CDC Interim Guidelines for COVID-19 Antibody Testing: What are the implications?[1]

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On January 7, 2020, Chinese health authorities confirmed that a cluster of pneumonia cases in the Hunan province was associated with a novel coronavirus, 2019-nCoV. On January 20, 2020, the first case of this novel coronavirus was reported in the United States.[2] On Memorial Day weekend, the United States mourned 100,000 deaths related to this pandemic. Public health and regulatory bodies moved fast to keep up with the needs to contain and respond to this rapidly moving pandemic. The Center of Disease Control and US FDA kept their guidance relevant with the changing knowledge about COVID-19 in the past few months.

The diagnostic needs of a new pandemic are being met as more diagnostic tools are developed and released with continuously improved performance accuracy. At the start of the pandemic, there was a high need for direct viral testing with molecular and PCR methods to diagnose acute infection. Very quickly the need for accurate antibody testing became evident, as questions around the scope of current and past infection and the development of immunity started to emerge. Many diagnostic manufacturers made available accurate antibody tests that qualitatively assess immune response to SARS-CoV-2.

While the clinical community and public health authorities are committed to complete large scale studies to better assess the questions around scope of infection at a population level as well as the nature of the immune response and immunity for SARS-CoV-2 at patient level, the CDC has issued Interim Guidelines for COVID-19 Antibody Testing.

DO THE CDC GUIDELINES SUPPORT THE USE OF ANTIBODY SEROLOGICAL TESTS?

The guidelines point to the importance of accurate serologic methods for public health and clinical uses to monitor and respond to the COVID-19 pandemic, but also point out limitations.

WHAT DO THESE GUIDELINES SAY ABOUT THE ACCURACY OF ANTIBODY SEROLOGICAL TESTS?

The guidelines stress on the accuracy of the tests in two ways:

1. **It is important to use high quality COVID-19 Antibody Tests with US FDA EUA:** CDC recommends that a test with US FDA EUA status should be chosen. On May 21, the US FDA announced that a list of antibody

tests that are being removed from the "notification list" of tests being offered under the Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency was posted. This "removal list" consisted of both antibody tests voluntarily withdrawn by the commercial manufacturer and those for which there is not a pending Emergency Use Authorization (EUA) request or issued EUA.[3] FDA expects that the tests on the removal list will not be marketed or distributed. This means that tests that remain on the US FDA's EUA list meet the FDA issued requirements and should be the only ones used.

2. **A test with high specificity reduces false positives and increases positive predictive value:** Experts believe that the Positive Predictive Value (PPV) is the most important metric of accuracy for COVID-19 antibody tests based on the current utilities. PPV is the probability that the test accurately detects the antibodies to COVID-19 and is dependent on the prevalence of the disease. High specificity tests provide a high PPV, and this is especially important for low prevalence diseases. Low specificity tests provide a lower PPV, which could be meaningless, especially in low prevalence setting. Specificities closer to 100% yield high PPV in low or high prevalence areas—so are ideal as prevalence changes.

The guidelines use an example to illustrate this: in a population where the prevalence is 5%, a test with 90% sensitivity and 95% specificity will yield a positive predictive value of 49%. In other words, less than half of those testing positive will truly have antibodies, which makes it difficult to interpret a positive result by this test.

In the example above, with a 5% prevalence, a test with 100% specificity will yield a PPV of 100%, and a test with 99.5% specificity will yield a PPV of 90.5%. So, even for laboratory tests with specificities ranging from 99.5%–100%, a small change in specificity makes a larger difference in PPV especially in lower prevalence settings.

DO THE GUIDELINES EXPLAIN A TESTING ALGORITHM TO INCREASE PPV?

Not directly. For tests with low specificity or poor PPV, they do explain that a two test "orthogonal" algorithm can be used to compensate for lower specificities to increase the PPV. However, one test is sufficient if it delivers greater than 99.5% specificity. It may pose challenges to adopt two different serological tests in every laboratory.

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WHAT DO THESE GUIDELINES SAY ABOUT HOW THESE ANTIBODY SEROLOGICAL TESTS CAN BE USED?

Per the guidelines, antibody serological tests may be used as follows:

As aiding in diagnosis: Antibody tests can be used in aid in diagnosis with a PCR test and patient's clinical information, but a diagnosis cannot be based solely on an antibody test result.

