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Understanding Antibody Testing for COVID-19

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ABSTRACT

The orthopedic community has seen the COVID-19 pandemic decimate elective surgical volumes in most geographies. Patients and essential workers, such as health care providers, remain rightfully concerned about how to appropriately begin to return to work and community activity in a safe and responsible manner. Many believe that testing for the presence of antibodies on a widespread scale could help drive evidence-based decision-making, both on an individual and societal scale. Much information, and an equal amount of misinformation, has been produced on antibody testing. Education about the role and science of such testing is critically important for programs to be effectively understood and managed.

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The orthopedic community has seen a pandemic decimate elective surgical volumes in most geographies. Patients and essential workers, such as health care providers, remain rightfully concerned about how to appropriately begin to return to work and community activity in a safe and responsible manner. Perhaps screening all staff and testing all preoperative elective patients for the presence of virus through molecular testing would help minimize a second spike in disease. Many believe that testing for the presence of antibodies on a widespread scale could help drive evidence-based decision-making, both on an individual and societal scale. Much information, and an equal amount of misinformation, has been produced on antibody testing. We believe that there is real value in such immune response testing, but also know that education about the role and science of such testing is critically important for programs to be effectively understood and managed.

On December 31, 2019, 27 cases of pneumonia of unknown etiology were identified in Wuhan city, Hubei province, China [1,2]. The causative agent was ultimately identified from throat swab

samples conducted by the Chinese Center for Disease Control and Prevention in January 2020 and was subsequently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease name was simplified to COVID-19 by the World Health Organization [3]. To date, many COVID-19-positive patients have developed mild symptoms such as dry cough, sore throat, and fever. Most cases have spontaneously resolved, and many infected patients have proven to be completely asymptomatic. However, some have developed various fatal complications including organ failure, septic shock, pulmonary edema, severe pneumonia, and atypical acute respiratory distress syndrome [4–6]. Typically, patients who require intensive care support were older and had multiple comorbidities including cardiovascular, cerebrovascular, endocrine, digestive, and respiratory disease [7].

There are currently few studies that define the pathophysiological characteristics of COVID-19, and there is great uncertainty regarding its mechanism of spread. Current knowledge is largely derived from similar coronaviruses, which are transmitted from human to human through respiratory fomites [8]. Typically, respiratory viruses are most contagious when a patient is symptomatic. However, there is an increasing body of evidence to suggest that human-to-human transmission may be occurring during the asymptomatic incubation period of COVID-19, which has been estimated to be between 2 and 10 days (“How Coronavirus Spreads | CDC,” n.d.) [9,10].

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As of April 12, 2020, there have been 1,853,155 cases of COVID-19 confirmed, and approximately one-third of cases are in the United States. The United States has suffered 22,071 of the 114,247 worldwide deaths secondary to the disease [11]. It is important to note that these figures are likely to be a gross underestimation because the data presented depicts laboratory-confirmed diagnoses only. In the United States, there have been strict guidelines in place regarding the severity of clinical symptoms before qualifying for a COVID-19 test, which most certainly underestimates the actual case load. The spread of COVID-19 may be interrupted by early detection, isolation, prompt treatment, and the implementation of a robust system to trace contacts [12]. Other strategic objectives include a means of ascertaining clinical severity, the extent of transmission, and optimizing treatment options. In addition, the United States has suspended all entry of immigrants and nonimmigrants having traveled to high-risk zones with the intention of halting further viral spread [13]. A key goal, unrelated to medical outcomes, is to also minimize the economic impact of the virus. Part of managing return to community activity, as well as minimizing the risk of a second spike in cases, includes robust testing strategies for both infected patients and those with some degree of conferred immunity due to prior exposure.

Regarding diagnostic testing, numerous companies from around the world have launched COVID-2019 testing kits for use [14,15]. Within each broad test category, there are multiple types of tests appropriate for various use cases. One type, which is becoming more widely available now, is molecular diagnostic testing that detects the presence of the viral genome. These tests are particularly useful for the diagnosis and triage of patients, monitoring the spread of disease, identifying strains and mutations (including next-generation sequencing), and testing the current infection status of a workforce segment. This polymerase chain reaction testing looks for the presence of the virus' genetic material (RNA) on a nasal or throat swab, and more recently via saliva. These tests can tell whether someone has an active, current infection. These tests are not intended to quantify the viral load presence but to amplify the presence of any viral material to determine the presence or absence of the virus in samples.

