

Serology Antibody Testing: Challenges and Best Practices

Testing Working Group Report

The COVID-19 Healthcare Coalition is a collaborative private-industry response to novel coronavirus. Our mission is to save lives by providing real-time learning to preserve healthcare delivery and protect populations. (<https://c19hcc.org>)

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Summary

A critical component of the COVID-19 response is a better understanding of how many people in the population have been infected. This has been particularly challenging with COVID-19 due to the lack of diagnostic testing capacity and the substantial occurrence of asymptomatic infections. The population-level data that can be gained from serology antibody tests will help decision makers understand the overall prevalence of the disease, calculate accurate disease and fatality rates, and plan for subsequent waves.

Serology antibody tests are blood-based tests that detect antibodies generated against foreign pathogens and can be used to identify whether a person has been exposed to that pathogen in the past. This is particularly helpful to understand the extent of infection in a population, including identifying people who might be asymptomatic or have recovered. The body produces different forms of antibodies, termed immunoglobulins (Ig), which perform specific functions during and after an infection. Antibodies mounted during an immune response to pathogen exposure include IgM, IgA, or IgG. Among the IgM and IgG antibodies, IgM typically appears mid-stage of infection while IgG develops ~2 weeks after infectionⁱ.

Current research suggests that antibody response to SARS-Cov-2 lags behind symptom onset by 7-12 daysⁱⁱ. While the immune response to SARS-CoV-2 is not well characterized at this time, current research seems to indicate that IgG might be the most specific antibody to test forⁱⁱⁱ. While these serologic tests can determine past infections, they provide minimal insight into active, acute stage infections. In the case of SARS-CoV-2 virus, positive results do not prove immunity, only an exposure.

Types of Serology Tests

There are two types of serologic antibody tests that are currently on the market—rapid point of care (POC) tests and enzyme-linked immunosorbent assays (ELISA).

- Serologic rapid **POC tests** are popular because they yield fast results (about 15 min), can be done at the point of care, and are easy to use. These qualitative tests are generally developed as lateral flow assays (LFA), similar to pregnancy tests, and report presence or absence of antibodies in blood or serum samples. Generally, many POC tests have received Clinical Laboratory Improvements Act of 1988 (CLIA) waivers, meaning that the tests are so simple to conduct, with low possibility for user error, that they can be performed outside of a traditional laboratory. That is, they can be performed in settings like pharmacies, physician offices, or even at home. In fact, the U.S. Food and Drug Administration (FDA) recently clarified that when it grants an Emergency Use Authorization (EUA) for a point-of-care test, that test is deemed to be CLIA-waived^{iv}.

While there are many benefits with serological testing, several challenges remain and will be covered in the upcoming sections of this document.

- **ELISA assays** are tests that are performed in an approved laboratory and can provide qualitative or semi-quantitative results (positive, negative, or indeterminate). Numerous quantitative ELISA tests are commercially available and can be used to determine the level of antibodies. The turnaround time for the results of the ELISA is determined by the personnel performing the testing. The ELISA results could be available in a matter of hours if performed in-house where the specimen was collected, or up to a few days later if being sent to an off-site reference laboratory.

For additional information about serology tests, please refer to this [educational](#) summary from FDA.

Challenges and Limitations of Serology Testing

Interpretation of antibody tests

Since serology testing is not recommended for diagnosing COVID-19 in an acutely ill patient, it is not considered a diagnostic test. This is because positive results, the presence of IgM, IgG, or IgA, generally provide insights into past exposure to SARS-CoV-2. Furthermore, negative results, the absence of IgM, IgG, or both, do not mean that the patient is not infectious nor that the patient does not have the virus or disease. A negative result could indicate that the patient did not mount a detectable immune response at the time of the testing.

Immune responses also vary by patient, in terms of how many antibodies they form and how quickly they make antibodies. *It is also important to note that currently there is no evidence that antibodies provide protection (lasting or short term) from the reinfection with SARS-CoV-2*

Accuracy

At this time, most serological tests currently available on the market have not been evaluated by the FDA. Individual tests have different performance characteristics based on the manufacturer design and quality control. Also, because of the nature of the tests, there will likely be false positives, as a result of cross-reaction with antibodies to more common human coronaviruses.

Elements used to determine the accuracy of serology tests include sensitivity (the tests' ability to detect antibodies, true positive) and specificity (the ability to distinguish people who are infected from those who are not, true negative). These reports can be used to compare accuracy across the available tests.

- A recent, small study published in the preprint server, *MedRxiv*^{vi}, evaluated the performance of three ELISA and six POC commercially available SARS-CoV-2-specific tests. Specificity and sensitivity of the ELISA tests ranged from 93-100% and 65-90%, respectively. Specificity for the POC tests ranged from 80-90%, and sensitivity between 80-100%.
- While this analysis only evaluated a subset of the available serological tests, it provides evidence that some tests perform better than others and that further analysis of all commercially available tests are needed.

Availability and FDA Validation

There are several serology tests being marketed online, particularly POC tests. All commercially available ELISA tests provide qualitative or semi-quantitative results (Positive, Negative, or Indeterminate).

