

Developing a National Strategy for Serology (Antibody Testing) in the United States



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I. Executive Summary

Serology (antibody) tests for the SARS-CoV-2 virus have the potential to inform good public health decision making during the pandemic. This report describes potential uses of the tests, areas of uncertainty where additional research is needed, and examples from other countries now beginning to make use of these tests. The priority for the United States now is to make validated, accurate tests available to: (1) public health authorities, to conduct surveillance and to estimate the numbers of people previously infected. Should antibody tests be determined to correlate with immunity to the disease, they should then be made available to: (2) essential workers, with priority for healthcare workers and those who interact with vulnerable populations (eg, nursing home residents); and (3) individuals who may use them to assess their personal risk of becoming infected with SARS-CoV-2 (COVID-19 disease). These tests will be in high demand, and manufacturing should be scaled appropriately, but the first steps will be to ensure accuracy, validity, and comparability of available tests.

Serology testing (or equivalently, serological testing) for COVID-19 may be used to identify whether people were previously infected by SARS-CoV-2. This is important to determine, because the polymerase chain reaction (PCR) and other rapid diagnostic tests now being used identify the presence of viral material, which is found only in people who are currently infected. Not everyone who has had the disease has had the opportunity to be tested before the virus was cleared from their bodies, and as many as 25% or more people are asymptomatic.^{1,2} Thus, it is now presumed that there is a significant population in the United States who likely have been infected with SARS-CoV-2, have recovered, and currently possess some degree of immunity. Because public health decision making depends in part on an understanding of the disease prevalence and the prevalence of likely immunity, extensive antibody/serology testing is needed to determine the true prevalence of SARS-CoV-2. Individuals want to be tested not only for their own peace of mind, but also because a positive result (ie, a history of having been infected and cleared the virus) may theoretically release them from the constraints of public health physical distancing measures. However, it is not known whether the presence of antibodies correlates with protection from disease.

While serology testing has the potential to provide valuable information to individuals and to public health authorities, there are significant areas of uncertainty that will need to be addressed in the coming weeks and months. The first and most urgent is serology test validation. There are dozens of serology tests being marketed in the United States that are not providing accurate information and that are not comparable to each other.³ Ensuring that tests are comparable and accurate requires a validation process with access to many patient samples, overseen by the Food and Drug Administration (FDA). While such a validation process is apparently under way, it is unclear when it will be completed. Second, while some degree of immunity to COVID-19 after recovery is assumed by most experts, determining whether there is a specific antibody level that correlates with immunity will require additional research. Third, even if protective immunity is successfully induced by infection, it is not clear how long that immunity

lasts. For SARS in 2003, antibodies were maintained in recovered patients for up to 2 years, but as the virus disappeared by mid-2004, protection from reinfection was never demonstrated.⁴

Because of the potential for relaxing physical distancing measures for those who have recovered, several governments and large employers are currently exploring the possible use of what has been termed “immunity certificates,” to release recovered people from physical distancing measures. Given the inaccuracies in available tests and the uncertainty about how the test results correlate to immunity, this is not a justifiable step at this time. In this report, we describe considerations surrounding the use of certificates, once accurate tests are available and validated and if they are correlated with immunity. A standard for COVID-19 immunity before a vaccine is available would need to be carefully constructed and monitored so as not to create perverse incentives. For instance, some people might imprudently try to get infected to evade physical distancing measures or to be hired by businesses that require immunity as a condition for employment. We address some of the ethical and legal implications of immunity certificates.

Serology tests will be an important tool for public health workers to estimate the prevalence of disease. These tests will be in high demand by individuals who hope to assess their risks of immunity to SARS-CoV-2. Serology testing, among other nonpharmaceutical interventions, can help to bridge the time before a vaccine is available. However, validated, accurate tests are currently in short supply. In this report, we seek to draw attention to the options for expanding access so that the potential benefits of serology tests can be realized as soon as practicable.

II. What Are Serology Tests and How Can They Be Used During COVID-19?

There are 2 principal types of testing used for infectious diseases: molecular and serological. *Molecular tests* detect the pathogen while it is circulating in the body, and they are used to diagnose and/or confirm cases for clinical treatment or surveillance purposes. Molecular tests may also detect fragments of the pathogen before it is fully cleared from the body, even if the pathogen is no longer able to replicate or cause disease. *Serological tests*—or “antibody tests”—on the other hand, detect evidence of the body’s immune response to an infection, which can provide information on both current and prior infection. Serological tests provide the capability to detect infections after the immune system has successfully eliminated the pathogen.

Currently, much of the SARS-CoV-2 testing worldwide is focused on molecular diagnostic tests. The diagnostic tests rely on a technique called reverse transcriptase-polymerase chain reaction (RT-PCR) to detect the presence of SARS-CoV-2 virus. These tests can provide qualitative results—that is, positive or negative—as well as quantitative information on the amount of circulating virus in a patient sample. Unfortunately, there are characteristic limitations to RT-PCR diagnostics when responding to a long-term outbreak: Since these tests detect the presence of the infecting agent, they cannot provide a diagnostic result for someone who was infected previously and has already cleared the infection. Serological tests, however, can answer this question and provide data critical to characterizing the scale of the pandemic, the severity of the disease, and, potentially, immune status after recovery.

What serology tests measure: antibody types IgM and IgG

Serological tests detect the presence of the body’s immune response to a pathogen. Antibodies, also known as immunoglobulins (Ig), are produced by B cells and are part of a highly specific defense against new antigens.⁵ Two classes of antibodies, immunoglobulin M (IgM) and immunoglobulin G (IgG), are common targets for serological tests because of their roles in targeting and destroying new infections. The immune system typically produces IgM soon after infection as a frontline defense, and IgG is generated later. Additionally, IgG persists in the body longer than IgM and contributes to longer-term immune memory, which enables the immune system to rapidly identify and respond to future infections by the same pathogen. IgA is another type of antibody, typically found in mucous membranes, that can be produced in high quantities during infections.

Antibodies are specific to components of each pathogen, and serological tests are used to identify the presence and/or quantity of antibodies that correspond to the pathogen of interest. Current evidence indicates that these antibodies begin to develop approximately 6 to 10 days after infection with SARS-CoV-2.⁶ IgM appears to peak approximately 12 days after SARS-CoV-2 infection and persists in sufficient quantities for as long as 35 days, after which the quantity declines rapidly.⁷ IgG has been observed

to peak approximately 17 days after SARS-CoV-2 infection and persist for at least 49 days (at which time the study was concluded).⁷ Further, IgG has been observed in patients 2 weeks after symptom onset.⁸ IgG has been found in patients up to 2 years after recovery from severe acute respiratory distress syndrome (SARS).⁴

Types of serology tests

There are 3 main types of serological tests. In the context of SARS-CoV-2, rapid diagnostic tests (RDTs) and enzyme-linked immunosorbent assays (ELISAs) have been designed to detect antibodies specific to the spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins of the SARS-CoV-2 virus. Tests that combine the number of targets, known as “multiplexing,” increase the specificity of the test. For example, 1 test could increase its specificity by looking for distinct antibodies that target the S protein and the N protein. Tests can also look for the presence of 1 or more types of antibody (IgG, IgM, or others). Diagrams demonstrating how these tests function can be found in [Appendix A](#).

- **Rapid diagnostic test (RDT)** relies on a lateral flow assay that returns qualitative (positive or negative) results within minutes. A small blood sample is placed at one end of the test strip, and the antibodies of interest in the blood sample interact with tagged proteins embedded in the test. The test displays colored lines at the end of the strip corresponding to a positive, negative, or inconclusive result with respect to the presence of the desired antibodies. RDTs are not capable of providing quantitative results indicating the *amount* of the antibodies in the specimen. They are small, portable, and can be used at point-of-care (POC). In the context of COVID-19, RDTs most frequently test for the presence of patient antibodies (IgM and IgG) specific to SARS-CoV-2.
- **Enzyme-linked immunosorbent assay (ELISA)** relies on specific binding of patient antibodies to a fixed viral protein of interest, often in a 96-well plate. ELISAs can return qualitative or quantitative results and are generally performed in a lab setting. These tests use whole blood, plasma, or serum samples. Patient samples are incubated with the viral protein of interest to allow antibody-protein binding. The resulting antibody-protein complexes are then exposed to a second antibody or a substrate that produces a color or fluorescent-based signal when bound to the complexes. The resulting signal reflects the presence and/or level of specific antibodies in the patient sample. In the context of COVID-19, ELISAs most frequently test for patient antibodies (IgM and IgG).
- **Neutralization assay** provides quantitative information on the ability of patient antibodies to confer protective immunity. Neutralization assays are the most time-consuming and skill-based of the 3 tests described. Using cell culture, live virus, and patient antibodies, researchers can visualize and quantify in a patient sample the level of antibodies capable of blocking viral replication. These tests require whole blood, serum, or plasma samples from the patient. Because these tests require live virus to challenge the antibodies, neutralization assays must be performed in the appropriate biosafety containment level (Biosafety Level 3, or BSL-3, or above) and require a week or longer to return results.