Another type of test assesses the development of the immune response to the virus in patients by detecting the presence of 3 types of antibodies (eg, IgG, IgM, and IgA) that the body produces in response to the infection. Immune response tests do not achieve the same detection rate as viral genome diagnoses in early infection, as the body needs time to respond to the antigenic viral invasion. Although these antibodies are less useful to monitor in the immediate response and are not indicated in the diagnosis and screening for active early infection, they will be essential in the event of a secondary recurrence of the virus in the community. Because the viral genome is no longer detectable after patient recovery, immune status and the status of a given population related to their ability to contract or resist infection will be based on their antibody status. This type of test is a serological (blood) test and documents the presence of antibodies produced by the immune system against SARS-CoV-2. More traditional laboratory-based tests may require traditional blood draws, whereas newer systems require only finger prick blood samples. Given the nature of these tests and the current need to ramp up testing, the United States Food and Drug Administration has been granting Emergency Use Authorization for testing modalities, including lateral immunoassay for COVID-19. It is important for physicians to understand that most marketed products in this category today still require a Clinical Laboratory Improvement Amendments laboratory certification for moderate- or high-complexity tests to be performed, and despite their simplicity cannot be done in a typical private office [16]. The details of the Emergency Use Authorization process and its

implications are outside the scope of this article and are rapidly changing. However, the Food and Drug Administration guidance is easy to find online with real-time updates.

By helping to detect the immunity of an exposed population, monitor the spread of disease, test the infection status of a workforce segment, and study the disease's progression, data can be used to make educated decisions about quarantine and social distancing regionally or locally and can be used to risk stratify health care providers, police, firefighters, utility workers, and other essential workforce employees. Testing also may provide peace of mind to surgeons who are unsure if they have been exposed to the virus, or if they ever developed immunity to the virus. Knowing these facts may educate decisions about the need for personal protective equipment and one's willingness to work with COVID-19-positive patients in the elective setting.

Antibody Function

An antibody, also called an immunoglobulin, is a protective protein produced by the immune system in response to the presence of a foreign substance (antigen), such as a pathogen. Antibodies recognize and latch onto antigens to remove them from the body. Antibodies are proteins produced and secreted by B cells (lymphocytes) [17].

Because a principal function of B lymphocytes is antibody production, it is important to understand the salient features of these defense molecules and describe their different isotypes or classes. Antibodies are glycosylated protein molecules present on the surface of B cells (surface immunoglobulins) serving as antigen receptors or are secreted into the extracellular space where they can bind and neutralize their target antigens [17]. A single antibody molecule consists of 4 protein chains: 2 "heavy" (H chains) and 2 "light" (L chains) linked to each other by disulfide bonds. The N-terminus regions of the heavy and light chains, which collectively make up the antigen-binding site, are where the variability between one antibody molecule and another resides, hence determining specificity. An important feature is that each antibody recognizes a specific antigen, a phenomenon called "antibody specificity." For example, an antibody that recognizes the mumps virus cannot recognize the measles virus and can only recognize one particular binding site on the mumps virus. There will likely be multiple antibodies to multiple different binding sites on an antigen such as a virus. For example, some antibodies to COVID-19 will target binding sites on proteins in the outer shell while some may target nucleic acid binding sites, but each will be specific and unique. Only when 2 different, but similar, viruses have identical structures will cross-reactivity occur. For example, if multiple strains of a coronavirus have maintained regions of nucleic acid that have not undergone mutation, an antibody that targets that region in one may target the identical region in another. Conversely, an antibody that recognizes the measles virus generally cannot recognize the mumps virus.

Five isotypes, or classes, of antibodies (IgM, IgD, IgG, IgA, and IgE) exist, and they are distinguished according to the C-terminus regions of the heavy chains, which are constant and therefore do not participate in antigen binding. Instead, these regions (designated Fc) are important for the common effector functions of antibodies, the means by which antibodies eliminate pathogens or alternatively cause tissue injury. It should be noted that there are 4 subclasses or isotypes of IgG antibodies (IgG1, IgG2, IgG3, and IgG4), the distinction of which is beyond the scope of this discussion. Antibodies exert effector functions in 3 principal ways [17]:

Antibodies are found in plasma and in extracellular fluid. Antibodies have 3 primary functions.

- 1) Antibodies are secreted into the blood and mucosa, where they bind to and inactivate foreign substances such as pathogens and toxins (neutralization). Antibody neutralization is important for protection from viruses, as it can prevent the virus from then being able to enter and infect cells. It is also important in binding to bacterial toxins and is the primary mechanism for protection conferred by successful vaccination.
- 2) Antibodies facilitate phagocytosis of foreign substances by phagocytic cells (opsonization). Antibody binding, for example, will not prevent bacterial replication. Rather, in this setting, the mechanism of enhanced protection through opsonization will increase phagocytosis by macrophages and neutrophils.
- 3) The third function is antibody activation of the complement system to destroy pathogens through lysis and enhanced chemotaxis.

