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## Background

Serology and other adaptive immune response tests can be used to determine a person's exposure and immunity to specific pathogens. Serology tests analyze serum—a component of whole blood—to identify whether a person has developed an immune response by measuring antibodies or antigens. In contrast, other adaptive immune response tests analyze whole blood to measure, for example, the activity of immune cells, such as T cells.

Antibodies are produced specific to antigens, which are particles of a pathogen that are perceived as foreign by the immune system. Serology tests are often used in viral infections to see if the patient has developed an immune response to a pathogen of interest, such as influenza. These tests cannot be used to diagnose infection or to ensure protective immunity. Serology and other adaptive immune response tests can only be used to understand the presence of an immune response to a certain antigen.

There are several types of serology tests.<sup>1</sup>

1. **Neutralization tests** can indicate whether the patient has active, functional antibodies to the pathogen in question by measuring how much the antibodies can inhibit viral growth in the lab ([Figure 1](#)). This can be used with SARS-CoV-2 virus in a biosafety level 3 setting or with pseudoviruses that express certain SARS-CoV-2 proteins in a lower biosafety level setting.<sup>2</sup> Surrogate neutralization assays may use a modified ELISA technique (see below) to establish levels of neutralizing antibodies.<sup>3</sup>
2. **Chemiluminescent immunoassays (CLIAs)** 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 serology tests, 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 functionally similar to pregnancy tests.

## COVID-19 detection and serology

Serology testing for COVID-19 is valuable because of its ability to identify an active immune response, even if the person was infected weeks to months ago. To date, a total of 76 serology and other adaptive immune response tests have received emergency use authorization (EUA) from the US Food and Drug Administration (FDA).<sup>4</sup> Of the 76 tests, 75 are serology tests and 1 is an adaptive immune response test that assays T-cell activity. Tests that have not received FDA EUA or approval should not be used.



# Serology and Other Adaptive Immune Response Testing for COVID-19

While serology testing is useful, the tests have variable sensitivity and specificity, and even EUA approved tests should not be used for diagnosis of COVID-19. Serology and other adaptive immune response tests should be primarily used as a public health tool, rather than for individual decision making.<sup>5</sup> The US Centers for Disease Control and Prevention currently recommends using molecular testing (nucleic acid amplification tests, antigen) to diagnose SARS-CoV-2 infections.<sup>6</sup>

Research has shown that spike and nucleocapsid proteins are the primary viral antigens against which antibodies are raised.<sup>7</sup> 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, 5 days after symptom onset. IgG antibodies are more tailored and typically emerge 10 days after symptom onset. Many serology tests detect both IgG and IgM, which increases specificity of the test. IgA antibodies may also increase during infection and are typically found in mucous (such as saliva).<sup>8</sup>

With serology tests now widely available, researchers continue to elucidate protective immunity. The presence of antibodies or reactive immune cells 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 never diagnosed. The presence of antibodies does not indicate that an individual is protected from reinfection. It is now well established that antibodies from natural infection can persist for up to 8 months postinfection, with IgG levels correlating well with neutralizing antibody levels.<sup>9,10</sup> While valuable for research and public health purposes, serology tests cannot currently inform an individual of their immunity to reinfection.

## Past coronavirus outbreaks and serology

Although serology assays were used in past coronavirus outbreaks that had major public health impacts, specifically, SARS and Middle East respiratory syndrome, they were time- and resource-intensive to create. Developing these serology assays took substantial time to develop, because proper animal models, protocols, and specific antibodies had yet to be identified, isolated, or created.<sup>11-13</sup> Previous work with SARS coronavirus identified an ELISA method that provided high sensitivity to detect infection in a monkey cell line. This was found to be more efficient than neutralization or indirect fluorescent antibody-based methods.<sup>13</sup>

## Next steps in serology testing

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.<sup>14</sup> These tests must also be independently validated, through an effort with the FDA and National Cancer Institute,<sup>15</sup> and 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 have been used to determine the true spread of the virus throughout populations and will continue to be an important element of the COVID-19 pandemic response. Currently, serology tests do not seem to be impacted by emerging variants of concern, but the FDA has alerted manufacturers of the need to monitor for any variant-related complications.<sup>16</sup> Validation of tests' sensitivity and specificity will bolster the utility of serology and other adaptive immune response testing.



# Serology and Other Adaptive Immune Response Testing for COVID-19

With the advent of vaccines with EUA, serology tests may have additional uses. At the time of writing, no serology test has been authorized to quantify or validate a vaccine-elicited immune response. Several serology tests with EUA have a target antigen other than spike protein, which is the only protein currently used in vaccine designs. In addition, no serology test has been validated against serum from a vaccinated individual; therefore, individuals should not currently use serology testing to validate their response to a vaccine.<sup>6</sup> Researchers examining vaccine efficacy may use specially designed serology tests, and in the future, serology tests may be used to examine antibody levels or status. However, this will require careful study and validation of these assays on sera from vaccinated individuals.

## References

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