Type of Test	Time to Results	What It Tells Us	What It Can't Tell Us	Expertise Needed
Rapid diagnostic test (RDT)	10-30 minutes	The presence or absence (qualitative) of antibodies against the virus present in patient serum.	The amount of antibodies in the patient serum, or whether these antibodies are able to inhibit virus growth	Point-of-care testing, usually handheld, minimal training needed
Enzyme-linked immunosorbent assay (ELISA)	2-5 hours	The presence or absence (quantitative) of antibodies against the virus present in patient serum	Whether the antibodies are able to inhibit virus growth	Lab space generally required; some technical training required
Neutralization assay	3-5 days	The presence of active antibodies in patient serum that are able to inhibit virus growth ex vivo, in a cell culture system	May miss antibodies that are specific for viral proteins not involved in replication	Lab space required, at least BSL-3 if using live SARS-CoV-2; extensive training needed

What makes a meaningful test?

Serology tests, like other clinical tests, involve some degree of error. Understanding the degree to which error occurs and the effect on individual- and population-level results is critical to using serological test results to inform public health policy and operational decision making.

Measuring test accuracy and error rates

The accuracy of a serological test can be directly related to the mechanism of the test itself, or it can be influenced by epidemiologic conditions, such as expected or known disease prevalence in the population.⁹ Sensitivity and specificity refer to the accuracy of the test in terms of accurately identifying positive and negative results. The sensitivity of a serological test is the ability of the test to correctly return a positive result for a sample that has the antibodies in question. Specificity refers to the ability of the test to correctly return a negative result for a sample that does not have the antibodies. In general, there is an inverse relationship between sensitivity and specificity: If the threshold required to return a positive result is lowered in order to detect more positive specimens, the test might provide positive results for more negative samples (“false positives”). Conversely, if a test is designed to return as few false-negative results as possible, it might also miss some positive tests.

Positive predictive value (PPV) and negative predictive value (NPV) provide insight into how accurate the positive and negative test results are expected to be in a given population, by factoring in both test accuracy and the prevalence of the disease in the population. PPV is the percent of the positive tests in a given population that will be correctly identified, and, similarly, NPV is the percent of negative tests that will be correctly identified. Both of these values are dependent on the proportion of the test population that is positive. For example, if the same test were used in 2 different populations—Population A with low disease prevalence and Population B with high disease prevalence—then the PPV would be lower in Population A than in Population B, because the number of false positives would be a higher percentage of the total number of positive tests in Population A. Conversely, the NPV would be higher in Population A than in Population B.

If serology testing is going to be used to make policy decisions or to guide individual actions around patient health and safety, it will be critical that both sensitivity and specificity are as high as possible to avoid false-positive and false-negative results. In addition to specificity and sensitivity, which are both independent of the disease prevalence in the population, PPV and NPV need to be considered. In the context of serological testing, PPV refers to how likely an individual is to have detectable antibodies given a positive test result, and NPV represents the likelihood that an individual that receives a negative test result does not have detectable antibodies. PPV and NPV are particularly important if serology testing is going to be used to determine an individual's immune status, because these measurements depend on the prevalence of disease in the population. Different serological tests might be needed in different populations, depending on the expected prevalence of SARS-CoV-2 infection, if individual test results are used.

In a clinical context, serology is part of a wider evaluation of a patient's disease state. PPV will be particularly important if governments or businesses plan to rely on serology for more than research, since tests with low positive predictive value will indicate that people have antibodies when they don't. A large number of false positives will severely strain public health efforts in isolation and contact tracing by drawing attention and resources away from true positives.

Evaluation of the utility of serology tests requires understanding of their predictive value. There are 4 categories of test results: true positives, false positives, true negatives, and false negatives. Serological tests of varying sensitivity and specificity lead to these 4 outcomes, which have consequences for making policy and public health decisions. Currently, serology tests in development have a range of sensitivity (87% to 93%) and specificity (95% to 100%), and the first EUA-approved RDT (Cellex) has a sensitivity of 93.8% and a specificity of 95.6%.

For example, consider a population of 1 million people (see table below). If we assume that 15% have been infected with SARS-CoV-2, there would be 150,000 infected individuals and 850,000 uninfected individuals.¹⁰ In this scenario, a serological test is

administered to everyone in the population. The test has a sensitivity of 95%—meaning the test will accurately provide a positive result for 95% of the infected individuals—and a specificity of 95%—meaning that the test will accurately provide a negative result for 95% of the uninfected individuals. In this example, we further assume that the presence of antibodies means that an individual is immune to SARS-CoV-2 infection.

Based on these conditions, 185,000 individuals will test positive; 142,500 will be true positives, and 42,500 (23%) of those will be false positives. These 42,500 individuals will receive a positive test result but are still susceptible to SARS-CoV-2 infection. Thus of 185,000 people who believe they are protected, 23% remain vulnerable.

Additionally, 815,000 individuals will receive negative test results, but 7,500 of those will be false negatives; these are individuals who are thought not to have antibodies but who may have actually already been infected and cleared the disease. In total, while the overall accuracy is high (95%), and 95% of the total population will receive accurate results, both positive and negative, functionally of those testing positive and presumed immune, 23% are still susceptible—almost 1 in 4. Only 77% of the positive results will be accurate—the positive predictive value of the test. Understanding the limitations of the test is critical to using it as a tool to make policy or operational decisions.

In summary, the consequences of a testing error—a false positive or a false negative—are not equivalent. A false negative may prevent an individual from returning to work; a false positive might lead to an epidemic chain.

Population	1 million				
15% Infected	150,000				
Sensitivity	95%				
Specificity	95%				
				Total Infected	150,000
				Total Positive Tests	185,000
				False Positives	42,500
				Percent of Positive Tests that Were Inaccurate	22.97%
				False Negatives	7,500
				Positive Predictive Values	77.03%

	Infected	Not Infected	
Seropositive	142,500	42,500	185,000
Seronegative	7,500	807,500	815,000
	150,000	850,000	

Validation and oversight

Validation of serological tests is critical to ensuring that the tests perform as they are intended. Lack of validation has led to a patchwork of false positives and false negatives across the country, interfering with estimates of seroprevalence.³ Imprecise information can affect decision making, such as relaxation of physical distancing and other public health measures. Validation will help in comparing tests.

On April 4, 2020, the Assistant Secretary for Health, ADM Brett Giroir, commented that validation efforts on antibody tests are being undertaken jointly by the FDA, the National Institutes of Health (NIH), and the Centers for Disease Control and Prevention (CDC).¹¹ The National Cancer Institute (NCI, within the NIH) will convert part of the activities of its Vaccine, Immunity and Cancer Program to validate serology tests, among other COVID-19 research initiatives.¹² NCI's planned validation activities will include comparing RDT results to neutralization assays to look for correlation between seroconversion and protective immunity. NCI will also use sera samples collected pre-COVID to determine the rate and risk of false positives due to preexisting exposure to related coronaviruses. The timeline for results is unknown.

SARS-CoV-2 unknowns that affect serology testing

There remain significant gaps in our understanding of SARS-CoV-2 and immunity, which affects the interpretation of serological test results. The presence of antibodies is not necessarily equivalent to protection from SARS-CoV-2. If antibodies are present, it will be important to understand if they are functional and at sufficient levels to be effective. The following are areas where further research is needed in order to accurately interpret results of serology tests:

- 1. Correlates of immunity:** It remains unclear if antibodies detected by serological tests are virus-neutralizing and what this means for protection from reinfection. We also do not know the amount of antibody needed for protection, or how important other parts of the immune system are, such as cell-mediated immunity (T cells) for protection against COVID-19.
- 2. Length of immunity after infection:** It is not known how long antibodies last in patients recovered from COVID-19, and how long those persisting antibodies remain effective. It is not known if SARS-CoV-2 infection elicits immune memory, which provides long-term protection.
- 3. Cross-reactivity of patient antibodies:** The COVID-19 serology tests are meant only to detect patient antibodies specific to SARS-CoV-2, but there are many circulating coronaviruses to which patients may have preexisting antibodies. It remains unclear if patients who have had other coronavirus infections could have antibodies that test positive on COVID-19 serology tests, and which parts of the SARS-CoV-2 virus should be used in serology tests to ensure that the patient antibodies detected are specific to that virus.

This report includes a review of current serology research on SARS-CoV-2, as well as past coronavirus epidemics. Further detail on research gaps and suggested future studies are found in [section VII](#).