After an infection, the cells producing pathogen-specific antibodies multiply and increase proportionally. As a result, the body is protected from repeated infection. This feature is called “immunological memory.” One of the most significant features of the immune response is its ability to retain a memory of previous infections, and this is also the principal behind the effectiveness of vaccinations. This both protects individuals from reinfection and limits the spread of infection in a community. Immune memory can be very long-lasting; immunological memory for the measles infection in adults decays so slowly, it would take over 3000 years to decrease by half. This therefore goes well beyond life-long protection for this particular antigen. These robust durable changes are the reason that, when we vaccinate, the protection this produces delivers long-term benefits. Within an individual, immune memory must be distributed throughout the body. Circulating antibodies travel in the blood, reaching everywhere the circulation does including extracellular spaces and in secretions. Natural killer (NK) cells can remain on guard within tissues “alert” but not activated, ready to attack rapidly if reinfection occurs. NK cells are activated in response to macrophage-derived cytokines and they serve to contain viral infections while the adaptive immune response generates antigen-specific cytotoxic T cells that can clear the infection. NK cells work to control viral infections by secreting interferon gamma and TNF α while also stimulating NK cells nearby with such secretions and destroying physiologically challenged cells due to viral infection or malignancy through complex mechanisms outside the scope of this review.

Finally, some infections have such a profound impact on a species that the imprint of individual pathogens can be seen in the tree of evolution. If an infection is lethal, only individuals who have genes that encode effective resistance will survive to produce the next generation, thereby driving herd immunity through natural selection rather than vaccination or infection. Modern methods of analyzing inheritance have demonstrated how the co-evolution of host and infection has shaped the makeup of the immune system and the receptors it uses for recognizing and fighting pathogens.

Structure and Characteristics of Antibody Isotypes

Before discussing antibody tests, it is worth understanding how human antibodies are classified into 5 isotypes (IgM, IgD, IgG, IgA, and IgE) according to their H chains, which provide each isotype with distinct characteristics and roles.

IgG

IgG is the most abundant antibody isotype in the blood (plasma), accounting for 70 to 75% of human immunoglobulins (antibodies). IgG binds antigen and drives the recognition of antigen-antibody complexes by leukocytes and macrophages. IgG is

transferred to the fetus through the placenta and protects the infant until its own immune system is functional. IgG is largely responsible for long-term immunity after infection or vaccination.

IgM

IgM usually circulates in the blood, accounting for about 10% of human immunoglobulins. IgM generally has a pentameric structure in which 5 basic Y-shaped molecules are linked together. B cells produce IgM first in response to microbial infection/antigen invasion. These are early phase immunoglobulins that will develop first during acute infection. Although IgM has a lower affinity for antigens than IgG, it has higher avidity for antigens because of its pentameric structure. IgM, by binding to the cell surface receptor, also activates cell signaling pathways.

IgA

IgA is abundant in serum, nasal mucus, saliva, breast milk, and intestinal fluid, accounting for 10 to 15% of human immunoglobulins. IgA forms dimers (ie, 2 IgA monomers joined together).

IgE

IgE is present in minute amounts, accounting for no more than 0.001% of human immunoglobulins. Its primary role is to protect against parasites. In regions where parasitic infection is rare, IgE is primarily involved in allergy.

IgD

IgD accounts for less than 1% of human immunoglobulins. IgD may be involved in the induction of antibody production in B cells, but its exact function remains unknown.

Molecular Testing for Viral RNA

Molecular test methods are considered the only reliable means of diagnosing an active case of COVID-19, particularly early in the infection course, and are the only means of determining if a patient is contagious to others. By detecting the presence of viral genetic material in the nasal, oral, and respiratory tracts, one can determine if a patient is actively shedding virus which can be spread to others. These tests are perhaps of greatest utility early in the course of infection as they can confirm viral presence up to 2 days before the onset of symptoms. Given that antibodies may not be detectable until 6-7 days after symptom onset, molecular tests can accelerate the diagnostic window by up to 9 days. The duration of viral shedding can be highly variable and depend on severity of symptoms, length of illness, and patient-specific immune response. Generally speaking, viral shedding is undetectable 21 to 35 days after symptom onset or 3 to 5 days after a patient becomes asymptomatic. At the end of the disease course, viral load ultimately becomes undetectable and therefore a molecular test will not detect a prior infection, even one that has recently resolved. Although point-of-care molecular tests for SARS-CoV-2 are becoming more widely available with faster result times, all currently available tests require a laboratory analyzer platform which are generally in short supply and on back order for several months. There remains a backlog of samples for molecular testing at many laboratories and public health officials have put in place strict guidelines requiring severe symptoms to qualify to receive a molecular test.

