COVID-19 Testing

SARS-CoV-2 causes COVID-19 and has led to a pandemic and a national public health emergency in the United States. The environment surrounding COVID-19 testing is continually evolving, and clinicians are encouraged to consult the CDC for the most current testing recommendations.[1]

Identification of patients infected with SARS-CoV-2 can help to isolate cases and prevent further person-to-person transmission, thus slowing the spread of infection, limiting the number of cases, and mitigating the impact on healthcare resources.[1]

Molecular diagnostic testing by nucleic acid amplification (NAA) is recommended for SARS-CoV-2 diagnosis.[2] Testing decisions should be based on local epidemiology, clinical signs and symptoms, and the course of illness.[1]

Saliva has recently been validated as an additional specimen type for the SARS-CoV-2 (COVID-19) by NAA test after having been determined in a study performed by ARUP and the University of Utah[3] to be an effective alternative to a nasopharyngeal swab specimen. Both saliva and nasopharyngeal swab specimens were found to be superior to anterior nasal swab specimens. Refer to the specimen collection instructions in the ARUP Lab Test Directory for additional information.

Serology testing is used to detect antibodies to SARS-CoV-2. This testing can be used to evaluate patients for exposure but is not recommended for COVID-19 diagnosis.[1] Early studies suggest that most patients seroconvert approximately 2 weeks after symptom onset.

False-positive results are possible in low-prevalence settings, even when an antibody test has >98.0% specificity. To reduce the likelihood of a false-positive result and to maximize the positive predictive value (PPV) of a test, the CDC Interim Guidelines for COVID-19 Antibody Testing[4] suggest testing individuals with a high pretest probability, choosing a test with a high specificity, or using an orthogonal testing algorithm so that individuals who are positive by one antibody test are retested with a second antibody test. The two antibody tests should have unique design characteristics (eg, different targets). The individual antibody tests offered by ARUP are complementary, as they target different proteins of SARS-CoV-2. As such, they can be used together in an orthogonal algorithm to maximize the PPV of testing and minimize false-positive results.

Disease Overview

Incidence

In early 2020, COVID-19 spread rapidly across the globe. Updated case counts can be found in the World Health Organization's Coronavirus Disease (COVID-2019) Weekly Epidemiological Update[5] and on the CDC's United States COVID-19 Cases and Deaths by State web page.[6]

Tests to Consider

SARS-CoV-2 (COVID-19) by NAA 3002638

Method: Qualitative Nucleic Acid Amplification

- Use to detect COVID-19 in specimens obtained during the acute phase of infection from individuals who meet COVID-19 clinical and/or epidemiologic criteria.
- This test is specific to SARS-CoV-2 and does not detect additional coronaviruses.
- This test is an FDA Emergency Use Authorization (EUA) assay when performed on nasopharyngeal, oropharyngeal, or nasal swab specimens; because of the FDA's recent statement on laboratory-developed tests, an EUA is not required for saliva testing.
- Refer to the SARS-CoV-2 (COVID-19) by NAA Specimen Collection and Shipping Instructions for specimen collection and transport information.

COVID-19 IgG, Qualitative by CIA 3002776

Method: Qualitative Chemiluminescent Immunoassay

- Use for the qualitative detection of IgG antibodies against the nucleocapsid protein of SARS-CoV-2 (COVID-19) that develop in response to natural infection with SARS-CoV-2.
- IgG antibodies do not develop as a result of a COVID-19 vaccination.
- There are no current recommendations for assessing COVID-19 vaccine response.
- This test is not recommended for COVID-19 diagnosis.
- The use of two different antibody assays in an orthogonal testing algorithm may reduce the likelihood of a false-positive result.
- The presence of IgG antibodies may not indicate protective immunity.
- This test is an FDA Emergency Use Authorization (EUA) assay.

COVID-19 IgG by ELISA 3002723

Method: Enzyme-Linked Immunosorbent Assay

- Use for the detection of IgG antibodies against the spike protein (S1) of SARS-CoV-2 (COVID-19) that develop in response to

Symptoms

Clinical presentation ranges from asymptomatic infection to mild symptoms to more severe illness. Symptoms may not appear until 14 days after exposure, but the median time from exposure to symptom onset is 4-5 days.[7,8]

Symptoms of possible COVID-19 include fever or chills, cough, shortness of breath or difficulty breathing, fatigue, myalgia, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and diarrhea.[7,8]

Emergency warning signs of COVID-19 include the following[8]:

- Difficulty breathing
- Persistent pain or pressure in the chest
- New confusion
- Inability to wake or remain awake
- Bluish lips or face

natural infection with SARS-CoV-2 or from COVID-19 vaccination.
- There are no current recommendations for assessing COVID-19 vaccine response.
- This test is not recommended for COVID-19 diagnosis.
- The use of two different antibody assays in an orthogonal testing algorithm may reduce the likelihood of a false-positive result.
- The presence of IgG antibodies may not indicate protective immunity.
- This test is an FDA Emergency Use Authorization (EUA) assay.

Transmission

- The virus spreads through respiratory droplets produced when an infected person coughs, sneezes, or talks.[9]
- These respiratory droplets can pass into the mouths or noses of people nearby or may be inhaled.[9]

Test Interpretation

Molecular Diagnostic Test: SARS-CoV-2 (COVID-19) by NAA

Due to high demand for this test, ARUP utilizes four different assays performed on three platforms (Thermo Fisher, Roche, and Hologic) to detect SARS-CoV-2. When specimens are received, they are routed to be tested using one of these four assays. This allows ARUP to meet high test demand and to manage the risk of unpredictable supply chains.