III. How Serology Is Currently Being Implemented for COVID-19 in the US and Globally

Germany initiated one of the first large-scale uses of serological testing for SARS-CoV-2, a study led by researchers from the Helmholtz Centre for Infection Research. German officials reportedly intended to use the data from these tests to distribute “immunity certificates,” which would allow individuals with positive serology tests (ie, those who previously have been infected) to resume normal activities. Additionally, data gathered from the tests would inform decisions about reopening schools, resuming mass gatherings, and relaxing other physical distancing measures.¹³ Researchers in Germany published preliminary results from 1 city, based on specimens provided by 500 residents.¹³ The study found that approximately 14% of the population had positive serological test results, compared to 2% of the population that had been diagnosed with COVID-19 using molecular diagnostic tests.¹⁰

As part of a 5-pillar plan to combat COVID-19, the **United Kingdom** included the planned development and implementation of widespread serological testing.¹⁴ Along with this announcement, reports emerged that the UK government aimed to use a system of “immunity passports” to allow the public to begin returning to work and other activities.¹⁵ The UK’s Secretary of State for Health and Social Care announced that the UK had ordered 3.5 million serological tests to support the response;¹⁶ however, these tests have yet to materialize, as effective and reliable tests are not yet available on a large scale.¹⁷ Elected and health officials in multiple other countries around the world—including **France, Italy, and the United States**—have also called for “immunity passport” programs or suggested that they were under consideration, but no programs have been formally announced or implemented.¹⁷

Singapore health officials used serological tests to identify epidemiologic links between cases that were not identified through traditional means. Based on contact tracing efforts, the officials identified 2 individuals who may have been the source of transmission for a cluster of 7 COVID-19 cases. The individuals had previously been ill but were not confirmed cases, and both were well at the time they were interviewed. The 2 individuals were tested using a serological test developed by the Duke-National University of Singapore Medical School, and the positive test results confirmed that both had been previously infected with SARS-CoV-2. These results provided the necessary information for health officials to determine that they were the likely source for the subsequent cluster.¹⁸

In the **United States**, a number of efforts are under way to initiate serological testing. For example, the Beaumont Health system, the largest in Michigan and principally operating in the **Detroit** area, announced that it will commence the country’s largest serological testing study among its employees. The study is voluntary and aims to characterize the scope of the local epidemic among its healthcare personnel and “relieve anxiety” about potential exposure. The study could also identify candidates for donating

convalescent serum, prioritize individuals for future vaccination, and help healthcare workers return to work sooner.¹⁹ The University of Southern California Price School of Public Policy partnered with the **Los Angeles** Department of Public Health to conduct a serological survey of 1,000 individuals in the LA area. The initial round of testing, conducted at drive-through testing sites, is reportedly complete, and preliminary results are expected in mid-April.^{20,21} The **US CDC** announced that it will roll out a 3-part serological testing strategy, focusing initially on priority hot spots and then expanding to broader geographic areas and, finally, specific priority populations like healthcare workers. The initial stage is currently under way, and subsequent stages will follow in the coming months.²²

Who is regulating serology tests?

The FDA is responsible for approval and regulation of serology tests available in the United States. Emergency Use Authorization (EUA) indicates that a test may be used for diagnostic purposes, after extensive FDA review of the serology test. Emergency Use Authorization is the FDA's method of approving serology tests for use in diagnostics—until that approval is received, a serology test should be used only for research. The FDA recently released their *Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency*, last updated March 16.²³ While there are several tests approved for diagnostic use outside the United States and 3 currently with EUA status in the US, all other serology tests available in the United States are currently for research use only (subsections IV.A-IV.D). This means that they cannot be used to diagnose a patient in a healthcare setting. The FDA has stated in this policy that it will not prevent development of novel commercial tests, but that these tests cannot be used as a diagnostic tool (they are only for research use: surveillance, epidemiology studies, etc). This is the FDA's position on all COVID-19 serology tests in development. This new policy was adopted to encourage development of COVID-19 serology tests while promoting transparency of companies' progress through their reporting of results to the FDA.

Section IV.A indicates a test is being developed by labs certified by the Clinical Laboratory Improvement Amendments (CLIA) and that the manufacturers are currently applying for EUA. While the kit may be used in these CLIA-approved labs, they have not yet been validated by the FDA. Initial testing results must be confirmed by an existing EUA test.

Section IV.B covers states that have been approved for diagnostic testing and are responsible for reporting results. The state does not have to submit an EUA request, but it must “take responsibility for COVID-19 testing by laboratories in its State during the COVID-19 outbreak.”

Section IV.C indicates a test has not yet been validated or approved by the FDA, but manufacturers are planning to prepare an EUA application. The test has been validated by the manufacturer and results communicated to the FDA. The tests may be distributed

to clinical labs and healthcare workers for point-of-care use, but results from this use must be continually posted and updated on the manufacturer’s website.

Section IV.D indicates a test is for research use only, and the manufacturers have not indicated that they will prepare an EUA application. They must include specific language in their product documentation, including that the FDA has not reviewed the test and that “results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.”

As these subsections allow for development of commercial serology tests without FDA approval, many serology tests that have been described in the news are for research use only. Only tests with explicit FDA EUA approval may be used for diagnosis.²⁴ It is important for tests in development to be widely used in research settings, generating data that can be used for validation. The data can then be presented in an EUA application to the FDA, subject to their review. Several serological studies initiated in the United States are reportedly using tests manufactured in China, but they are not approved for use by the US FDA or China’s Center for Medical Device Evaluation.²⁵ China has banned the export of the tests, but 2 US companies are distributing them—presumably they were purchased prior to the export restrictions being implemented. The Association of Public Health Laboratories (APHL) has reportedly expressed concern about the reliability of unlicensed serological tests being sold in the United States.²⁶ Multiple federal agencies, including the FDA, the CDC, the Biomedical Advanced Research and Development Authority (BARDA), and the NIH, are evaluating the tests’ performance; however, current FDA policy does not prohibit the sale of these products.

IV. Serology as One of Many Public Health Tools

Serological testing has been hailed as essential for decisions to relax physical distancing measures and reopen businesses. Yet, other public health tools should be also considered in making decisions about reopening businesses, including engineering controls (eg, barriers between individuals, ventilation in work spaces), frequency of disinfection, number of contacts in the facility, intensity of exposure between contacts, and up-to-date information on the local or regional trend in cases. Serology tests will not be perfect and may not be available to everyone in the immediate future. Thus, these other considerations in conjunction with serology will be important for keeping people safe and limiting transmission.

Using serology to inform regional physical distancing

Serology could be useful for making policy decisions at the regional level. However, there is uncertainty in its potential to help individuals understand their personal risk. Serology studies will be an important component of the overall public health response, providing critical information on the incidence of SARS-CoV-2 infection in the population. These data will permit more accurate measurements of the case fatality ratio and other parameters, improve modeling efforts, and help epidemiologists better understand disease transmission. Understanding who has previously been infected may help with contact tracing efforts by filling in gaps that questionnaires alone cannot fill. In this sense, with caveats about false-negative test results and the current lack of evidence for long-lasting immunity, serology will be helpful for understanding the breadth of spread of SARS-CoV-2 in communities.

Limitations of serology as a “one size fits all” tool

For a variety of reasons, serology cannot be used alone in planning to reopen businesses or ending shelter-in-place orders. First, it is not clear that infection by SARS-CoV-2 will result in long-term immunity. If people who have previously had COVID-19 are not immune to reinfection for more than a few months, if at all, then it will not matter if a serology test is performed from the standpoint of individual risk. In that case, employees and individuals will still be susceptible to disease after a period of time. Serology tests provide information about antibodies at the time of testing. They cannot say how long those antibodies will last or indicate titers of antibodies in the future. Furthermore, some individuals may possess more protective immunity than others. For example, one individual’s immunity might not be as strong or long lasting as that of another person. This kind of variation must be taken into consideration before serology can be used in decision making.

If serology is going to be used as part of a reopening strategy for COVID-19, and it is determined that the presence of antibodies correlates with immunity, there will be a high demand for tests, as was seen with molecular diagnostic tests. However, there will likely be delays in expanding serological tests across the country. The development of a reliable test requires validation, followed by the scaling up of manufacturing and

distribution. Healthcare workers (including staff at long-term care facilities) should be prioritized, followed by essential public-facing jobs, including food service workers, delivery drivers, and grocery or pharmacy staff. Nonessential workers who cannot work from home may be prioritized over nonessential workers who can work from home. Finally, serology testing will likely need to be repeated for each individual; an individual may test negative when first exposed to the virus but then develop antibodies after the initial test.