Therefore, there is a clear role for antibody testing as an important tool in the diagnostic toolbox for COVID-19. Antibody testing can provide important insight to individuals about their functional immunity to the ongoing pandemic, giving peace of mind and assisting with decisions about return to community activities and the workplace. These tests also provide valuable

information to public health officials about the spread of virus in different communities, especially in light of the high reported numbers of asymptomatic cases. Furthermore, although never a primary diagnostic tool, antibody status can be used to aid the clinical diagnosis of suspected noncritical cases that present 7 days or more after the start of symptoms. In these cases, the use of simple, cost-effective, point-of-care antibody cassette systems can offload the pressure on molecular testing throughput to make those resources more immediately available to the acutely ill and critical patients.

Principle of Antibody Assays

With the rapid acceleration of the COVID-19 pandemic, there has been a rush to develop tests that can detect the presence of antibodies produced by the body in response to exposure/infection with the SARS-CoV-2 virus. These testing methodologies rely on the antigen-antibody binding affinity described previously. The test principle relies on a recombinant antigen produced in the laboratory which is designed to mimic specific structures of the virus, causing any antibodies present in whole blood or serum with a binding affinity to attach to the antigen. The specificity of the test can be highly dependent on the target antigen that is chosen as some viral structures can be highly conserved across broad families of virus and others can be highly derived and specific to a given strain. Therefore, it is important to understand the cross reactivity to other viruses of the test being used so as to avoid misinterpretation of a false positive result that may be detecting an antigen from a similar virus, yet not confer immunity to the SARS-CoV-2. These tests fall into 2 broad categories: laboratory-based immunoassays which require a reader or analyzer to detect the reaction, and cassette-based systems which can be read at the point of care through a change in color in an indicator region visible to the naked eye.

Laboratory-based immunoassays have several technical advantages when compared with cassette-based systems. Because they are read by sensitive laboratory instrumentation and a controlled aliquot is delivered to the test system, they can be considered quantitative or semiquantitative tests. Therefore, they can determine how much of a given antibody (titer level) is present per unit volume of serum and, when operating at the margins of detection, their sensitivity and specificity can be higher than cassette-based systems. However, these systems have real disadvantages. Infrastructure costs, requirements for venous puncture, additional steps in sample processing, time to obtain results, and practical throughput challenges are problematic and substantially increase cost per test.

Cassette-based systems rely on a color change that is visible to the human eye and is more appropriate for use at the point of care with almost immediate results available to health care workers and patients. Cassette systems use the principle of lateral flow immunoassay or immunochromatography. The cassette contains a shallow well into which approximately 10 to 15 μ l (eg, 2 small drops from a finger prick) of whole blood, serum, or plasma are placed along with a small quantity of buffer specific to the test kit. The blood and buffer are absorbed into a porous test strip which is impregnated with recombinant viral antigens doped with an indicator (eg, colloidal gold, latex particles, europium, or quantum dots). Antibodies from the serum bind to antigens in the test strip and are wicked laterally along the length of the test strip. In the indicator regions of the test kit, anti-human antibodies which are immobilized in the test strip will bind to the antigen-antibody complex and hold them in the indicator region. The colloidal gold, or other colorant, accumulates in the indicator region leading to a visible change in color along a narrow band of the wicking substrate. There may be one or more indicator regions with anti-

human antibodies that are specific to IgM, IgG, or other immunoglobulins. All tests should also contain a “control” indicator line to confirm that the test sample has appropriately wicked along the length of the assay and antigens in the test kit remain viable, therefore confirming a valid test procedure (Fig. 1). This entire process, from finger-stick to result, occurs in less than 15 minutes.

These point-of-care test cassettes are intended to be read as a binary outcome (presence/absence) for each antibody indicator region. Although the degree of color change may be an indicator of the quantity of antibody present in a sample, variability between antibody concentrations in whole blood, plasma, and serum along with a buffer that is added in “drops” make it unreliable to attempt interpretation of color change intensity with most currently available technology. Kits that use fluorescent indicators can provide semiquantitative results with a hand-held or tabletop reader and appropriate sample preparation techniques but cannot be read by eye. The advantages of lateral flow immunoassay kits are that they can be produced at low cost and in large quantities, they can be used point of care with finger-stick whole blood samples, require low human and facility resource utilization, and can provide very rapid test results—while a patient remains on site. This makes lateral flow immunoassay cassettes an ideal choice for population level sampling and workforce sampling for small and large employers, as discussed further.

