PUBLIC HEALTH CONSIDERATIONS: Serologic Testing for COVID-19

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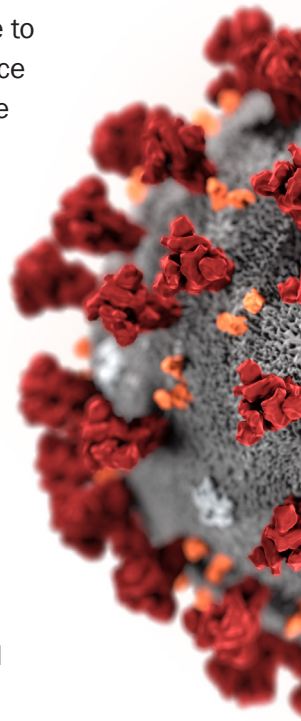
www.aphl.org
eoc@aphl.org

www.cste.org

Serologic assays have several important public health applications in the current coronavirus disease (COVID-19) response. Despite their importance, serologic assays do not replace molecular methods as the primary tool for the diagnosis of acute or active infection. One essential application is the use of high-quality serologic test methods to estimate the prevalence of *past* viral infection or estimate the cumulative incidence of infection in the US population. Serologic testing can improve our understanding of disease transmission patterns and data from serologic surveys can be used to understand the proportion of persons previously infected, among various populations.

In order for these methods to be used effectively for both population level studies and individual use scientists need more data on the performance characteristics of these tests and the human immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. This includes the persistence and protection offered by antibodies. Without this information, results from these methods cannot be properly interpreted.



With those caveats, potential public health applications include:

- Determining how widespread COVID-19 infection has been in a community or population to both understand the scale of the current pandemic and in preparation for future vaccine development and deployment.
- Identification of persons with an antibody response to serve as convalescent plasma donors.
- Determining if a person had an immune response to SARS-CoV-2, irrespective of whether they had symptoms or not.
    - At this time there are not enough data to determine whether or not an immune response confers immunity or for how long.
    - Until more evidence about protective immunity is available, serologic test results should not be used to make staffing decisions (return to work), decisions regarding the need for personal protective equipment or need to discontinue social distancing measures.
- If used in conjunction with other diagnostic tests and clinical history etc. serologic tests may be used as part of the testing algorithm to establish a diagnosis of COVID-19 and identify probable cases.

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Overview of COVID-19 Serologic Methods