Guidance from the FDA has changed over the past two months. On March 16, 2020, the FDA stated that it did not intend to object to the development and distribution by commercial manufacturers nor the development and use by laboratories of serology tests to identify antibodies to SARS-CoV-2, thus providing regulatory flexibility and limited oversight on the validation of these tests^{vii}. Figure 1 shows the distribution of serology tests on the market in the United States; as of June 01, 2020, there were 15 tests with EUA— three LFAs and nine ELISAs.

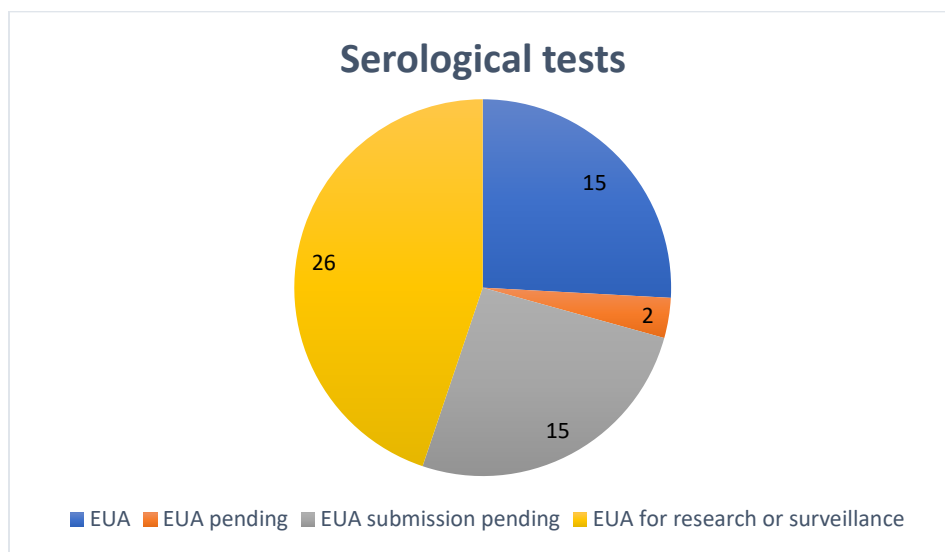


Figure 1: Summary of serological tests with EUA status as of 06/01/2020

The FDA released new guidance on May 4, 2020, which requires manufacturers marketing their tests to file applications for EUA and submit validation data^{viii}. This new policy is meant to give FDA more oversight. However, it still allows unapproved medical products to be used without being subjected to a full-fledged review.

Additionally, the FDA recently posted test [performance data](#)^{ix} for serology tests with EUA which provides explanations and context for test results, including Positive and Negative Predictive values (PPV and NPV). These values take into account the percentage of individuals in the population who have antibodies to SARS-CoV-2, termed prevalence. Given how prevalent individuals with antibodies are in a population, the PPV and NPV values provides how likely it is that a person who receives a positive or negative result from a test truly does or does not have antibodies to SARS-CoV-2, respectively.

Best Practices

Using LFA antibody tests for COVID-19 has gained traction over the past few weeks due to the rapid turnaround time as decision makers strive to quickly understand the extent of SARS-CoV-2 infections in their communities. However, recent advice from the [World Health Organization](#)^x has indicated these antibody **point of care tests should only be used in research settings and not for making clinical decisions**. This WHO report cites the delayed development of antibodies to SARS-CoV-2 during the course of infection.

Additionally, FDA has [recommended](#)^{xi} that **serological tests be used only to detect antibodies to SARS-CoV-2 in order to identify people with past exposures and those recovered from COVID-19**, rather than as the sole basis for diagnosis of COVID-19. Molecular (RT-PCR) based testing continues to be recommended for diagnosis of SARS-CoV-2.

Path Forward

More work is needed to:

- Understand the clinical and population significance of IgM and IgG titers as it relates to conferring long-term immunity and protecting against reinfection of SARS-CoV-2.
- Understand the performance characteristics of commercially available serologic tests; and
- To scale PCR-based diagnostic testing widely in the community and other settings to support federal and state plans to safely re-open local economies.

ⁱ Hangartner, L., Zinkernagel, R. M., & Hangartner, H. (2006). Antiviral antibody responses: the two extremes of a wide spectrum. *Nature Reviews Immunology*, 6(3), 231-243.

ⁱⁱ Liu, L., Liu, W., Wang, S., & Zheng, S. (2020). A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients. *medRxiv*.

ⁱⁱⁱ Jin, Y., Wang, M., Zuo, Z., Fan, C., Ye, F., Cai, Z., ... & Xu, A. (2020). Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019. *International Journal of Infectious Diseases*.

^{iv} https://www.cdc.gov/csels/dls/locs/2020/fda_clarifies_clia-waived_status.html

^v <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-serological-test-validation-and-education-efforts>

^{vi} Lassaunière, R., Frische, A., Harboe, Z. B., Nielsen, A. C., Fomsgaard, A., Krogfelt, K. A., & Jørgensen, C. S. (2020). Evaluation of nine commercial SARS-CoV-2 immunoassays. *Medrxiv*.

^{vii} <https://www.fda.gov/media/135659/download>

^{viii} <https://www.fda.gov/news-events/fda-voices/insight-fdas-revised-policy-antibody-tests-prioritizing-access-and-accuracy>

^{ix} <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance>

^x https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19#.Xo_1jdsCuUE.twitter

^{xi} <https://www.fda.gov/medical-devices/letters-health-care-providers/important-information-use-serological-antibody-tests-covid-19-letter-health-care-providers>