Interpreting Point-of-Care Antibody Tests

Because most test kits contain indicator lines for both IgM and IgG antibodies, there are several different combinations and corresponding interpretations of results that may occur. Results themselves should always be interpreted in accordance with the

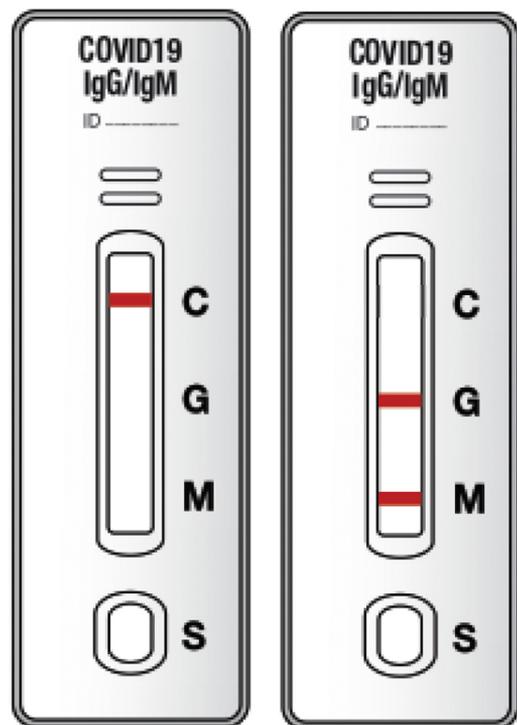


Fig. 1. The typical lateral flow immunoassay cassette with a control band “C” and two antibody indicators. The “M” band indicates the presence of IgM and “G” band indicates IgG is detected. For a test to be considered valid, the control band must be present. Therefore, the test cassette on the left would be considered a negative test result (control band and no antibody bands), whereas the cassette on the right would be an invalid result (lack of control band, despite both antibody bands being present).

package insert and instructions for use with the specific test kit being utilized, but the following discussion is helpful in linking the binary results of the typical antibody test to clinical meaning and making informed recommendations for patients and the workforce. As discussed earlier, IgM antibodies are produced in the short term after infection, whereas IgG are produced in a more delayed timescale. The use of both is advantageous [18–20]. In a recent study, of 535 plasma samples taken from 173 patients with SARS-CoV-2, the median seroconversion time for total antibody (Ab), IgM, and then IgG were day 11, day 12, and day 14, separately. The presence of antibodies was <40% among patients within 1-week since onset, and rapidly increased to 100.0% (Ab), 94.3% (IgM), and 79.8% (IgG) 15 days after onset. By contrast, RNA detectability decreased from 66.7% in samples collected before day 7 to 45.5% during day 15–39. Combining RNA and antibody detections significantly improved the sensitivity of pathogenic diagnosis for COVID-19 ($P < .001$), even in early phase of 1 week since onset ($P = .007$). Moreover, a higher titer of Ab was independently associated with a worse clinical classification ($P = .006$) consistent with the understanding that more symptomatic patients are likely to have higher antibody responses, and higher inflammatory markers, on average. Using the SARS coronavirus as an example, IgM antibodies generally rise above the detectable threshold for these point-of-care tests in approximately 5 to 7 days after the initial onset of symptoms (assuming a patient does develop symptoms after infection). The IgM then remains above the detection threshold for 14 to 21 days from symptom onset. About midway through the rise and fall of IgM production, around day 14 after symptom onset, IgG will rise above detection levels. IgG production will generally continue to rise for 28 to 35 days after symptom onset, peaking around or after clinical recovery. IgG typically has a long half-life and will remain above detectable thresholds for months or even years after the resolution of infection.

The clinical level of functional immunity imparted by SARS-CoV-2 IgG antibodies, and their critical concentration cutoffs, have not been determined for this virus. Therefore, recommendations must be based on similar viruses and knowledge of the immune system. Ultimately, this information will be available and quantitative tests will be valuable in determining immunity titers months and years after COVID-19 infection or vaccination, once vaccines exist. Based on experience with other viruses, if reinfection does occur with the same pathogen one would expect symptom severity and duration to be greatly reduced because of the inherent memory of the immune system. Furthermore, the hyper-immune response that leads to acute respiratory distress syndrome and which is associated with the high mortality rate of this virus would expect to be mitigated by the presence of a prior immune response.