Consideration must be given to vulnerable and underserved populations. People in these groups may have less access to testing sites or may be unable to afford transportation fees. As with rapid diagnostic testing for COVID-19, validated serology testing for COVID-19 should be free if it is reported to a national database, and it would theoretically be free if done as part of a research study. Once decisions are made to actively promote a certain type(s) of serology tests, strategies for extensive, equitable testing could include going into a community to provide testing rather than requiring people to travel to a testing site; language interpreters should be available when testing and providing results. Importantly, special efforts should be made to ensure the privacy of test results for these populations. Privacy of test results and personal information will be particularly important when expanding testing to communities with high proportions of undocumented workers.

Considerations for an “immunity certificate”

There have been calls in some countries to develop “immunity certificate” programs. These certificates would be given to individuals who had a positive serology test to allow them to go back to work or reenter society. The lack of accurate, validated, available tests makes this option premature, but even after tests become available, there are significant technical, logistical, and ethical challenges with creating such a certificate that need to be addressed.

There are several research questions that must be addressed before immunity certificates are issued. Technical challenges for immunity certificates include ensuring the test used to create such a certificate has high negative predictive value and high specificity to ensure certificates are not given out to people who do not have antibodies, which could lead to a false assurance of individual health and safety. High numbers of false positives with immunity certificates would have the potential to increase epidemic risks and, at the population research level, lead to false survey information. A serology test with 100% accuracy would likely be required for the equitable and precise use of immune status to determine whether an individual can return to work. Even more important, it is still not known if having COVID-19 leads to immunity and how long this immunity lasts. If having COVID-19 does not lead to immunity, or immunity is not long lasting, then there is no reason to have immunity certificates.

The example described previously in which we evaluated the positive predictive value (PPV) of a hypothetical serology test illustrates the considerable challenge of using

such a test to determine eligibility to return to work or other community activities. In that example, 15% of a population of 1 million people were infected with SARS-CoV-2 at some point. Using a serological test with 95% sensitivity and 95% specificity for the entire population, 185,000 people would receive positive results and, therefore, an immunity certificate. Notably, however, 42,500 (22.97%) of those individuals were false positives, meaning that nearly a quarter of those with immunity certificates would not carry detectable antibodies to SARS-CoV-2 and would be, therefore, not actually immune to SARS-CoV-2 infection (assuming detectable antibodies correlate with immunity). Additionally, 7,500 individuals would receive false-negative results, rendering them unable to resume their normal activities, despite being immune to the virus. Allowing susceptible individuals to return to normal activity could facilitate a resurgence of SARS-CoV-2 transmission in the community, including to those individuals still maintaining physical distancing (eg, in the same household). Further, the transmission could potentially be exacerbated by a false sense of security afforded by the positive test results and immunity certificate, both among those with false-positive results and their susceptible contacts.

Immunity certificates would rely on serology testing, which would be limited by the number of tests available. It will take time to manufacture a sufficient supply of tests, and each person may need more than 1 test, as they could be negative at one time but positive a week later. Tests would need to be prioritized. If more than one serology test is given approval, each test would need to be validated and compared to all others to make sure the test being used does not disproportionately affect individuals' chances of being allowed to return to work compared to individuals using a different test (and perhaps receiving different results). Until it is known how long immunity lasts, people would need to be retested at regular intervals to ensure they continue to have immunity. There would need to be a registry of people who have been given the immunity certificate, which would need to be managed by an organization capable of handling that data securely and without conflicts of interest. Patient confidentiality and privacy would be especially important considerations for any test. Special consideration would need to be given for vulnerable populations, many of whom already distrust the government, to ensure equitable access to testing across socioeconomic and racial groups.

There are ethical and legal challenges associated with using immunity certificates to allow people to return to work or leave their homes for nonessential trips. Before an immunity certificate strategy is adopted, multiple ethical and legal challenges must be carefully evaluated. Pressures to return to work would include the need for continued pay as well as pressure from workplace leadership; either driver might induce people to willfully expose themselves or their families to infection. A "black market" for immunity cards might develop. There is the risk of blocking employment to or rejecting those who are not immune. The US Equal Employment Opportunity Commission pandemic plan does not mention this contingency, but employers have considerable leeway for medical screening in a pandemic.²⁷

Historically, the notion of immunity certificates has led to discrimination and inequity. During the nineteenth century in the Deep South of the United States, those who recovered from yellow fever gained access to employment and housing, while the “unacclimated” were denied employment and entrance to society.²⁸ These restrictions led to the fearsome practice of gathering in small houses to exchange the virus among groups, a practice that has been unfortunately mirrored in the current pandemic.²⁹

More recently, during the 2014-2016 West Africa Ebola epidemic, Liberia provided immunity certificates as part of its community reintegration program for Ebola survivors.³⁰ Presenting survivors with a Certificate of Medical Clearance (immunity certificate) was an integral component of the reintegration program to ensure Ebola survivors were welcomed back into their communities. The immunity certificate was part of a package a survivor received after leaving an Ebola treatment unit and an essential component of the education campaign conducted in the community to mitigate fear and stigmatization around returning survivors. While primarily used as an educational and reintegration tool for affected communities, certificates of medical clearance were also required by some countries for travelers returning from Ebola-affected countries.³¹ However, it was unclear whether medical clearance certificates for travel were based on serological assays or on other diagnostic and screening tests for Ebola. It is also important to note that there are many decades of research on Ebola infection–induced immunity, as compared to the few months of research on SARS-CoV-2, and additional data and analysis are required to better characterize SARS-CoV-2, serological tests, and immunity. Ebola survivors could be reasonably well assured that their recovery, and subsequent development of antibodies, did protect them against reinfection. Such assurances are currently not possible for survivors of COVID-19.

V. Resource Needs for a Robust US Serology Strategy

In order to ensure the robust use of serology for public health, there are 3 key resource questions that must be answered: Are there enough tests? Are there enough people to run the tests? Is there space to run them? There is a limited supply of tests approved for diagnostic use. Currently, only 4 serology tests have been approved for an Emergency Use Authorization in the United States, including an RDT from Cellex and an ELISA from Mount Sinai Laboratory. There are approximately 20 tests developed by companies and academic labs in the United States that are either approved for research use only or are being developed in preclinical settings.³² There are also more than 35 tests available worldwide, with 6 approved for diagnostic use in countries outside the United States. In the FDA's latest *Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency*, they have explicitly stated that they will not prohibit or prevent companies from developing in vitro diagnostic tests.²³ However, many of these tests are not in the pipeline for an EUA and should not be marketed as "FDA-approved." The CDC also is preparing serology-based testing that is now in clinical trials.²²

Based on available data and press releases, companies may be prepared to produce millions of kits, but it will be essential to validate approved tests, ensure proper packaging and storage of kits, and distribute the tests to state and local testing facilities, including clinics and hospitals.³³ RDTs require far fewer resources and less training for interpretation than ELISAs, and this should be considered when planning personnel and equipment needs at testing sites. Kits involving RDTs are likely more suited for screening efforts, including "drive-through" testing sites. ELISAs provide more quantitative information regarding antibody titers, although these typically require lab space and properly trained personnel to perform the tests and interpret the results. BARDA has provided funding to molecular diagnostics for COVID-19 and could also use funding to support serology test development.³⁴

Scaling up and implementing serological testing

Tests are in high demand by public health authorities and by individuals, but it is important that the tests first be validated for accuracy, and for individuals, that they provide meaningful information about immunity, which is not yet known. Availability of validated serological tests will need to increase significantly in the coming months, with priority given to health departments to do seroprevalence studies and then to individuals. The priority for the United States now is to make validated, accurate tests available to (1) public health authorities to conduct surveillance and to estimate the population of previously infected individuals. Should the presence of antibodies correlate with protection of the virus, they should be made available to (2) essential workers, with priority given to healthcare workers and those who interact with vulnerable populations (eg, nursing home residents); and (3) individuals who may use them to assess their personal risk of contracting SARS-CoV-2 disease. These tests will be in high demand and manufacturing should be scaled appropriately, but the first steps will be to ensure accuracy, validity, and comparability of available tests. Federal and

state governments should look for ways to incentivize manufacturers to increase their capacity and to validate the tests.

The efficiency of implementation and availability of testing will depend on the methodology of the test—that is, point-of-care or laboratory-based. To facilitate population-scale testing, tests that can be run using fingerstick blood specimens, such as RDTs, would be top choices. If fingerstick tests are validated and deemed appropriate, current set-ups for drive-through testing could be converted for these purposes.

The involvement of a large and representative sample of the US population will be necessary to inform policy decisions as the COVID-19 pandemic progresses. There are challenges with ensuring that a representative portion of the population is included in serological surveillance studies. Obstacles include aversion to needles or blood draws and limited access to testing sites. Efforts must be made by the appropriate authorities working with community stakeholders to distribute testing equitably. The involvement of community leaders will facilitate community buy-in, an especially important factor in areas where underserved people may be less willing to participate in a government-led screening campaign.

