

Cite as: F. Krammer, V. Simon, *Science* 10.1126/science.abc1227 (2020).

Serology assays to manage COVID-19

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Measurement of antibodies to SARS-CoV-2 will improve disease management if used correctly

In late 2019, China reported a cluster of atypical pneumonia cases of unknown etiology in Wuhan. The causative agent was identified as a new betacoronavirus, called severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2), that causes coronavirus disease 2019 (COVID-19) (1). The virus rapidly spread across the globe and caused a pandemic. Swift sequencing of the viral genome allowed for the development of nucleic acid–based tests that have since been widely used for the diagnosis of acute (current) SARS-CoV-2 infections (2). Development of serological assays, which measure the antibody responses induced by SARS-CoV-2 infection (past but not current infections), took longer. This is in part due to bottlenecks with availability of positive control sera and the need for extensive specificity and sensitivity testing in the context of preexisting immunity to seasonal coronaviruses. Serological assays are important for understanding prevalence of and immunity to SARS-CoV-2.

Many types of serological assays have been developed over the past decades to measure antibody responses to pathogens in bodily fluids, especially blood serum or plasma. These assays use different platforms, including binding assays such as enzyme-linked immunosorbent assays (ELISAs), lateral flow assays, or Western blot–based assays. In addition, functional assays that test for virus neutralization, enzyme inhibition, or bactericidal assays can also inform on antibody-mediated immune responses. Collectively, serological assays are essential tools in the management of infectious diseases, including diagnosis of infection, measurements of protective antibody titers upon vaccination, and seroprevalence assessments of immunity in a population.

Serological assays for SARS-CoV-2 are now becoming widely available and include ELISAs (3–7), lateral flow assays (5, 8, 9) (see the figure), and virus neutralization assays. ELISA and lateral flow assays are performed with recombinant antigens, such as the spike protein (the main surface glycoprotein that is used to attach and enter cells) of SARS-CoV-2, the receptor-binding domain (RBD), which is part of the spike protein, or the viral nucleoprotein. Of note, using the SARS-CoV-2 nucleoprotein is expected to induce more cross-reactivity (antibodies that bind to multiple strains of coronavirus) than the spike protein, owing to sequence homology of the viral nucleoprotein. These assays can be

handled at biosafety level 2 (and therefore can be carried out more widely) given the recombinant nature of the selected antigens. By contrast, neutralization assays with replication-competent SARS-CoV-2 have to be performed in biosafety level 3 facilities, which limits their application. Safer and more high-throughput alternatives to using infectious virus are under development and include the use of pseudotyped viral particle assays, in which the SARS-CoV-2 spike protein is grafted on harmless viruses or virus-like particles.

A limited number of ELISA and lateral flow assays have recently received emergency use authorization from the U.S. Food and Drug Administration (FDA). In addition, many lateral flow assays from different companies are available, but their usefulness is questionable given the lack of official performance validation with respect to sensitivity (how many true positives are detected) and specificity (the proportion of false positives) (9–11). Using serological assays with validated sensitivity and specificity performance is critical for obtaining meaningful results. For some applications, such as serosurveys in high-prevalence populations, somewhat lower specificity is acceptable, whereas sensitivity should be high. For uses where a false-positive test result would be consequential, very high specificity is essential. In general, both sensitivity and specificity should be as high as possible.

An important application of serological tests is to understand the antibody responses mounted upon SARS-CoV-2 infection and vaccination. Assays that inform on antibody titer and/or show antibody functionality (e.g., virus neutralization) will be extremely useful to answer important scientific questions about immune protection from reinfection. For example, do all infected individuals mount a robust antibody response to SARS-CoV-2 infection? It is unclear whether there is a difference in the antibody responses found in individuals presenting with severe, mild, and asymptomatic COVID-19 and how long antibody responses last. Moreover, it is unknown if the presence of binding antibody to the spike or RBD antigens correlates with virus neutralization. Whether antibody titers (binding or neutralizing) correlate with protection from reinfection is also unclear. Such data will be important when dissecting antibody responses generated by natural infection compared to vaccination.

Serological testing can also inform on the prevalence of

SARS-CoV-2 infections in different populations. Although it is impractical to test the whole population, well-designed serosurveys are essential to determine how prevalent COVID-19 is in the general population, in selected subsections of the population (e.g., health care workers), or in specific risk groups. Both quantitative assays and assays with a binary outcome can be used for these surveys. Quantitative assays may provide more reliable results [e.g., two-step ELISAs (12)], but they are also harder to scale because they often have to be performed in specialized laboratories. By contrast, assays with binary outcomes (e.g., lateral flow assays) can be easily scaled and implemented because they are often point-of-care tests. Analyses of the results of serosurveys need to account for the sensitivity and specificity of the assay used as well as the estimated prevalence of infections in a population. In addition, biological variables resulting from in-depth characterization of the immune responses such as, but not limited to, the duration of the immune responses and the dynamic nature of antibody titers linked to severe, mild, and asymptomatic COVID-19 manifestations will need to be factored into calculating prevalence based on serosurveys. Currently, many of these critical variables are unknown, and any serosurvey analysis generated in the immediate future should be interpreted with caution.