- Unlike molecular methods, serologic methods do not directly detect presence of the virus but instead detect evidence of viral infection at some time point in the past.
- Serologic methods should not be the sole-basis for diagnosis of COVID-19 infection (*see Considerations for Test Result Interpretation*). Specifically, antibodies may not develop until one-two weeks post symptom onset which means serologic tests may not detect current SARS-CoV-2 infections and should not be used to diagnose current COVID-19.[1,2]
- Serologic methods detect and/or measure the amount of antibody to SARS-CoV-2. A qualitative method would detect antibody, a quantitative method would allow you to calculate the amount of antibody present in the sample. This can be useful to monitor immune status over time, as is done for other infectious diseases. There are three major categories of serologic test methods available or in development for SARS-CoV-2: [3,4]
    - Rapid Serologic Test, also called a Rapid Diagnostic Test, most frequently use a lateral flow design are typically qualitative (positive or negative), single-use and rely on visual interpretation (subjective). The time to result is frequently as short, 15–30 minutes and are commercially available. Sample types: fingerstick blood, saliva or nasal swab fluid.
    - **Laboratory–Based Immunoassays**
        - Enzyme-linked immunosorbent assay (ELISA), viral protein(s) are immobilized on a solid phase surface, commonly a microwell and patient samples are added. If the patient specimen contains antibodies to that protein they can be detected as change in color or fluorescence by a plate reader (objective). These methods are commercially available and produce either a qualitative (positive or negative) or quantitative result, can be automated, can test multiple patient samples at once and the time to result varies by test method. Sample types: whole blood, plasma, or serum.
        - Chemiluminescent immunoassay (CIA or CLIA), similar to an ELISA viral protein(s) are incubated with patient samples to determine if the specimen contains antibodies. The viral protein can be used to coat magnetic micro particles and the reaction creates a change in light or luminescence which is detected by a reader (objective). These methods are commercially available and tend to produce a quantitative result, are automated, can test multiple patient samples at once and the time to result varies by test method. Sample types: whole blood, plasma, or serum.
    - Virus Neutralization Test, or plaque reduction neutralization test (PRNT), includes three main elements, cultured cells (requires a method to grow cells in a laboratory), viable virus (SARS-CoV-2 virus, which requires BSL3 laboratory facility), and patient specimens to determine if the patient has neutralizing antibodies which are antibodies that "neutralize" or prevent the virus from infecting cells in cell culture. This test may take 3-5 days to complete and requires several dilutions of patient specimen to determine a virus neutralizing titer. Sample types: whole blood, plasma or serum.
- Most serologic methods currently available in the US detect IgG antibodies (infection at some point in the past). Some methods may also detect other types of antibodies such as IgM (indicate recent infection) or IgA (associated with immunity in mucosal membranes). When more than one antibody type can be detected the method may be referred to as a "total antibody" test, most commonly IgM/IgG. Serologic methods can also include detection of viral antigen. Detection of viral antigen would be a marker of current infection.
    - Total antibody tests are unlikely to discriminate between the different types antibodies. The result will be detection (or not) of antibodies indicating infection at some point in the past.
    - Total antibody tests that include both IgM and IgG may have a greater sensitivity than a method that detects only one or the other. However, they may also have a lower specificity since IgM antibodies tend to be less specific than IgG antibodies.
- The currently available serologic tests for SARS-CoV-2 also utilize different antigenic targets which can impact the sensitivity and specificity of the test.
- Several other organizations have developed much more in-depth explanations of serologic test methods. Please refer to the links in Additional COVID-19 Serology resources at end of document.

Current Test Availability

- There are well over 100 manufacturers marketing serologic tests for SARS-CoV-2 available for purchase in the US. These tests fall into different categories.
  - FDA Authorized: the FDA reviewed and issued an Emergency Use Authorization (EUA) for the test and can be performed in authorized laboratories (*Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. 263a, to perform moderate and high complexity tests*)
  - Not FDA Authorized — Manufacturer Developed: the FDA has **not** reviewed validation data for the test and has **not yet** issued an EUA.[5] Manufacturers are allowed to market and sell these tests for 10 days prior to submission of an EUA package to FDA but EUA submission is required. These tests can only be performed in high complexity laboratories, where the test has been adequately validated and test reports include specific language, as provided by the FDA.
    - Note that this is part of an updated FDA policy issued on May 4, 2020. All manufacturers marketing a test that was available under the March 16, Early Market Access FDA policy must submit an EUA package or cease selling their test.
  - Not FDA Authorized — Laboratory Developed: the FDA has **not** reviewed validation data for the test and has **not** issued an EUA. These tests may only be developed and performed in high complexity laboratories and are not available for purchase by other laboratories. Laboratories testing under this policy must adequately validate the test, notify FDA and include specific language provided by FDA on their test reports.
- As of May 7, 2020, FDA has not authorized any serologic test for use in CLIA waived settings. All serologic tests require that staff performing the testing meet the educational qualifications required for moderate complexity testing or greater and thus can only be performed in a traditional laboratory setting not at point of care or near-patient settings.
- All clinical tests, regardless of whether they have been reviewed by FDA or not, should be verified in each laboratory prior to implementation.
- The performance of these assays are highly variable; users should review performance characteristics of the test as outlined in the Instructions for Use (IFU) or FDA's webpage, EUA Authorized Serology Test Performance.