VI. Actions for Leaders

National level

Coordinate validation and availability of serology tests. Serology tests are in high demand by public health authorities. The priority should be to provide accurate, validated tests. Therefore, the federal government should continue to validate these tests.³⁵ The government should also coordinate with industry to ensure appropriate scale-up of manufacturing and distribution of tests. A task force should be created to ensure equitable access to and use of serology testing.

Collect data on serological surveillance. The primary purpose of serological studies should be to determine seroprevalence, and these efforts should be initiated by every state health department as well as major city health departments. The federal government, through CDC, should collect data from serological surveillance studies conducted by state and local public health officials, as well as from studies conducted by researchers, as quickly as possible and at close regular intervals to assess how quickly the population has become exposed to SARs-COV2. This information should be made publicly available on the CDC website. Protections will be needed to ensure that data are collected for appropriate purposes, including epidemiologic studies, and that data collection and use comply with applicable regulations and FDA guidance.

Determine whether antibodies correlate with protection. Individuals would like to use these tests to assess personal risk from COVID-19. However, it is unknown whether the presence of antibodies to SARS-CoV-2 correlates with immune protection. Without knowing whether the presence or the level of antibodies indicates reduced risk of infection or transmission, a serological test to determine the presence of antibodies would not be meaningful.

Create testing prioritization guidelines for individuals, if testing is meaningful. Extensive serology testing may lead to a shortage of tests. A prioritization strategy for using a limited number of serology tests should be to provide tests first to public health authorities to do seroprevalence studies, and, if positive tests correlate to immunity, tests should then be given to individuals deemed essential employees who may be at greater risk of infection because of their increased interactions with others in their communities. Essential employees constitute 30% of the workforce, so priority should be given to healthcare workers and others who work with vulnerable populations (eg, in nursing homes).

Provide clear guidance on appropriate uses and interpretation of serology tests. Because of underlying complexities and gaps in the immunological understanding of SAR-CoV-2 infection, misinterpretation and misapplication of serology tests must be monitored as their use becomes widespread. The federal government should clearly and accurately communicate the risks, benefits, use-cases, and interpretations of emerging evidence from serological surveillance studies. The FDA currently monitors advertising claims made by companies with tests on the market.

Create and fund a research strategy. The federal government should develop a strategic plan for COVID-19 and SARS-CoV-2 serology and immunity research, so that results from tests can be appropriately considered over time. Questions related to serology to be addressed include the rate of false positives of individual tests, antibody neutralization capacity, the possibility of reinfection, and duration of immunity. These questions are under study by various laboratories and groups, but there is a lack of coordination and national leadership in directing the research. The research strategy should include longitudinal studies to follow COVID-19 survivors and people who are seropositive but never develop disease. Longitudinal seroprevalence studies will also be important for understanding the impact COVID-19 will have on the population and on loosening physical distancing measures.

State, territorial, tribal, and local levels

Plan, conduct, and oversee public and private testing. State and local governments should work with healthcare providers, hospitals, and academic institutions to conduct population-wide testing when possible. Should antibody tests correlate with immunity, then the order of testing of individuals should follow the current CDC guidelines for molecular testing, with priority given to healthcare workers, first responders, and individuals in contact with infected individuals.³⁶ As private testing initiatives become available, the state should oversee that all appropriate health, privacy, and public safety regulations are followed.

Use serology to inform ongoing contact tracing. In collaboration with academic and public health institutions, testing of patients with COVID-19 infections could provide valuable insight into antibody persistence over time. If patients test positive, healthcare professionals could collaborate with public health researchers to provide follow-up serology testing. Follow-up serology testing could also alert a healthcare provider if a patient's antibodies drop below detection limits, which could indicate that the patient's immunity is waning. Importantly, these records should not be publicly available, to prevent discrimination (in the workplace or community) against people who do not have or are losing immunity.

Ensure that serology surveillance is available to vulnerable and underserved populations. The CDC has already released guidance for community leaders on preventing spread of COVID-19.³⁷ The pandemic has had a severe impact on underserved and vulnerable populations, but more extensive testing has been detected in more affluent communities.^{38,39} Testing logistics and obstacles must be carefully and fully addressed early on in local responses, so that trust is maintained in public health leaders. Local health departments should also actively coordinate with the Indian Health Service (IHS) to ensure that indigenous communities are receiving adequate resources and testing. These efforts could also further inform public health communication that is effective and sensitive to the groups being tested. Engaging trusted community leaders can help assuage concerns over testing invasiveness, while also educating public health leaders on priorities for certain communities (eg, resource limitations, important social events).

VII. A Research Agenda to Close Gaps in Immunity Knowledge

A coordinated research agenda is needed to supplement the current limited understanding of immunity to SARS-CoV-2. Research is already under way in critical areas, but developments in these areas must inform parallel efforts in seroprevalence surveys and diagnostic test validation. Preliminary studies find that COVID-19 patients develop antibodies as soon as 5 days post-infection and that antibodies may persist at least 49 days post-symptoms. In laboratory settings, antibodies from COVID-19 patients were found to neutralize SARS-CoV-2 virus. However, our understanding of how long these antibodies last, and whether they are truly protective, is not complete. Past coronavirus outbreaks suggest that neutralizing antibodies are generated following infection and that antibodies and other immune components are important to maintaining immunity. Duration of immunity and levels of antibodies needed for protection are unknown. Further research must also address the potential for antibody-dependent enhancement (ADE), a condition that occurs when low levels or non-neutralizing antibodies can worsen disease upon reinfection, as well as the thresholds required for herd immunity to COVID-19. Major scientific knowledge gaps are in 3 areas:

1. the temporal dynamics of antibodies to SARS-CoV-2 in patients
2. the protective immunity provided by antibodies
3. the threshold of antibody titers required for protective immunity

How long do antibodies to SARS-Cov-2 last?

Not all infections provide long-lasting, specific immunity. Measles and chickenpox infections produce decades-long immunity. Malaria and HIV trigger an immune response at the time of infection, but the antibodies that result are ineffective, or the infection does not induce sufficient immune memory. Influenza immunity lies between these; antibodies are produced but the predominant strain of flu is different from year to year, so the immunity from previous strains is not useful.

SARS-CoV-2 is a novel pathogen, so there are limited data on the duration of immunity. There are 2 main considerations for antibody dynamics in patients: the levels of antibodies (titers) produced over time, and the level of immune memory that is produced. Antibody titers change rapidly over the course of infection and afterwards.

SARS-CoV-2 antibody dynamics are incompletely understood. Studies to characterize antibody levels over time take significant time and require following patients after recovery from infection for weeks to months. Consequently, there are very limited data on COVID-19. In 34 patients in China, researchers characterized antibody levels (IgM and IgG) to the virus over the course of 7 weeks.⁴⁰ In a single patient examined 36 days post-symptom onset, IgM and IgG antibodies were detected, with higher levels of IgG1. IgG1 is the most abundant of the IgG subclasses, and these antibodies are commonly

produced by immune cells that encounter foreign proteins, like the spike protein of SARS-CoV-2.⁴¹

Another study of 23 patients demonstrated that in both severe and mild COVID-19 cases, IgM and IgG antibodies were produced against the virus nucleocapsid protein and the receptor binding domain of the spike protein.⁸ These peak around day 10 and persist through day 25 post-symptom onset. In this cohort, IgM peaked after IgG in the majority (53%) of cases.

A further study demonstrated that a group of 65 patients first developed IgM antibodies to the nucleocapsid protein early on, peaking at day 12 and dropping below 50% positivity by day 35.⁷ IgG antibodies developed later, peaking at day 17 but persisting in at least 80% of patients by day 49. However, this study lost patients to follow up over time, ending with only 5 patients examined by day 49.

A clinical trial sponsored by Indiana University is now attempting to address antibody prevalence in healthcare workers using a new SARS-CoV-2 IgG antibody test.⁴² This trial is an effort to identify individuals eligible for convalescent plasma prophylaxis, although it is not yet recruiting and is set to begin in November 2020. Another clinical trial, sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), is attempting to characterize prevalence of individuals with antibodies to SARS-CoV-2 who have not experienced symptoms.⁴³ This trial is cross-sectional and will not examine the prevalence of antibodies over time. None of these studies examined the neutralizing ability of these patient antibodies.

Further studies of antibody persistence in COVID-19 are necessary. These studies should examine antibodies to the nucleocapsid protein, as well as the spike protein. Research suggests that the full-length spike protein provides more sensitivity in serology tests than the receptor-binding domain alone.⁴¹ The spike protein is important to include in these tests because virus-neutralizing antibodies have been shown to bind to this protein.⁴²

How long do patient antibodies last in other coronavirus diseases, like SARS or MERS?