For the interpretation of results in an effort to make meaningful recommendations on community behavior to test recipients, there are several factors that must be considered:

1. Is the subject symptomatic or asymptomatic at the time of antibody testing?
2. If symptomatic, how long since symptom onset?
3. IgM positive or negative?
4. IgG positive or negative?

Interpreting Results When the Subject is Asymptomatic at the Time of Testing

Both IgM and IgG are Negative

The subject's immune system has not produced any antibodies to the target viral antigen and is not suspected of having COVID-19

(Fig. 1, left image). It is not likely the subject has had the infection in the past and the subject is not immune to getting the infection in the future. This result does not rule out that the subject was recently exposed to the virus and is not yet producing antibodies. A subject that was recently exposed to the virus could spread the disease even if this test is negative and they are asymptomatic. As long as the virus is spreading in the community, the subject should continue to practice social distancing or current guidelines to protect themselves and those around them. If the subject does develop symptoms, they should seek medical care and repeat testing based on future potential exposure or symptoms.

IgG is Positive and IgM is Negative

The subject's immune system has produced longer acting antibodies to the target viral antigen (Fig. 2). The subject likely had the infection several weeks ago, even if no symptoms were present. The subject has some degree of functional immunity to the virus. Depending on the time that has passed since exposure, it is unlikely that the subject is spreading virus. A molecular test may be used to rule out viral shedding (as discussed in greater detail in the next section).

IgM is Positive and IgG is Negative

This test result indicates that the immune system is actively producing antibodies to a recent infection (Fig. 3). The subject should immediately isolate from healthy individuals and seek further medical advice if symptoms develop. This subject can likely spread disease to others, even when asymptomatic. The subject may remain symptom free, experience minor symptoms, or worsening symptoms as the disease course progresses. The subject should be vigilant and seek care appropriate to the symptoms they experience. After at least 14 to 21 days, the subject should consider

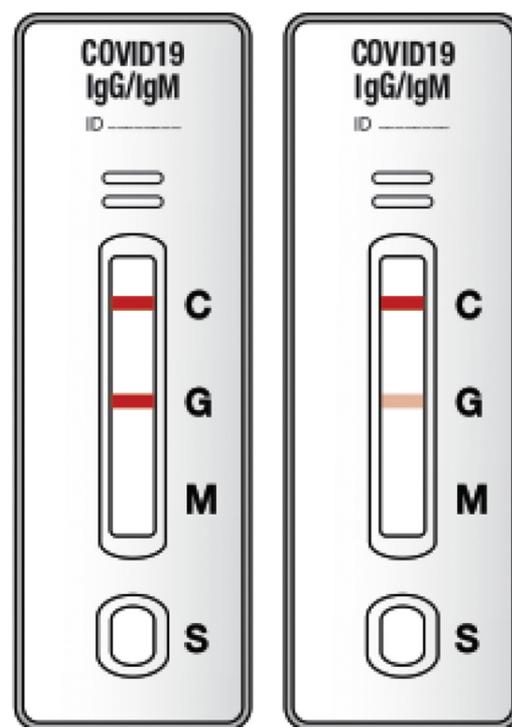


Fig. 2. Lateral flow immunoassay cassette with positive IgG. In both of the cassettes, the IgG band is detectable and the control band is present. This indicates a valid test confirming the presence of IgG to the target antigen. Color intensity should not be interpreted quantitatively and both images are considered a positive result.

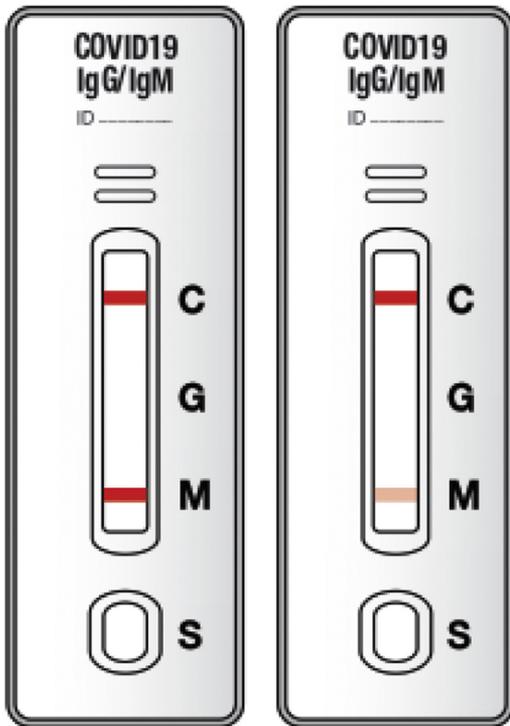


Fig. 3. Lateral flow immunoassay cassette with positive IgM. In both of the cassettes, the IgM band is detectable and the control band is present. This indicates a valid test confirming the presence of IgM to the target antigen. Color intensity should not be interpreted quantitatively and both images are considered a positive result.

