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. 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.
2. Immunofluorescent assay (IFA) shows whether a patient has antibodies to a pathogen by displaying a fluorescent signal when patient antibodies interact with virus proteins.
3. Enzyme-linked immunosorbent assays (ELISAs) are more rapid serology tests 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- (see Figure 1).
4. Western blot tests, which can be used in laboratory settings to detect the presence of a protein of interest by the emission of a colored or fluorescent reporter when an active antibody interacts with the viral protein.

COVID-19 detection and serology

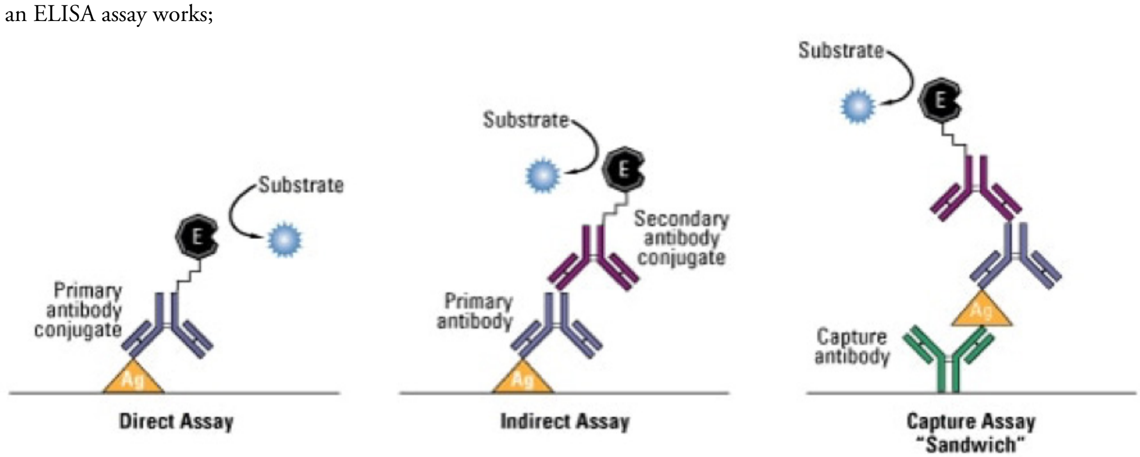
Serology testing for COVID-19 is attractive because of the relatively short time to diagnosis and the ability to test for an active immune response against the virus. Currently, however, no serology tests are available for COVID-19. The CDC uses and currently recommends a PCR method to diagnose infection based on the presence of viral RNA. While PCR is a very sensitive assay, combining PCR with serology would theoretically increase sensitivity of testing. Having 2 tests would allow confirmation of infection, rather than relying on a single test.

The 2 potential serology tests for COVID-19 would be (1) a test for viral proteins (likely a Western blot), with antibodies raised in animals that would allow for detection; or, more commonly, (2) an ELISA that detects the patient's antibodies to the virus. These would allow for rapid detection of viral proteins, or antibodies to those proteins, using serum alone.

Before any serology tests can be developed, a better understanding of the viral proteins to which immune cells react is required. Coronavirus has only 4 structural proteins: the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. In the case of COVID-19, the spike protein appears to be the primary protein interacting with host cells. Hence, the spike protein is likely the protein to which antibodies are raised, but this is not clear at this time. Once we know the antigenic protein, we can potentially raise antibodies to this protein (or the entire virus) in animals. This can be used to produce antibodies that would react to any viral proteins in a Western blot or provide the protein "anchor" for the ELISA.

Figure 1

How an ELISA assay works;



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.[1-3] 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.[4]

Next steps in serology testing

Once identified, the viral protein of interest must be produced (likely in *E. coli* or *S. cerevisiae*) in large quantities to provide the "anchor" of the ELISA. Now that the viral sequence is known, this provides a path to produce the protein. However, the proper antibody binding, antigen presentation, and protein folding need to be further elucidated. The protein must be presented on the ELISA as it is presented in the body, or the antibodies may not bind and can lead to a false negative. Once we have this anchor, a secondary antibody can be created that reacts to human antibodies bound to the anchor, which will create a colorimetric or fluorometric output that can be quantified. A recent study identified that the spike protein of COVID-19 can be recognized by monoclonal antibodies, known as CR3022 to SARS-CoV.[5]

Hopefully, we will begin to understand the viral proteins and immune response to COVID-19 in the coming weeks. Companies and researchers are now working to produce antibodies and antigens of COVID-19. The Native Antigen Company is selling spike protein antigens from COVID-19, starting at about $540 for 100 µg.[6] Sona Nanotech has partnered with this company to create a rapid screening test that could quickly diagnose patients.[7] The synthetic antigens, produced in a Native Antigen cell line, could be used to raise antibodies in a suitable animal model once this is established. Researchers at the Hong Kong University of Science and Technology have identified putative segments of the COVID-19 spike protein that seem to be immunogenic, based on past studies with SARS coronavirus.[8] With continued studies to improve our understanding, we can develop serology testing for this virus.

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