Past coronavirus infections can inform hypotheses about antibody dynamics in COVID-19, but SARS-CoV-2–specific studies must be performed in order to answer these questions in the current context. Previous studies of coronavirus 229E, one of the commonly circulating coronaviruses in humans that does not normally cause severe human illness, showed that 1 year post-infection, volunteers had raised levels of nasal IgA antibodies to the coronavirus.⁴⁴ Serum IgA levels were not raised. In other infections, such as influenza, nasal IgA can be sufficient to protect patients from infection—even when serum IgA is low. This suggests that measuring nasal IgA to SARS-CoV-2 could be important in understanding immunity to the virus. Serum IgG levels were slightly raised relative to controls after 1 year. When re-challenged at 1 year with the exact same virus, 67% (6/9) became reinfected.

In more recent studies of patients infected with SARS-CoV, 89.6% of patients infected maintained IgG antibodies for 2 years.⁴ Antibodies to the virus peaked after 3 to 4 weeks. This study did not examine whether these antibodies were protective. In MERS, a group of 34 patients were shown to produce antibodies after 2 to 3 weeks post-symptom onset. However, these IgG and neutralizing antibody loads were not correlated with viral clearance.⁴⁵

Recent work in SARS-CoV-2 showed that in a rhesus macaque model, 4 monkeys that were infected mounted immune responses that were persistent through 28 days post-infection. When 2 monkeys were rechallenged at 28 days post-infection, no viral replication was found by nasopharyngeal swab or by autopsy.⁴⁶ Given conflicting results from other coronavirus diseases, it is unclear how long antibodies would persist following SARS-CoV-2 infection.

Do survivors of COVID-19 have long-lasting immune responses to the virus?

There are currently no study results examining immune memory and SARS-CoV-2, primarily because the virus has not been circulating long enough to investigate this. It is unclear whether the immune response during COVID-19 induces memory in the adaptive immune response (antibodies, B cells, T cells, etc). Immune memory refers to a preexisting pool of immune cells that recognize and are specialized for a pathogen, so that upon reinfection, the immune response is rapid and effective.⁴⁷ Studies in animal models and patients who have recovered from COVID-19 and are reinfected are necessary to understand immune memory. Past coronavirus epidemics have shown that immune memory is induced and protective. In SARS survivors, researchers found memory T cells that responded to the membrane protein of SARS-CoV 1 year after infection.⁴⁸ In mice, memory T cells responsive to the nucleocapsid protein were able to induce protective immune responses to both SARS and MERS infections.⁴⁹ Studies such as these will be useful in future studies of SARS-CoV-2 in understanding immune memory. If the immune system has sufficient memory to respond to and control reinfection by SARS-CoV-2, this could link the presence of antibodies in patient serum to immunity over longer periods of time.

Moving forward, studies must address the longitudinal dynamics of antibodies in patients with COVID-19. For serology test results to be meaningful, researchers must demonstrate how long antibodies persist after infection in both mild and severe cases of COVID-19. It is currently unclear how long antibodies to SARS-CoV-2 remain in the serum, although preliminary studies suggest it could be 49 days or longer. If possible, current and future trials examining patients with COVID-19 should seek to isolate serum at multiple time points over the course of disease and after recovery for as long as feasible. Patients should be stratified by age, sex, and severity of disease, among other factors. ELISAs that detect antibodies to the N and S proteins, at minimum, could be used to quantify antibody titers in the serum over time. If possible, antibodies should be isolated and examined for neutralization assays against viruses in lab culture. These assays will inform antibody dynamics against SARS-CoV-2 over infection and

recovery and can provide context to serological test results. As time progresses and patients recover, studies of immune cells isolated from the patients will be essential in understanding immune memory. Although animal models are limited at this time, trials involving multiple challenges of the animals with SARS-CoV-2 can provide data on immune memory in COVID-19.

Does the presence of antibodies indicate that someone cannot be reinfected by SARS-CoV-2?

The presence of antibodies alone does not necessarily translate to immunity, as not all antibodies are neutralizing against the virus. Protective immunity refers to having an immune response that can clear infections of SARS-CoV-2. Serology tests can provide a qualitative (yes/no) or quantitative (titers) readout of antibodies to a specific part of the virus. However, the ability of antibodies to prevent viral replication and clear infection is determined through neutralization assays. These are essential to understand to which part of the virus the effective antibodies are targeted; those specific parts can then be used as “bait” in diagnostics. Consequently, a positive result could indicate that the person has protective, neutralizing antibodies. To date, it appears that truly neutralizing antibodies are specific to parts of the spike (S) protein and nucleocapsid (N) protein in SARS-CoV-2.^{6,50} Ultimately, neutralization assays are the gold standard for determining if a patient has effective antibodies and protective immunity against SARS-CoV-2. Researchers are beginning to understand neutralizing antibodies in the context of COVID-19, but significant work is still needed. A recent study of 175 Chinese patients examined neutralizing antibodies to the receptor binding domain, spike protein subunit S1, and spike protein subunit S2 of the spike protein in patients with mild COVID-19.⁵¹ In an immune response, several different antibodies may bind to the same protein but different regions (epitopes). Certain epitopes can be more essential to the viral infection cycle than others; if an antibody binds the right epitope, they can neutralize the virus by stopping viral entry to the cell. Other epitopes may bind antibodies, but the binding is not enough to impede the virus from entering cells. Understanding which epitopes are most important for SARS-CoV-2 immunity can help inform development of diagnostics and therapeutics.

One study found that older patients had higher titers of neutralizing antibodies despite lower levels of immune cells (lymphocytes).⁵¹ They also found that patient antibodies did cross-bind to SARS-CoV in ELISAs, but that these antibodies were not neutralizing.⁵² However, this study did not perform neutralization assays on live SARS-CoV-2, but on a modified virus, called a pseudovirus. A pseudovirus is essentially a virus that researchers can work with in lower biosafety levels—in this case, vesicular stomatitis virus—that has essential pieces of the SARS-CoV-2 virus such as the envelope and spike proteins. This way, the virus is safer for researchers to work with, but the interactions between viral proteins and host cells are similar to a SARS-CoV-2 infection. A minority (30%) of patients had very low levels of neutralizing antibodies, despite having recovered from the disease. In addition, 52% of female patients had low to medium-low levels of neutralizing antibodies, whereas only 39% of males were in

these categories. This study indicates that neutralizing antibody levels are variable and warrant further study. Specifically, studies should test patient antibodies against live SARS-CoV-2, although this would require at least BSL-3 facilities. Future studies should also include such stratifications as age, sex, severity of disease, and comorbidities. Factors associated with certain levels of neutralizing antibodies could then be identified in each population.

What do we know about neutralizing antibodies to other coronaviruses?

Previous work in human coronaviruses and neutralizing antibodies can also inform future studies. Neutralizing antibodies can be present in the animal reservoir of the virus, including with MERS. A surveillance study of camels from Eastern Africa from 1983 to 1997 showed that MERS-specific neutralizing antibodies were present in the serum in 85% of camels tested.⁵² This indicated that the virus had been circulating in camels over that period of time, preceding MERS outbreaks. Using an engineered antibody screen to certain parts of the MERS spike protein, researchers then identified 3 important regions of the spike protein that elicited neutralizing antibodies.⁵³ Importantly, the researchers found that virus mutations that allowed viruses to “escape” from those antibodies (not be detected and destroyed) reduced viral fitness.

Using patient serum, a study of an outbreak of MERS demonstrated that the majority of patients (6/7) that had antibodies to MERS-CoV had neutralizing antibodies.⁵⁴ In SARS-CoV infections, it appears that the majority (89%) of patients had neutralizing antibodies to SARS, and that these neutralizing antibodies were primarily IgG.⁵⁵ In another study, 56 survivors were found to have neutralizing antibodies peak approximately 4 months after infection, although these declined after 16 months.⁵⁶ At a 2-year follow-up, the majority had neutralizing antibodies but it was unclear if these were at high enough titers to provide protection.

These studies indicate that patients with coronavirus diseases appear to mount neutralizing antibody responses to infection and that these can last weeks to months. However, further studies of SARS-CoV-2 infections are necessary. Future studies should seek to characterize the presence and titers of neutralizing antibodies in COVID-19 patient sera. Patients should be stratified by, at minimum, severity of disease, age, and sex to determine if any of these factors correlate with neutralizing antibody titers. While neutralization assays are time consuming, they are valuable to understand the immune response to SARS-CoV-2. Once these neutralizing antibodies are identified, researchers can screen the antibodies against panels of viral protein fragments to better understand the exact region to which the neutralizing antibodies are binding.

Are antibodies the ultimate measure of immunity? Are there other cells involved?

The immune response to infection is multifaceted, and the humoral response is often complemented by cell-mediated immunity. Cell-mediated immunity refers to the branch of the adaptive immune response that does not depend on antibodies but instead uses

immune cells that recognize, target, and clear infected host cells. We will not provide an exhaustive review of cell-mediated immunity, but we will highlight important roles it plays in the response to COVID-19.