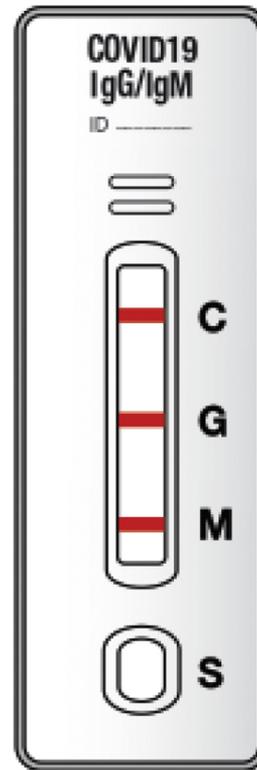


Fig. 4. Lateral flow immunoassay cassette with both IgM and IgG positive. This cassette shows a case where both IgM and IgG bands are present, in addition to the control band. This is a valid test that has detected the presence of two different antigens, one with affinity to IgM and a second with affinity to IgG antibodies.

repeat testing to confirm their IgG antibody status has become positive and they are outside the expected window to shed virus before returning to normal activities. If more rapid return to community activities is warranted, for example, for critical industry workers, a molecular test should be performed to assess viral shedding status.

Both IgM and IgG are Positive

The subject's immune system is actively producing antibodies to an ongoing infection that likely began more than 14 days ago (Fig. 4). The subject should immediately isolate from healthy individuals and seek further medical advice if symptoms develop. The subject is likely experiencing an asymptomatic disease course but may still be able to spread the disease to others. Consider repeat testing in 7 to 14 days to confirm IgG only status before returning to normal activities. If more rapid return to community activities is warranted, for example, for critical industry workers, a molecular test should be performed to assess viral shedding status.

Interpreting Results When Symptomatic at the Time of Testing

Both IgM and IgG are Negative

The subject's immune system has not produced any antibodies to the target viral antigen. If it has been greater than 7 days since the onset of fever or other symptoms, the disease is unlikely to be COVID-19, but a full-panel test including COVID-19, influenza, and bacterial bronchitis could be performed if available and recommended based on history and symptoms. If it has been less than 7 days since the onset of symptoms, COVID-19 cannot be ruled out with antibody testing alone. The subject should isolate from others and could repeat antibody testing at least 7 days after the onset of symptoms unless a molecular test was available for confirmation. If

symptoms are severe and COVID-19 is suspected based on clinical signs, a molecular test is indicated for the detection of viral genetic material in respiratory samples. The subject should not return to normal activities until SARS-CoV-2 infection is ruled out through alternate diagnoses, molecular testing or repeat antibody testing, or appropriate time has elapsed after symptom development to rule out ongoing viral shedding (shedding can occur up to approximately 31 days from exposure).

IgG is Positive and IgM is Negative

The subject's immune system has produced antibodies to the target viral antigen. The subject is likely in the later stages of the disease course but may still be contagious to others and capable of spreading the virus. The subject should remain isolated from disease-negative population for at least 14 days to minimize the chance of spreading the virus. If more rapid return to community activities is warranted, for example, for critical industry workers, a molecular test should be performed to assess viral shedding status.

IgM is Positive and IgG is Negative

The subject's immune system is actively producing antibodies to a recent infection with the target virus. The subject should immediately isolate from healthy individuals and seek medical care appropriate to the symptom severity they experience. The subject can spread the disease to others at this point in disease course. After at least 14 to 21 days, the subject should consider repeat testing to determine IgG status before returning to normal activities.

Both IgM and IgG are Positive

The subject's immune system is actively producing antibodies to an ongoing infection that likely began more than 14 days ago. The

subject should immediately isolate from healthy individuals and seek further medical care appropriate to the symptom severity they experience. The subject can likely still spread the disease to others. Consider repeat testing in 7 to 14 days to confirm IgG only status before returning to normal activities. If more rapid return to community activities is warranted, for example, for critical industry workers, a molecular test should be performed to assess viral shedding status.

