tests are not intended for asymptomatic persons. However, due to their rapid turn-around time and ease of use, they may be useful as for routine screening of residents and staff of congregate settings or individuals who reside or work in a setting or area with an outbreak or increased prevalence of COVID-19, and must always be done in conjunction with NAA-based test confirmation.

VI. Interpretation of SARS-CoV-2 test results in vaccinated persons

Prior receipt of the Pfizer-BioNTech or Moderna COVID-19 vaccine will not affect the results of SARS-CoV-2 NAA or antigen tests. Currently available serologic antibody tests for SARS-CoV-2 assess IgM and/or IgG to one of two viral proteins: spike or nucleocapsid. Because the Pfizer-BioNTech and Moderna COVID-19 vaccines contains mRNA that encodes the spike protein, a positive test for spike protein IgM/IgG could indicate either prior infection or vaccination. To evaluate for evidence of prior infection in an individual who has received COVID-19 vaccine, laboratory evaluation of IgM/IgG to the nucleocapsid protein should be performed. Antibody testing is not currently recommended to assess for immunity to COVID-19 following Pfizer-BioNTech or Moderna COVID-19 vaccination.

VII. Additional Information and Guidance

- New York State Wadsworth Center:
  - Coronavirus Testing Guidance on testing, pooling results, and handling specimens.
- Centers for Disease Control and Prevention:
  - Interim Guidance for Rapid Antigen Testing for SARS-CoV-2
  - Considerations for Use of SARS-CoV-2 Antigen Testing in Nursing Homes
- Food and Drug Administration:
  - FAQs on Testing for SARS-CoV-2
  - SARS-CoV-2 Reference Panel Comparative Data
- World Health Organization:

    - Antigen-Detection in the Diagnosis of SARS-CoV-2 Infection Using Rapid Immunoassays
- Interpreting a COVID-19 Test Result (Watson and Brush, BMJ, May 2020)
- Harvard University Center for Systems Biology:
    - COVID-19 Diagnostics in Context
- American Society for Microbiology:
    - Why Pre-Test Probability Matters

The NYC Health Department may change recommendations as the situation evolves. 12.23.20