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COVID-19 Clinical Testing – Frequently Asked Questions

What is molecular diagnostic COVID-19 testing?

- Molecular diagnostic COVID-19 tests identify the SARS-CoV-2 viral RNA in respiratory samples, typically a nasopharyngeal swab.
- More recently, newly designed nasal swabs have been validated for COVID-19 diagnostic testing.
- These tests identify active SARS-CoV-2 infection.
- See Table for additional details.

What is serologic COVID-19 testing?

- Laboratory tests that identify antibodies made during an immune response to infection with SARS-CoV-2 virus in blood samples.
- These tests identify acute (immunoglobulin M [IgM] antibody–positive only) or past (immunoglobulin G [IgG] antibody–positive only) infection.
- However, while IgG positivity may indicate immunity to re-infection, this has **not been proven**. Even if IgG positivity does correlate with immunity, **it is unknown how long immunity lasts**.
- See Table for additional details.

What is COVID-19 antigen testing?

- Laboratory tests that identify active infection.
- Antigen tests detect viral proteins specific to SARS-CoV-2 in respiratory samples.
- The first antigen test recently received Emergency Use Authorization (EUA) and may be run in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) or at point of care.
- Sensitivity may be low; antigen testing is not currently recommended by the World Health Organization (WHO) for patient care.

What is FDA EUA for COVID-19 diagnostic testing?

- Diagnostic tests for COVID-19 require Food and Drug Administration (FDA) EUA or similar FDA-recognized authorization from the overseeing State.
- The FDA conducts a limited review of data submitted by test developers and grants an EUA if no problems are identified (for a list of EUA tests, see “Molecular” listings).
- EUA is a **temporary** medical device authorization process that is valid only during the COVID-19 public health emergency.
    - The purpose is to speed the development of clinical testing capacity to meet emergency needs.
    - Only the most important aspects of the usual, more rigorous, approval requirements are retained for EUA.
    - Once the emergency declaration is lifted, tests will need to meet standard review requirements for FDA clearance or approval.

- Limited review consists of manufacturer-supplied data demonstrating analytic validity and accurate identification of contrived reactive (e.g., viral RNA spiked into negative clinical specimens) and non-reactive clinical specimens.
    - Click on "IFU" link for each test, scroll for performance information.
- No real-world clinical validation study was initially required.
    - As of May 4, 2020, the FDA has re-issued EUA guidance recommending clinical validation in the form of a minimum of 30 known positive and 30 known negative samples as determined by an authorized assay.
    - Acceptable results are 95% agreement at 1 to 2 times the limit of detection and 100% agreement at all other concentrations.

What does the FDA require for COVID-19 EUA serologic testing?

- Initially, laboratories developing in-house tests and manufacturers had only to notify the FDA of in-house validation, test distribution and use, and meet certain results reporting requirements.
- As of May 4, 2020, the FDA updated its EUA guidance regarding serologic testing as follows:
    - Commercial manufacturers of a new test must submit EUA requests and validation data within 10 business days from the date of FDA notification or from May 4, 2020, whichever is later. This also applies to manufacturers who notified the FDA under the policies of earlier versions of the EUA guidance.
    - An "umbrella" EUA route is also available, whereby developers may submit their tests to an independent validation study at the National Cancer Institute or similarly designated agency, which will meet the same EUA guidance criteria.
        - A list of EUA serology tests is available (see "Technology-Serology" listings).
        - A list of manufacturers who have previously notified FDA but have not yet submitted an EUA for a serologic test can be found here (Section IV.D, manufacturers).
    - CLIA-certified high-complexity clinical laboratories developing in-house serologic tests must provide their own validation and notify the FDA; they are encouraged to seek EUA.
        - List of CLIA-laboratory FDA-notified tests, Section IV.D.
    - All developers must meet newly defined threshold recommendations for positive and negative percent agreement in a clinical study.
    - Available summary performance data for specific serologic tests is provided by the FDA along with a calculator that allows adjustment for estimated antibody prevalence in the population.
- FDA continues "to take appropriate action against firms unlawfully marketing their tests."

How do the molecular and serologic responses work together?

- This recently published article discusses estimated time intervals and rates of viral and antibody detection.