As outlined previously, regardless of the testing methodology used to detect antibodies, these tests are not intended to be used as a means to diagnose an active infection. This is primarily because 1) there is a temporal lag between exposure to the virus and the development of antibodies by the immune system and 2) antibodies will persist long after the body has cleared an infection. These tests are an excellent tool to be used in parallel with molecular testing, patient history and clinical presentation in symptomatic patients, or used in asymptomatic patients over a period to understand return to activity recommendations. The temporal framework related to exposure, onset of symptoms, production of IgM and IgG, symptom resolution, and virus shedding throughout the course of disease has not been well quantified for SARS-CoV-2 specifically. At this time, most of the data and recommendations have been extrapolated from the studies performed on the first SARS outbreak in the early 21st century. It is also important to understand, that molecular testing is imperfect as well, with up to 30% false negative rates in some series. Therefore, when looking at the sensitivity and specificity of antibody testing, one must be academic enough to recognize the inherent weaknesses of using such a test to evaluate another test. It will be hard to know whether a positive antibody test in a patient with a negative molecular test means the former test is a false positive or the latter was a false negative. Physicians must be truly diligent with these products and be satisfied with the data, as well as the track record of the company marketing it. We expect a lot more information to be forthcoming in the peer-reviewed literature over the next 6 to 18 months which will improve the interpretation and utility of antibody tests.

Long-Term Immunity—Does It Exist With COVID-19?

A study in the wake of SARS, the similar coronavirus that triggered an epidemic in 2003, showed that survivors maintained neutralizing antibodies for 2 years on average, with the number of antibodies declining thereafter. Other coronaviruses in circulation in the human species also lead to at least partial immunity for some period. The immunity question has implications for whether COVID-19 follows an annual cycle like seasonal influenza or becomes dormant for multiple years and then erupts again, according to a recent publication in the journal *Science*. The authors noted that 2 other coronaviruses in circulation (OC43 and HKU1), which cause common colds, result in about 45 weeks of immunity on average. If the new virus follows that pattern, it would probably create annual outbreaks, they found [21]. However, generally speaking, the sicker you become due to an infection and the robust immune system response to it, the more robust the immunity provided. Therefore, one might assume that those people who get the most ill have the longest and most effective immunity after a re-exposure to the same virus. In a study by Callow et al. [22], people volunteered to have coronavirus 229E, which also causes common cold symptoms, inoculated up their nares. Ten became infected, and 8 developed cold symptoms. One year later, all but one of them returned to be reinfected again. Most reinfected, but those who had been ill before did not develop cold symptoms. Moreover, the period during which the patients shed the virus, and were potentially contagious, was shorter. The new virus, SARS-CoV-2, is

genetically similar to the first SARS virus—hence the “2”—but it affects people differently. It is not as lethal as the original SARS but is more easily spread (R_0 is higher) [23]. Many people who are infected do not develop symptoms at all and yet can potentially transmit the virus to others.

A very recent report from China that has not yet been peer-reviewed found a wide range of antibodies among people with mild cases of the virus. Most strikingly, younger people had fewer antibodies in the wake of the disease—and 30 percent of those sampled had low levels. Some individuals had no trace of antibodies. That has raised the question of whether a person with a mild or asymptomatic infection, but confirmed by the sensitive polymerase chain reaction test, might still be susceptible to a second infection. The neutralizing antibody titers were positively correlated with plasma CRP levels but negatively correlated with the lymphocyte counts of patients at the time of admission, indicating an association between degree of humoral response and cellular immune response. However, those with mild disease who do not develop high levels of antibodies may be at increased risk of reinfection, but are not likely to get significant symptoms, although they may be contagious. Therefore, those without antibody production should likely be treated as if they never had the disease from a social distancing perspective, even if they were proven to be positive with molecular testing.

Conclusion

Longitudinal serological studies are urgently needed to determine the extent and duration of immunity to SARS-CoV-2. It will undoubtedly take time for these data to become available. Despite the hyperbolic media coverage regarding antibody testing, initially positive, and then a week later somewhat negative, physicians must continue to do what we have always done. We must accept that data, especially early data, are rarely complete or perfect and we must utilize the existing body of knowledge to make evidence-based decisions. We believe that there exists a real value in antibody testing when performed properly with quality devices and a good understanding of how to interpret the clinical meaning of the results. Such testing can help educate decisions regarding social distancing and the use of personal protective equipment and can help risk stratify essential workforce members. As physicians, we know that there is no “perfect” test, and it behooves us all to make certain that we utilize as many data points as possible while trying to safely recover from both this pandemic and the possible secondary spikes in COVID-19 incidence that may appear before widespread vaccination.

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