The roles of T cells (both CD4+ and CD8+) should be considered in COVID-19, as these cells have been important mediators of infection in other coronavirus diseases. Knowledge of the cell-mediated immune response in SARS-CoV-2 infection is incomplete at this time. Recent work has elucidated the fate of T cells in a retrospective study of 522 patients with severe COVID-19 disease. In patients with more severe disease, and in elderly patients (over 60), both CD4+ and CD8+ T cell numbers are reduced relative to mild COVID-19 cases.⁵⁷ Overall numbers of T cells were reduced by approximately 2.5-fold. In non-ICU patients, T cell counts below 800/ μ L were associated with fatality, indicating that T cell counts could serve as a marker of disease severity. The study also found evidence of T cell exhaustion in patients with severe COVID-19. T cells can undergo exhaustion in an immune response when too many signaling molecules have stimulated the cell for too long.⁵⁸ The T cell is then less effective and controls infection poorly. These results, though preliminary, indicate that T cells may play an important role in the severity of COVID-19 disease. Studies of patients with severe COVID-19 in Wuhan also show evidence that T cells are the most significantly reduced immune cell population.⁵⁹ CD4+ T cells appeared more severely reduced than CD8+ T cells in this study. Clearly, more work is needed to understand the role of T cells in protective immunity in patients and how this relates to severity of disease.

What are the minimum amounts of antibody necessary for protection?

Immunity to infection must be considered at the individual and community levels. Specifically, further work is needed to elucidate the threshold of antibody titers or T cell counts necessary for protection against COVID-19. At the community level, the proportion of the population that must be immune to SARS-CoV-2 to provide herd immunity should also be examined.

In any infection, a certain threshold of antibody titers is necessary for protective immunity. Current COVID-19 serology tests in the development pipeline include RDTs and ELISAs, although only ELISA assays can provide quantitative data on antibody titers in patient serum. Currently, only 4 serological tests have an FDA EUA. Many of these tests are RDTs and provide qualitative (yes/no) information on presence of antibodies but not the exact titers in the patient sample. Understanding the threshold of antibody titers necessary for protection is essential in predicting probability of reinfection in patients, as demonstrated in previous vaccine efforts.⁶⁰ Work in malaria underscores that the presence of antibodies alone does not translate to protection; different populations may have differing thresholds of antibody titers necessary for protection.⁶¹ Currently, no studies have attempted to determine the minimum threshold of antibodies to SARS-CoV-2 necessary for protection. A study found that in a group of 10 patients, IgG and IgM levels declined over the course of infection, and titers could theoretically be used as a marker of disease progression.⁶²

The need to establish thresholds could also extend to cell-mediated immunity. Preliminary work in COVID-19 has demonstrated that patients with fewer than 800 T cells/ μ L have a higher probability of mortality, although this is from a single study.⁵⁷ Determining these thresholds is essential in understanding how to triage patients and in understanding immunity in recovered individuals. Further work should also address differing antibody thresholds for protection in individuals at higher risk for severe clinical manifestations of COVID-19, including elderly individuals and patients with diabetes and cardiovascular disease. If serology tests are to be used to determine immune status to COVID-19, there must be further characterization of the minimum antibody titers needed for true protection.

Are all antibody levels protective against SARS-CoV-2 reinfection, or is there potential for worsened disease?

Understanding thresholds relevant for COVID-19 complications is also important. The presence of antibodies can be detrimental in the case of antibody-dependent enhancement (ADE), where antibody levels are too low to provide protection but high enough that the antibodies enable the virus to spread. ADE has been demonstrated in SARS-CoV, where antibodies to the spike protein of 1 strain improve the ability of novel strains of the virus to enter cells in cell culture.⁶³ ADE is particularly important in diseases such as dengue fever, where secondary infection by a different serotype of virus (1, 2, 3, or 4) induces very severe disease relative to the primary infection.⁶⁴ Researchers are calling for further studies of ADE in SARS-CoV-2.⁶⁵ This underscores the importance of determining minimum protective antibody titers. If ADE occurs in COVID-19 infection, any individual with titers below this threshold could experience more severe disease upon reinfection. In addition, if the virus evolves over time to develop different serotypes, as seen in dengue virus, reinfection with another serotype could also result in ADE. It should be noted that, at this time, there is no evidence of multiple serotypes of SARS-CoV-2 or ADE occurring in patients. But this is a research gap that should be addressed, as it has significant implications for patient outcomes.

How important is herd immunity to stopping the COVID-19 pandemic?

At the community level, research is needed regarding thresholds of immune individuals needed to provide herd immunity. Herd immunity refers to the minimum proportion of nonsusceptible (immune) individuals in a population needed to provide protection from viral spread. For a very infectious disease such as measles, an extremely high proportion (92% to 95%) of the population must be immune in order for herd immunity to prevent epidemics.⁶⁶ Currently, it is unclear what proportion is needed to provide herd immunity for COVID-19. The threshold for herd immunity depends in part on the R_0 , increasing as R_0 increases. Researchers have attempted to estimate the threshold needed for herd immunity, although it appears extremely variable by country, with 69.6% in the United States and 56.1% in Iceland.⁶⁷ It should be noted that these are merely predictions based on mathematical models, and further characterization of R_0 in populations can better inform the minimum threshold needed.

VIII. Conclusions

Serology testing has the potential to provide valuable information for individuals and for public health authorities. Seroprevalence studies will be important for public health decision making, including physical distancing measures, and as a complement to viral testing.

There will also be a demand from individuals and employers for the test, to determine perceived immunity to SARS-CoV-2. It will be important for the government to validate the tests as well as provide information about the uncertainties of the results. There are dozens of serology tests being marketed in the United States, but ensuring that they are comparable and accurate requires a validation process with access to many patient samples, preferably overseen by the FDA. As COVID-19 is a new disease, there are important areas of uncertainty that need to be addressed through research: how long immunity lasts after infection, whether and for how long that immune response is protective, and whether there is a specific antibody level that correlates with immunity.

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Appendix A

Types of Serological Tests

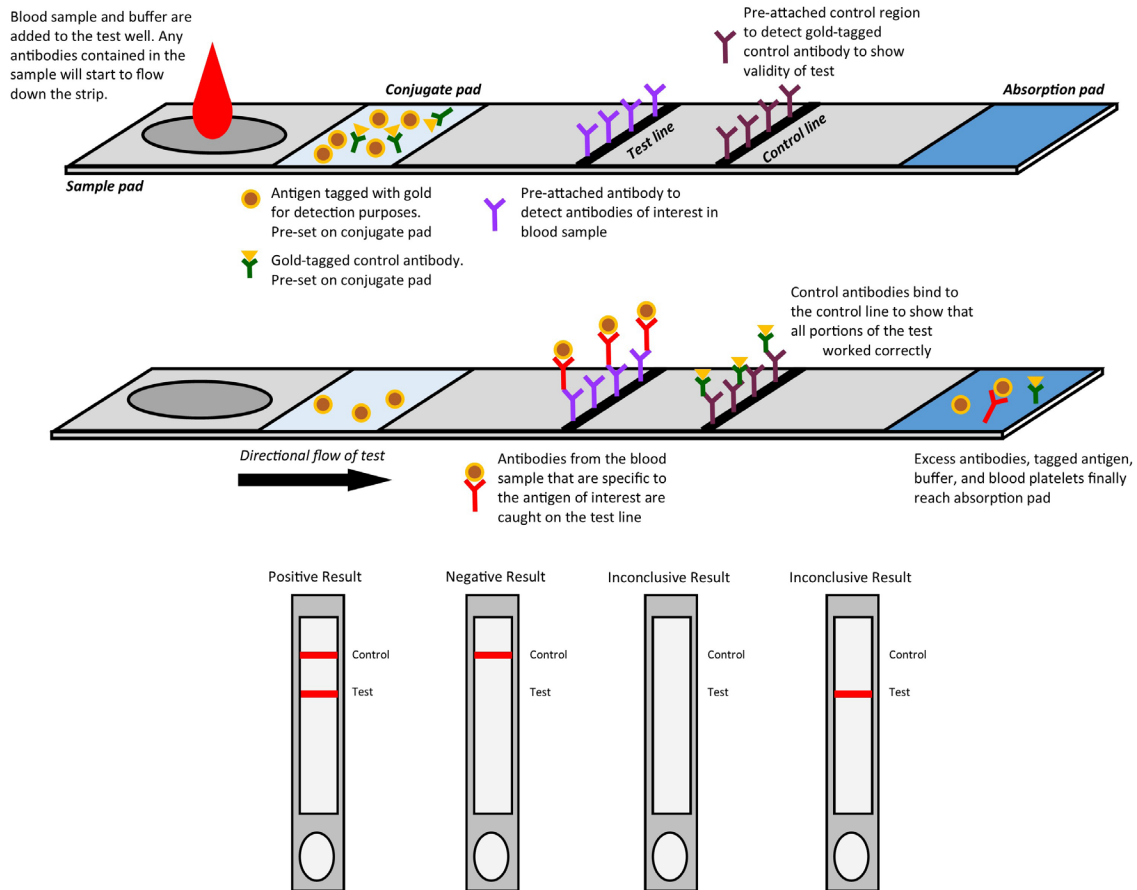


Figure 1. Rapid diagnostic test (RDT) based on lateral flow. An RDT relies on the binding antibodies specific for the antigen of interest in the patient sample. As the sample moves laterally along the test strip, patient antibodies encounter the conjugate pad, where they specifically bind to the antigen of interest. These antibody-antigen complexes then move down the test strip and are “caught” or bound by capture antibodies at the test region, thus giving off a color signature at the test line. Control antibodies (that are not designed to bind to the antigen of interest) are “caught” at the control line, showing that the test result is valid. A failure of the control line to show up indicates that the test did not perform as expected and must be invalidated.

