Update on SARS-CoV-2 / COVID-19: Testing

April 15th, 2020

Summary

Widespread testing has been at the forefront of our priorities for several weeks. Increased testing would serve to combat the spread of COVID19, determine the degree of asymptomatic transmission, and eventually determine theoretical immunity from the virus so that individuals can begin to re-enter public places and work environments. However, there are impediments to this effort, and we are encumbered by both test availability and accuracy. In terms of availability, the only way Americans can currently get tested--for active infection or antibodies alike--is at hospitals, clinics or drive-through sites and with a doctor's order. This is because there is a continued shortage of test kits, reagents, and specimen collection materials. In terms of testing accuracy, we are hindered both by the constraints and variability of the tests themselves, as well as our ability to interpret them when we don't know the underlying percentage of the population (prevalence) that has had the disease. Even though widespread testing is the *right* strategy in principle, we must account for test limitations in our interpretation of their results.

There are two types of tests related to COVID-19 that are currently used. A polymerase chain reaction (PCR) test detects viral particles directly and indicates whether or not someone currently has the active viral infection, while the second type of test, called a serologic or antibody test, reveals if someone has antibodies to the virus. This is further complicated by the fact that some antibodies appear early (IgM), while others appear later (IgG). These antibody tests will be essential to determine when and how to relax social isolation. Given the nascent stage of test accuracy data, antibody tests are best used in widespread testing of high-risk and high exposure populations to get a sense of the disease prevalence, and are less useful on an individual level.

Critical to determining the "accuracy" of a diagnostic test, it is important to understand the role of prevalence. For example, let's use a test that has 85% sensitivity, (85 of 100 infected people test positive and are correctly identified as positive, with 15 false negatives) and 97% specificity (97 of 100 non-infected people are correctly identified as negative, with 3 false positives). If a city revealed that 5% of citizens had the disease, and you had a positive test, there is only a 60% probability that you actually had the disease (barely better than flipping a coin!), while you could be 99% certain that a negative test was really negative. Alternatively, assume that we are underestimating the number of infections in NYC, and 25% of the population has already been infected. If you then took the same test and received a positive result, the chance that you actually had the virus jumps to 90%, while your confidence in a negative test falls slightly to 95%. For more information please refer to the recent video for which there is an accompanying downloadable spreadsheet located here that will allow you to better understand the relationship between sensitivity, specificity, prevalence, and positive- and negative-predictive value.

FAQ

Q: How do PCR and antibody/serology tests compare?

There are currently two types of tests that are used to test for the virus (Table 1). Diagnostic polymerase chain reaction (PCR) tests detect genetic material from the virus in respiratory secretions. PCR tests determine if the virus is present at the time of testing. FDA-approved rapid tests can return results at point of care (POC) in as little as 15 minutes. Currently, four of the EUA rapid tests give results in under an hour, while three of which can be performed at the point of care (rather than requiring a trip to a lab). For non-rapid tests, results take 4-6 hours, or more than a day if shipped to a lab. Serology testing are blood-based tests that identify whether someone has been exposed to the virus by looking at the immune response. The tests detect two types of antibodies: IgM antibodies, which are made early in an immune response and whose levels quickly wane, and IgG antibodies, whose levels rise more slowly after infection but usually persist after viral infection has passed. Antibody tests for COVID-19 exist in two formats: an enzyme-linked immunosorbent (ELISA) antibody assay and a rapid, POC antibody assay. ELISA assay is a lab-based, quantitative test (results take 2-3 day to return from the lab) and POC antibody tests are qualitative (positive or negative), only requiring a finger prick (results at POC within 15 minutes).

As of April 1, 2020, only one antibody POC test (made by Cellex and designed for medical professional use only) was under EUA, but the FDA stated that it would authorize the distribution of other unreviewed tests as long as "they are clinically validated and not used as the sole method to diagnose an infection."