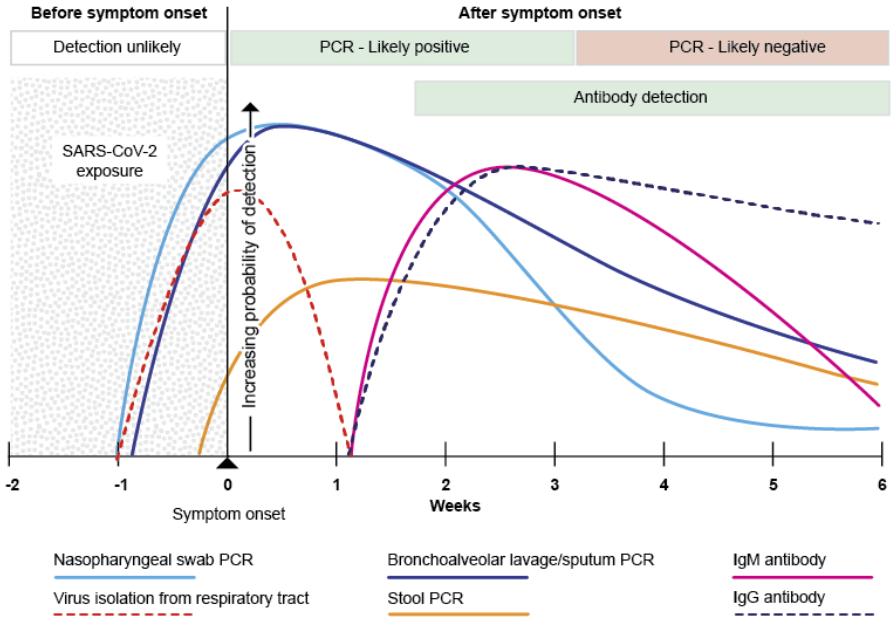

Figure. Variation over time in molecular and serologic tests for detection of SARS-CoV-2 infection relative to symptom onset. Time intervals and rates of viral detection are estimates based on several published reports and should be considered approximations.

Adapted from Sethuraman N, Jeremiah SS, and Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. 2020. Epub ahead of print. May 6, 2020. Available at: https://jamanetwork.com/journals/jama/fullarticle/2765837?guestAccessKey=c26c8c00-a470-4777-8abe-f2857a184887&utm_source=silverchair&utm_medium=email&utm_campaign=article_alert-jama&utm_content=olf&utm_term=050620. Accessed May 11, 2020.

Who can run COVID-19 tests and report results for patients?

- Most tests must be run by clinical laboratories certified under CLIA for high or, in some cases, moderate complexity testing.
- Tests without an EUA must be run in a CLIA-certified high complexity laboratory.
- If granted an EUA for a point-of-care test, then the test is CLIA-waived and can be performed in a CLIA-certified patient care setting with a certificate of waiver.
- See this CDC website for additional information.

What about at-home COVID-19 testing?

- The FDA authorized an at-home sample collection kit that can be sent to specified laboratories for COVID-19 diagnostic testing.
- The nasal swab is placed in a supplied tube containing saline and mailed in the supplied package for testing at the laboratory site.
- Similar arrangements for additional home collection kits for specific laboratory tests using nasal swabs and saliva samples have also been authorized.

What are test "kits" and why is test capacity limited?

- Diagnostic test collection kits: the test laboratory/manufacturer specifies the collection device(s) that have been validated for use with the test. Only the specified devices should be used for sample

collection for each test. Includes, for example, nasopharyngeal swab, transport media in tube, packaging for shipment.

- RNA extraction kits isolate RNA from the collected sample in preparation for some reverse transcription (RT)-PCR–based diagnostic tests.
- The diagnostic test kit contains the ingredients and disposables to be used for one assay, usually of many samples, that is run on an automated instrument.
- Each of these types of kits, or kit components, have been in short supply at times during the COVID-19 crisis, limiting overall testing capacity. The availability of personal protective equipment (PPE) for healthcare professionals collecting and processing samples may also be limiting.
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Table. Overview of Diagnostic and Serologic Testing for COVID-19

	Molecular Diagnostic Tests	Serologic Tests
What the test detects	Presence of SARS-CoV-2 viral RNA-specific sequences	Antibody specific to SARS-CoV-2
Interpretation of positive result	Current infection; individual is potentially infectious	• IgM+ Current infection likely • IgG+ Past infection (immune?*) • IgM+/IgG+ In transition
Interpretation of negative result	No active infection Other possibilities (consider in context of overall clinical presentation): • Sample inadequate for detection (false-negative) • Assay failure • Sample mix-up	No past infection; no immunity Other possibilities: • Test performed too early in infection, prior to measurable immune response • Assay failure • Sample mixup
Primary technology	Reverse transcription (RT)-PCR + fluorescence detection	Enzyme-linked immunosorbant assay (ELISA)

Alternate technologies	• Isothermal amplification + fluorescence detection
	• RT-PCR + alternate detection methods†
		• Lateral flow + dye detection
		  ○ immunochromatographic assay
		  ○ low volume/single-use casette

Test selection considerations	• High vs low/single-use volume • Laboratory-based vs point of care • Stability of entire supply chain • At-home patient collection allowed? • Are there data available from a real-world clinical study?	• High vs low/single-use volume • Stability of supply chain • No point-of-care tests‡ • Are there data available from a real-world clinical study?

*It is not known at this time if antibody positivity confers immunity to re-infection or, if so, for how long.
†Lateral flow detection; amplicon melt curve detection; competitive hybridization with electrochemical detection.
‡As of May 19, 2020, per FDA; beware of false claims.