Considerations When Selecting an Assay for Seroprevalence Studies

The immune response to SARS-CoV-2 infection is still being investigated and is not well understood which makes interpretation of results challenging. At this time, it is not known whether the detection of antibodies indicates protective immunity and if so, how long protective immunity might last, or how complete the immunity is. For example, less than complete immunity could mean (1) you still get infected and sick if exposed, but less sick than you would have without the presence of antibodies; or (2) your chances of getting infected are reduced, but not by 100%. Additionally, it is unknown how much antibody must be present to be protective or if there is a type of antibody that must be present to have protective immunity. Despite this lack of information, here are some general considerations for selecting an assay for conducting serologic testing.

- Choose a method with the best possible performance characteristics including the highest possible sensitivity and specificity, and minimal cross-reactivity:
  - **Sensitivity** measures the ability of the test to correctly identify persons who have SARS-CoV-2 antibody.
  - **Specificity** measures the ability of the test to correctly identify persons who do not have SARS-CoV-2 antibody, ideally should be greater than 97% given the low prevalence of infection in the US.[2]
  - **Cross-reactivity** is the detection of other related antibodies (e.g., produced by past infection with other coronaviruses) which would produce a

false-positive result. It is estimated that 90% of persons age 50 and older have antibodies to the common non-SARS-CoV-2 human coronaviruses HKU1, NL63, OC43, or 229E.[6] Ideally a method should have minimal to no cross-reaction with non-SARS-CoV-2 antibodies.

- Be aware of the impact of prevalence on the positive and negative predictive value of the test. A low prevalence of infection will decrease the positive predictive value of any method.[4] Positive predictive value is a function of test specificity, test sensitivity, and population prevalence. At low prevalence values (in the range of 1 to 3%), with sensitivity of .99, a specificity of .98 can still yield a positive predictive value of 30 to 60%.
    - **Prevalence** is the proportion of the population that have been infected with SARS-CoV-2. This will be based on the number of cases identified and models may be used as well to get a more likely estimate.
    - **Positive predictive value** is the probability that a person with a positive SARS-CoV-2 serologic test result actually has SARS-CoV-2 antibodies and was infected.
    - **Negative predictive value** is the probability that a person with a negative SARS-CoV-2 serologic test result does not have SARS-CoV-2 antibodies and was not infected.
- For a population level study, many hundreds to thousands of samples will be tested so a method must be chosen that aligns with the study design of the project with considerations for sample collection and transport as well as finding efficiencies in time and cost. For example, a test that uses fingerstick blood might be easiest to obtain from persons at various locations compared to venipuncture, it may also be difficult to perform such a high volume of individual use tests.

Considerations for Test Result Interpretation

The caveats presented above regarding our lack of knowledge about protective immunity and the importance of selecting an assay that performs well also apply to tests used to inform individual decision making. If these methods are used to detect antibody response on an individual or population level there are considerations for interpreting the results.

- A negative result using one of these methods does not rule-out SARS-CoV-2 infection.
    - A negative result may be due to performing the method prior to the development of antibodies. It may take 1–2 weeks post symptom onset before an individual infected with SARS-CoV-2 has detectable antibodies.[1] (*Note: it often takes about a week from infection to symptom onset, so antibodies may not develop for 2–3 weeks after infection). This initial window when individuals may be serologically negative usually overlaps with when infected individuals are also infectious.
    - A negative result also may be due to a test method with low sensitivity (i.e., patient antibody levels are below the limit of detection of the method) which would be considered a false negative result.
    - Consideration should be given to following up a negative result with molecular diagnostic testing to rule out current infection.
- A positive result indicates past and/or present infection with SARS-CoV-2.
    - A positive result also may be due to detection of cross-reactivity (i.e., antibodies to non-SARS-CoV-2 strains may cross-react with SARS-CoV-2 antibodies) which would be considered a false positive test result.[6]
    - A positive result **does not mean that the individual is immune to COVID-19 infection**.
    - A positive result **does not mean that the person is no longer shedding virus or is no longer infectious**.

