JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH | Center for Health Security

Serology testing for COVID-19

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Background

Serology tests are blood-based tests that can be used to identify whether people have been exposed to a particular pathogen. Serology-based tests analyze the serum component of whole blood. The serum includes antibodies to specific components of pathogens, called antigens. These antigens are recognized by the immune system as foreign and are targeted by the immune response. These types of tests are often used in viral infections to see if the patient has an immune response to a pathogen of interest, such as influenza. The tests can be used to diagnose infection.

There are several types of serology tests.[1]

1. Neutralization tests can indicate whether the patient has active, functional antibodies to the pathogen in question by measuring how much the patient antibodies can inhibit viral growth in the lab (Figure 1). This can be used with SARS-CoV-2 virus in a BSL-3 setting, or pseudoviruses that express certain SARS-CoV-2 proteins in a lower BSL setting.
2. Chemiluminescent immunoassay (CLIA) shows whether a patient has antibodies to a pathogen by displaying a fluorescent signal when patient antibodies interact with virus proteins (Figure 2).
3. Enzyme-linked immunosorbent assays (ELISAs) are more rapid serology tests performed in a lab that provide a readout of antigen- antibody interactions. Essentially, patient antibodies are "sandwiched" between the viral protein of interest and reporter antibodies, so that any active patient antibodies are detected- (Figure 3).
4. Lateral flow assays (LFAs), also called rapid diagnostic tests (RDTs) display a colorimetric, qualitative readout of the presence of antibodies. These are often used in point-of-care settings. The patient sample is flowed over a membrane that has the target antigen anchored. If the sample contains antibodies specific to that antigen, they form a complex that results in a colored band on the strip (Figure 4). These are similar to pregnancy tests.

COVID-19 detection and serology

Serology testing for COVID-19 is attractive because of the relatively short time to diagnosis and the ability to test for an active immune response against the virus. While hundreds of serology tests are currently on the market, only 21 have received Emergency Use Authorization (EUA) from the FDA.[2] Tests that have not received EUA are not FDA approved, and should not be used for diagnosis of COVID-19. Serology tests have variable sensitivity and specificity, and even EUA approved tests should not be the sole basis of diagnosis. The CDC currently recommends using molecular testing (PCR, antigen) to diagnose SARS-CoV-2 infections.[3]

Research has demonstrated that the spike (S) and nucleocapsid (N) proteins are the primary viral antigens against which antibodies are raised.[4] These antigens are the most commonly used in serology tests. During infection, several types of antibodies are raised to the virus. IgM antibodies emerge first, after 5 days post-symptom onset. IgG antibodies are more tailored, and typically emerge after 10 days post-symptom onset. Many serology tests detect both IgG and IgM, which increases the specificity of the test. IgA antibodies may also increase during infection, and are typically found in mucous (such as saliva).[5]

While serology tests are now widely available, the correlates of immunity are incompletely understood. The presence of antibodies only indicates previous SARS-CoV-2 infection. The results of serology tests can then be used to estimate the true spread of the virus through a population, even if individuals were asymptomatic or were never diagnosed. The presence of antibodies does not indicate that an individual is protected from reinfection. There is limited understanding of the levels and persistence of antibodies necessary for protective immunity. Therefore, serology tests cannot inform an individual of their immunity to reinfection.

Past coronavirus outbreaks and serology

Serology assays were used in past coronavirus outbreaks, but these have been time- and resource-intensive to create. SARS and MERS were both coronavirus-based diseases that had major public health impacts. Serology assays developed for these diseases took significant time, because proper animal models, protocols, and specific antibodies had to be developed. In other words, the reagents needed to create serology assays were non-trivial to isolate.[6-8] Previous work with SARS coronavirus identified an ELISA method that provided high sensitivity to detect SARS coronavirus infection in a monkey cell line. This was found to be more efficient than neutralization or IFA-based methods.[9]

Next steps in serology testing

Further research of correlates of immunity will improve our understanding of serology test results. The persistence of antibodies must be characterized. Researchers have characterized IgM and IgG responses in patients, showing that IgG appears to persist for weeks to months. However, IgM and IgG both decline over time, especially after 4 weeks post-symptom onset.[10] Neutralizing antibody levels also appear to correlate with IgG levels, though further work is needed to establish levels of antibodies necessary for protection from reinfection.[11] Antibody levels alone may not induce protective immunity, and the cellular immune response should be further characterized for its role in immunity.

The immense number of serology tests now available warrants careful validation of test performance. Currently, all tests receiving EUA must submit validation studies performed by the manufacturer, with thresholds for sensitivity and specificity.[12] These tests must also be independently validated, through an effort with the FDA and National Cancer Institute (NCI).[13] These independent validation studies should be published to better inform consumer decision making. There should also be a continued effort to clarify the status of many tests that fraudulently claim FDA approval. Serology tests will be used to determine the true spread of the virus throughout populations, and the tests used must be as reliable as possible. Extensive validation of tests sensitivity and specificity will bolster this.

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