Results

Results are reported as SARS-CoV-2 detected, not detected, presumptive positive, invalid, or inconclusive.

Specificity

All assays used to perform this test detect the 2019 novel coronavirus strain (SARS-CoV-2). The assays were shown by the manufacturers via direct testing or *in silico* analysis to not cross-react with a large number of other bacteria and viruses. ARUP and the test manufacturers are monitoring SARS-CoV-2 variants, including the novel UK variant, to understand what impact, if any, these variants have on the specificity of these assays.

Each of ARUP's four assays targets several gene targets (see table for details). The utilization of multiple targets helps these assays detect genetic variants by adding redundancy.

ARUP SARS-CoV-2 Assay Gene Targets	
Assay	**Gene Targets**

RT-PCR, reverse transcription polymerase chain reaction; TMA, transcription-mediated amplification

Assay	Gene Targets
Thermo Fisher RT-PCR	Orf1ab/O-methyltransferase gene N gene S gene
Roche RT-PCR	Orf1ab/O-methyltransferase (2 different sites) Env E-gene/pan-sarbecovirus
Hologic RT-PCR	Orf1ab/O-methyltransferase (2 different sites)
Hologic TMA	Orf1ab/O-methyltransferase (2 different sites)

RT-PCR, reverse transcription polymerase chain reaction; TMA, transcription-mediated amplification

Limitations

- A negative result does not preclude SARS-CoV-2 and should not be used as the sole basis for patient management decisions.
- A negative result must be combined with other clinical observations, patient history, and epidemiologic information.
- A positive result indicates the detection of nucleic acid from the relevant virus.
  - Nucleic acid may persist even after the virus is no longer viable.
- Positive results do not rule out bacterial infection or coinfection with other viruses.
- Reliable results are dependent on adequate specimen collection, transport, storage, and processing.
- Theoretically, assay sensitivity might be decreased in viral variants with mutations in several regions targeted by the assays. The assay manufacturers are continuously monitoring available variant sequences to ensure their assays are detecting emerging variants.

COVID-19 IgG (Serology)

COVID-19 IgG, Qualitative by CIA

This CIA assay, developed by Abbott and performed on the Architect platform, detects IgG antibodies specific to the nucleocapsid protein of SARS-CoV-2 that form as a result of natural SARS-CoV-2 infection. This test is reported as negative or positive.

This and other serology tests for COVID-19 offered by ARUP have been evaluated by both the manufacturer and the U.S. Food and Drug Administration (FDA) in partnership with the National Institutes of Health (NIH), the CDC, and the Biomedical Advanced Research and Development Authority (BARDA). Please visit the FDA web page, EUA Authorized Serology Test Performance, for more information.[10]

Performance of COVID-19 IgG, Qualitative by CIA			
Antibody	Performance Measure	Estimate of Performance	95% CI
IgG	Sensitivity	100% (88/88)	95.8% to 100%
IgG	Specificity	99.6% (1,066/1,070)	99.0% to 99.9%
IgG	PPV at prevalence = 5%	93.4%	84.0% to 97.3%
IgG	NPV at prevalence = 5%	100%	99.8% to 100%

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value

Data source: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance[10]

COVID-19 IgG by ELISA

This ELISA assay, developed by EUROIMMUN, detects IgG antibodies specific to the S1 domain of the spike protein of SARS-CoV-2 that develop in response to natural SARS-CoV-2 infection or to COVID-19 vaccination. This test is reported as negative, indeterminate, or positive, and will include an index value. The American Association for Clinical Chemistry (AACC) does not recommend the use of serology for assessing COVID-19 vaccine response.[11]

This and other serology tests for COVID-19 offered by ARUP have been evaluated by both the manufacturer and the FDA in partnership with the NIH, the CDC, and BARDA. Please visit the FDA web page, EUA Authorized Serology Test Performance, for more information.[10]

Performance of COVID-19 IgG by ELISA

Antibody	Performance Measure	Estimate of Performance	95% CI
IgG	Sensitivity	90.0% (27/30)	74.4% to 96.5%
IgG	Specificity	100% (80/80)	95.4% to 100%
IgG	PPV at prevalence = 5%	100%	46.1% to 100%
IgG	NPV at prevalence = 5%	99.5%	98.6% to 99.8%

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value

Data source: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance[10]

Serology Limitations

- Antibody test results should not be used as the sole criterion to confirm or rule out SARS-CoV-2 infection or to assess infection status.
- There are no current recommendations for assessing COVID-19 vaccine response.[11]
- Negative results do not exclude infection with SARS-CoV-2, especially in individuals with known exposure to the virus.
  - Follow-up molecular diagnostic testing should be considered in those with recent exposure to COVID-19.
  - Immunocompromised patients infected with COVID-19 may have a delayed antibody response or antibody levels too low to result in a positive test.
- Positive results suggest exposure to SARS-CoV-2 but may not indicate immunity.
- False-positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- False-positive results are possible in low-prevalence settings, even when an antibody test has >98.0% specificity; to reduce the likelihood of a false-positive result, the use of a second, different antibody assay is recommended if an initial antibody test is positive (refer to the CDC Interim Guidelines for COVID-19 Antibody Testing[4]).
- COVID-19 serology tests are not for use in screening donated blood.

References

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Additional Resources

Panther Fusion SARS-CoV-2–Hologic, Inc. Fact sheet for healthcare providers. [Updated: Sep 24, 2020; Accessed: Dec 29, 2020]

Related Information

Community-Acquired Pneumonia - CAP
Influenza Virus
COVID-19 - SARS-CoV-2
Respiratory Viruses

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