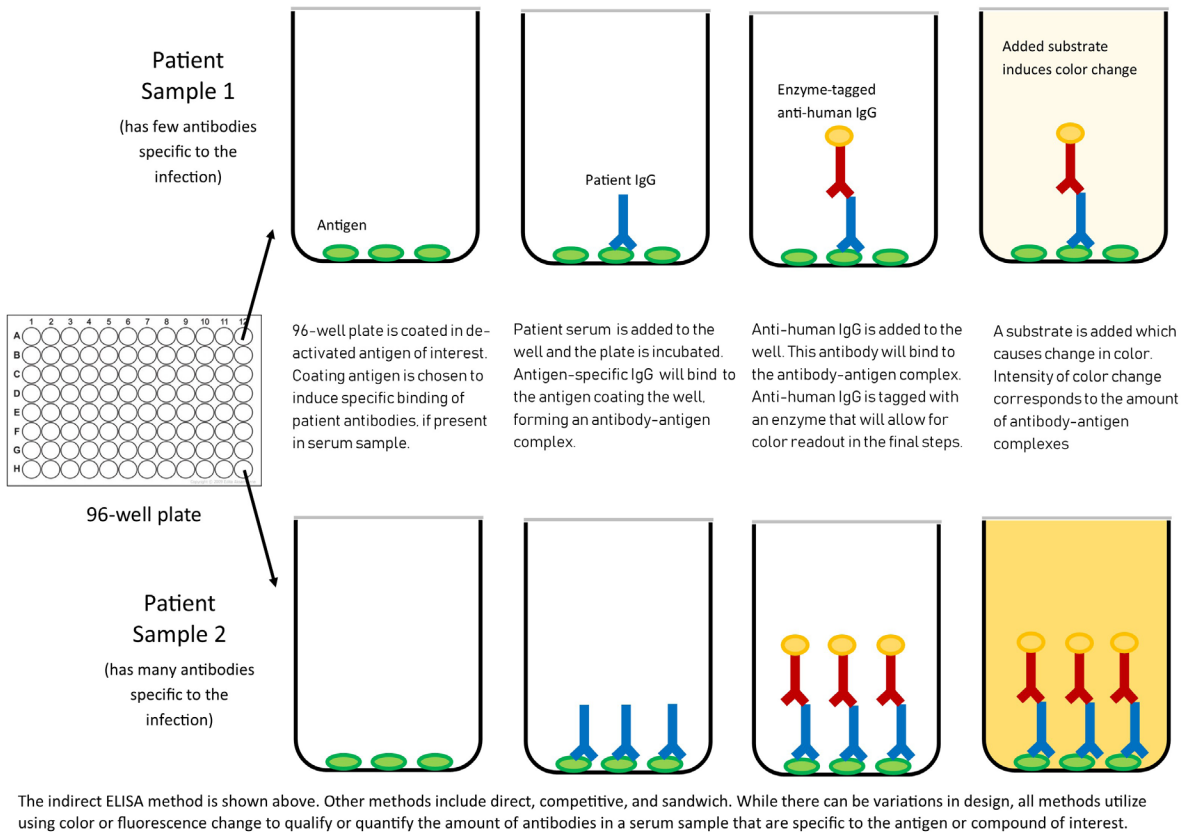


Figure 2. Enzyme-linked immunosorbent assay (ELISA). There are several different methods of designing an ELISA assay. Shown above is the indirect ELISA method. In this method, the 96-well plate is coated in the antigen of interest. In all methods, antibodies in patient serum are added to the coated 96-well plate, and the plate is incubated to allow binding between the patient antibodies, antigen, and color signaling elements. After excess material is washed away, the 96-well plate is then read by technicians under a spectrophotometer to read the relative change in color that corresponds to the amount of antibodies in the sample. ELISAs can also quantify the amount of antibodies in a sample when performed with a standard ladder.

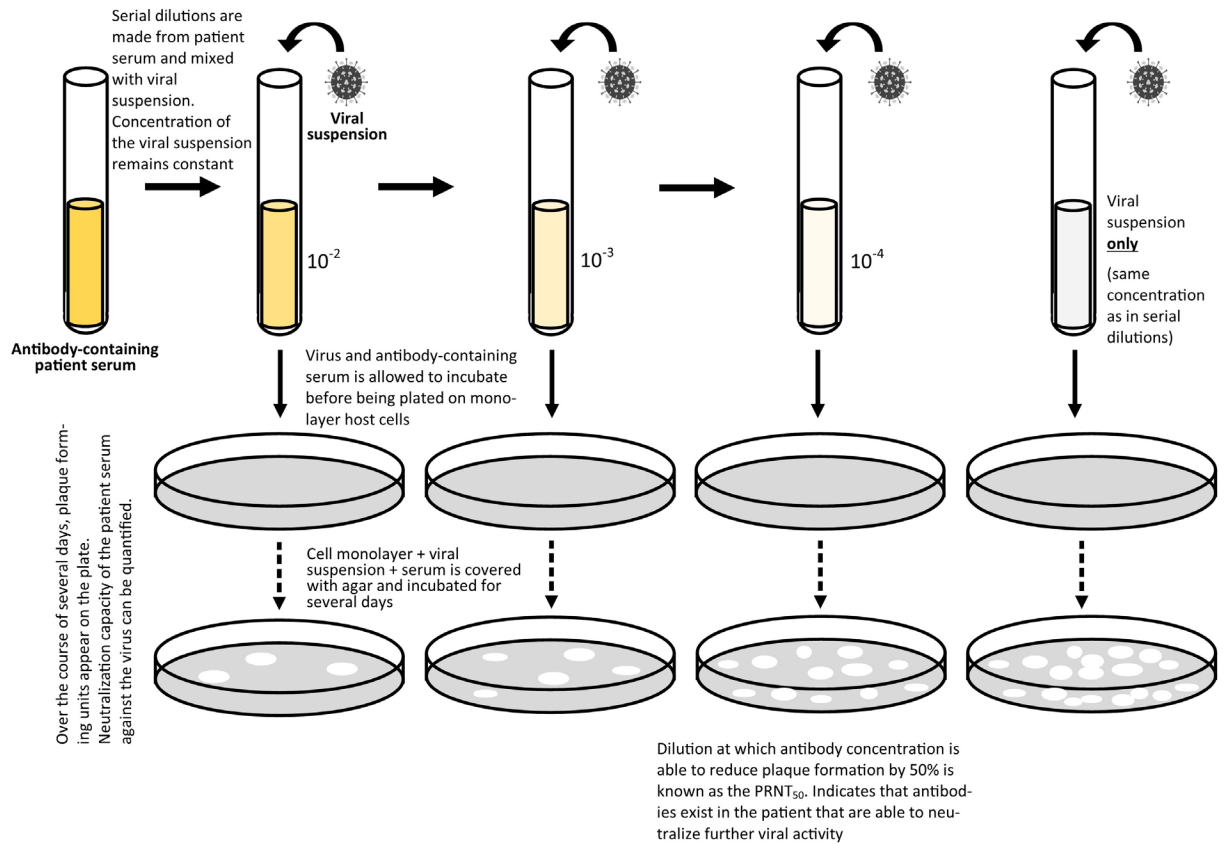


Figure 3. Neutralization assay (shown above: plaque reduction neutralization test, or PRNT). Live virus is added to serial dilutions of patient serum and then cultured in a cell line on an agar plate. Over several days, patient antibodies with neutralizing capacity will prevent the virus from creating “plaques,” or small areas of no cell growth, on the plates. Researchers can quantify the neutralizing capacity of patient antibodies by comparing the serial dilution plates and the control plates.

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