Additional Considerations and Outstanding Research Needs

- Independent evaluation of serologic assays using a well-characterized set of samples to determine performance characteristics including sensitivity, specificity, accuracy and cross-reactivity
  - The FDA, National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC), and the Biomedical Advanced Research and Development Authority (BARDA) are collaborating to assess the performance of various serological tests.[7]
  - The Foundation of Innovative Diagnostics (FIND) is also evaluating SARS-CoV-2 immunoassays- though not all assays in their evaluation may be available for purchase in the US at this time.[8]
- Research studies to determine whether presence of antibodies (type and amount) confer protection or immunity to future exposure/infection with SARS-CoV2, for how long antibodies remain protective, and how protective they are (e.g., prevent disease entirely or reduce severity of disease).
- High-quality representative seroprevalence surveys to provide an accurate assessment of community or population level immunity (i.e., how many susceptible versus immune individuals are in a given population): attention to selection of representative sample of individuals to be tested, field methods, recruitment methods, incentives, community participation, etc.
- Ongoing monitoring of the availability of new tests and performance data including how that could impact national case definitions for surveillance.
- Creation and access to a well-characterized set of samples that is available for clinical and public health laboratories to validate and verify the performance of serologic assays, a requirement to perform clinical testing.
- Monitor ongoing discussions on use of different testing algorithms to improve overall confidence in test results and consider how any forthcoming guidance on testing algorithms should be incorporated into serologic testing plans.
- Communication(s) regarding the need for additional testing for SARS-CoV-2 should be clear and specific and include the types of testing (molecular detection vs. antibody) and purpose for testing (detection of acute infections vs. improving understanding of population level immunity) that is needed.

References

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Additional COVID-19 Serology Resources

IDSA COVID-19 Antibody Testing Primer, Infectious Diseases Society of America

COVID-19 Serologic (Antibody) Testing, CDC

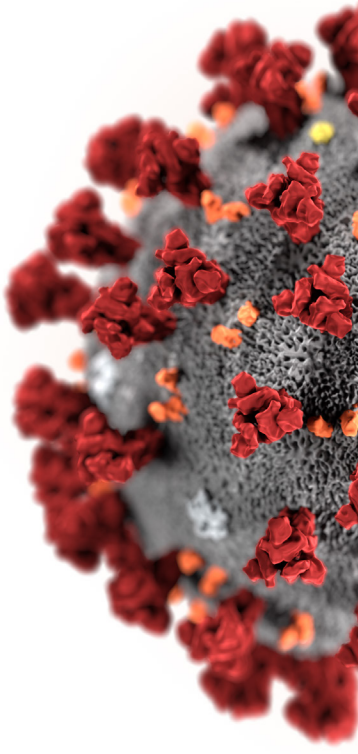
Coronavirus Disease 2019 (COVID-19) EUA Information, FDA

FAQs on Diagnostic Testing for SARS-CoV-2, List of Serology Assays Submitted under Section IV.D of the FDA Policy for Diagnostic Tests for Coronavirus Disease-2019

> **Section:** What Laboratories and Manufacturers are Offering Tests for COVID-19?
>
> **Not FDA Authorized- Manufacturer Developed:**
> Question: What commercial manufacturers are distributing serology test kits under the policy outlined in Section IV.D of the Policy for Coronavirus Disease-2019 Tests? (Updated 5/5)
>
> **Not FDA Authorized- Laboratory Developed:**
> Question: What laboratories are offering serology tests under the policy outlined in Section IV.D of the Policy for Coronavirus Disease-2019 Tests? (Updated 5/5)

FIND Interactive Dashboard, Contains performance data on sensitivity and specificity as assessed in evaluation studies. Data are presented as submitted to FIND. FIND has not verified quality and accuracy of data provided by third parties.

EUA Authorized Test Performance, FDA webpage that contains performance data from the manufacturers' instructions for use and adds information on positive and negative predictive values for each test.



APHL™

eoc@aphl.org
8515 Georgia Ave, Suite 700
Silver Spring, MD 20910

CSTE

www.cste.org
2635 Century Parkway NE, Suite 700
Atlanta, GA 30345

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