Donors for convalescent plasma therapy can be identified with serology testing. Antibody-rich plasma or serum from convalescent individuals (or animals) has been used to treat many infections as well as snake bites. One of the earliest examples is the treatment of diphtheria with antiserum obtained from horses for which Emil von Behring received the Nobel Prize in 1901. More recently, antiserum has been used for the treatment of a range of viral infections (e.g., infections with Hantaan virus, Junin virus, measles virus, and ebolavirus, as well as potential rabies infections). Individuals who recover from COVID-19 develop antibodies to SARS-CoV-2. During the initial stages of the COVID-19 epidemic in China, convalescent plasma therapy was used compassionately (13) and has since been implemented in the United States and elsewhere. Success of this intervention likely increases with the antibody titer of the donor. It is, therefore, important to screen potential convalescent donors so that individuals with the highest antibody titers can be selected. This screening can be accomplished by measuring virus-neutralizing activity of the plasma, which is a lengthy process (several days) and needs to be performed in a biosafety level 3-laboratory. ELISA-based antibody testing that produces a titer is quick (hours) and easy to perform. Quantitative measurements of antibody titers from at least two different ELISAs have been shown to correlate well with neutralizing titers (3, 4).

Identifying individuals who are immune is an important but also complex and politically charged application of

serological assays. Individuals who were infected with “common cold” human coronaviruses develop antibody responses and are protected from reinfection for a certain period of time, likely for years (14). If reinfection occurs, it is often mild or asymptomatic. In addition, infection with SARS-CoV-1 was shown to induce neutralizing antibody responses that last for several years (14). On the basis of these data, individuals with antibodies to SARS-CoV-2 are assumed to be less susceptible to reinfection, reducing the risk of severe COVID-19 and also limiting the possibility of spreading the virus. Therefore, it has been proposed that individuals with robust antibody responses could safely return to normal life and work, slowly starting the economy on a path to recovery. Detection of protective immune responses is also an important consideration for health care workers. In addition, people immune to SARS-CoV-2 could be spared from quarantine and social distancing measures during a potential second or third wave of SARS-CoV-2 infections in the winter of 2020. Accordingly, some countries have proposed an “immune passport” for such individuals.

However, there are numerous caveats that should be carefully considered before proceeding. It needs to be demonstrated that individuals who have developed antibodies to SARS-CoV-2 are protected. If antibodies provide immunity and protection, it is not (yet) known how long they will persist at the needed titer. A person protected today might no longer be protected in 6 months. It is, therefore, a matter of urgency to conduct studies aimed at dissecting the magnitude, duration, and functionality of the immune responses induced by SARS-CoV-2 infection, including antibodies, as well as cellular (adaptive) immune responses, and to determine the correlation between immune response and protection. In the absence of this knowledge, decisions about deploying the workforce may be based on incomplete information and guided by incorrect assumptions.

A known antibody titer that correlates with protection would also be extremely beneficial for vaccine development. Protective titers and/or correlates of immune protection have been established for many virus infections, including influenza virus, hepatitis A virus, hepatitis B virus, and measles virus. For several of these infections, the dynamics of the immune responses are well understood and the duration of protection based on antibody titers has been successfully modeled (15). For these types of studies, serological assays that measure a quantitative antibody titer have been instrumental. However, when converting the concept of an “immune passport” to practice, point-of-care serological assays that produce a binary response may also be useful. A combined strategic approach may be the safest while also being feasible. To account for sensitivity and false positives, if every positive lateral flow test result is confirmed with a second test that produces a titer—which also indicates the robustness of

the response and could be linked to the presence and duration of protection—the number of false-positive results would be greatly reduced. Such a targeted sequential approach would provide reliable information on immunity and avoid putting individuals at risk.

Several academic laboratories have developed robust, specific serological assays, and high-quality commercial options are becoming available. In accordance with academic grass-roots traditions, a toolkit to set up antibody assays has been distributed to more than 200 laboratories across the world, and a detailed protocol to facilitate local implementation has been published (12). With high-quality serological assays now available, the key challenge will be to apply and deploy these tests in a strategic manner to safely bring communities out of the current pandemic response back to the realm of “normal” life.

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ACKNOWLEDGMENTS

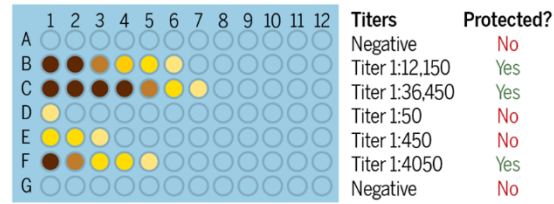
Thanks to colleagues and laboratory members who have worked nonstop to help control this pandemic. The authors are supported by the National Institute of Allergy and Infectious Disease (NIAID) Collaborative Influenza Vaccine Innovation Centers (CIVIC; 75N93019C00051), the NIAID Centers of Excellence for Influenza Research and Surveillance (CEIRS; HHSN272201400008C), institutional funding, and philanthropic donations. The SARS-CoV-2 reagents developed in our laboratories are freely available to the scientific community (www.beiresources.org/). Mount Sinai is in the process of commercial licensing of assays described here and has filed for patent protection.

Published online 15 May 2020
10.1126/science.abc1227

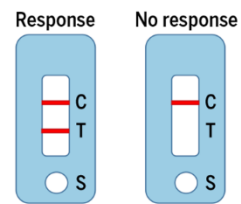
Quantitative and binary readouts in serology assays

Quantitative and binary serology tests can provide important information about infection.

Quantitative assays [e.g., enzyme-linked immunosorbent assay (ELISA)]



Assay with binary result (e.g., lateral flow assay)



| Result | Quantitative titer | Yes or no |
|---|--|---|
| Linked to protection? | A quantitative titer can be linked to protection | A positive result can be loosely associated with protection |
| Could predict protection duration? | Yes | No |
| Scalability | Moderate | High |
| Ease of use | Performed in specialized laboratories | Easy to use, even as point-of-care test |

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published online May 15, 2020

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