To understand a patient's clinical picture: Especially in complicated clinical cases when there are multisystem inflammatory syndromes.

To assess seroprevalence: These tests can help determine the proportion of a population previously infected with SARS-CoV-2 and provide information about populations that may be immune and potentially protected. Thus, demographic and geographic patterns of serologic test results can help determine which communities may have experienced a higher infection rate and therefore may have higher rates of herd immunity.

To determine who may qualify to donate convalescent plasma: Serologic test results may assist with identifying persons potentially infected with SARS-CoV-2 and determining who may qualify to donate blood that can be used to manufacture convalescent plasma as a possible treatment for those who are seriously ill from COVID-19.

WHAT DO THESE GUIDELINES SAY ABOUT WHICH TYPE OF TEST SHOULD BE USED?

The guidelines explain that there are two broad antibody test types:

1. Binding antibody tests which can be further divided into:
   - Point-of-care (POC) tests on lateral flow devices
   - Laboratory tests based on ELISA Enzyme-Linked Immunosorbent Assay or CIA
2. Neutralizing antibody tests that determine the ability of antibodies to prevent infection in vitro.

Binding antibody tests that detect IgM, IgG and Total antibodies are available. However, the guidelines state that there is no identified advantage of one of these types over another. They explain that antibodies in some persons can be detected within the first week of illness onset. However, SARS-CoV-2 infections are somewhat unusual because IgM and IgG antibodies arise nearly simultaneously in serum within 2 to 3 weeks after illness onset. This combined with the fact that how long IgM and IgG antibodies remain detectable following infection is not known.

Although the guideline states that "there is no identified advantage of one of these types over another," based on the reported heterogeneity of IgG and IgM seroconversion among COVID-19 patients, a Total assay detecting all antibody isotypes is more sensitive in comparison to a IgG or a IgM only test.

WHAT DO THESE GUIDELINES SAY ABOUT ASSAY DESIGN?

The guidelines state that the two major antigenic targets of SARS-CoV-2 virus against which antibodies are detected are spike glycoprotein (S) and nucleocapsid phosphoprotein (N). While S protein is essential for virus entry and is present on the viral surface, N protein is the most abundantly expressed immunodominant protein.

It follows that assays targeting S1 protein should show higher specificity.

The guidelines state that "S1" is the least "conserved," implying that the S1 protein is more unique (less conserved) in comparison to N or RBD, thus, less likely to cause cross-reactivity with antibodies to the corresponding proteins of other coronaviruses. It follows that assays targeting S1 protein should show higher specificity.

WHAT DO THE GUIDELINES SAY ABOUT HOW TO TEST FOR IMMUNITY?

The guidelines clearly mention that no antibody tests currently can test for immunity.

Antibodies most commonly become detectable 1–3 weeks after symptom onset—and at this time infectiousness likely is greatly decreased and that some degree of immunity from future infection has developed. Additional data are needed before modifying public health recommendations based on serologic test results.

Multiple agencies (FDA, NCI/NIH, CDC, BARDA) are collaborating with members of academia and the medical community to evaluate several serology tests using a well-characterized set of clinical samples (serum or plasma) collected before and during the current COVID-19 outbreak.

Representatives from BARDA, CDC, FDA, NIH, OASH, DoD, and White House Office of Science and Technology Policy are working with members of academia and the medical community to determine whether positive serologic tests are indicative of protective immunity against SARS-CoV-2.

WHERE CAN THE CDC GUIDELINES BE FOUND? WHEN WERE THEY UPDATED?

The CDC Guidelines were updated on May 23, 2020, and should be consulted regularly. They can be found on the following links:

Antibody Testing At-A-Glance Recommendations for Professionals: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-professional.html

Interim Guidelines for COVID-19 Antibody Testing in Clinical and Public Health Settings: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html

REFERENCES

1. Center for Disease Control and Prevention, May 2020. Interim Guidelines for COVID-19 Antibody Testing.

2. The epidemic of 2019-novel-coronavirus (2019-nCoV) pneumonia and insights for emerging infectious diseases in the future, Li et al, Microbes Infect. 2020 Mar; 22(2): 80–85.

3. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-provides-promised-transparency-antibody-tests (21 May 2020)