Type of SARS-CoV-2 Test	PCR Test	Home antibody test
What does it tell you?	If you are currently infected	If you were previously infected, eventually to indicate immunity
Result format	Yes or no answer to whether infected	Home kit gives yes/no on IgG and IgM antibodies
How accurate and easy to use?	Very sensitive and specific; requires lab equipment	Home kits available, currently less accurate than PCR, not as accurate as quantitative ELISA (a lab antibody test)
Time to get results	Rapid: 1 hour Non-rapid: 4-6 hours If shipped to lab: 1 day+	10-15 minutes
Sensitivity (how confident are we that a positive test is really positive?)	RT-PCR tests under EUA do not report	80 to 90%+, can depend on severity of illness and time since symptoms started
Specificity (how confident are we that a negative test is really negative?)	RT-PCR tests under EUA do not report	90 to 99%, can depend on severity of illness and time since symptoms started
Disadvantage	Not available outside healthcare setting	May also detect infection by other coronaviruses in addition to SARS-CoV-2

Table 1. A comparison of PCR tests and at-home antibody tests.

Q: When and where can I get tested for SARS-CoV-2?

- For both PCR and antibody tests, there are currently no kits for at-home use. Patients being treated at a medical facility who are suspected of having COVID-19 are given priority and will be tested if kits are available. As these tests become available to private medical offices, you can contact your primary care physician if you have a fever and respiratory symptoms, or if you have had contact with someone who is known or suspected to have the virus.
- When the FDA does authorize distribution of home-based antibody test kits, you will be able to self test to see if you have been infected with SARS-CoV-2. However, PCR tests require special equipment, so even if tested at home, samples will need to be sent to a laboratory.

Q: What does a PCR test tell us?

- PCR tests are used to directly detect the presence of an antigen by detecting viral RNA, which will be present in the body before symptoms of the disease are present. PCR tests can tell whether or not someone is currently infected with the virus.

Q: Are there any reports on PCR test accuracy?

- PCR tests approved under emergency use authorization (EUA) do not report true clinical sensitivity (true positive)/ specificity (true negative) values, due to the absence of a reference standard.
- FIND, a WHO Collaborating Centre for Laboratory Strengthening and Diagnostic Technology Evaluation, is in the process of independently evaluating these tests.
- Given the absence of data on sensitivity and specificity as well as widespread reports that PCR tests have a high false negative rate, there is concern about their reliability.
- BGI, in particular, has reported that its PCR test showed an 89.9% agreement with clinical diagnosis when positive (sensitivity) for virus and a 100% agreement where negative for virus with clinical diagnosis (specificity). Moreover, it did not cross react with 54 tested pathogens or the human genome.

Q: How and what does an antibody (IgM and IgG) test measure?

- These tests, called serological tests, search the blood for antibodies to the virus and can identify if someone has been infected, even after the infection has faded. IgM and IgG are antibodies that work together to produce short-term and long-term protection against infection.
- The available antibody test type is either a qualitative rapid diagnostic test (RDT), which tells us if antibodies are present, or a quantitative ELISA (serological enzyme-linked immunosorbent assay) test, which tells us the amount of antibodies present against the virus in patient serum.
- There are four possible results: negative, positive IgM only, positive IgG only, and positive for both IgM and IgG (Figure 1).

Q: What is the availability of antibody tests?

- Antibody tests are currently only available at medical facilities and drive-through testing sites with a physician's order, but they are becoming more widely accessible. The Mayo Clinic expects to have them available this week. One company, BD, has just produced a point-of-care test that involves only a finger prick and can provide results in 15 minutes. It plans to make the test available later this month. Although companies must have the FDA's permission to distribute antibody tests, they are less strictly regulated than PCR tests and during this pandemic the agency is allowing ~50 companies to distribute them without going through the formal approval process. Another company, Cellex, recently became the first (and as of 7 April, only) company under EUA for its antibody test.

Q: When can I get an at-home antibody test?

- The FDA has not currently approved the sale of any at-home antibody tests, but it has indicated a willingness to work with companies that are developing at-home tests for future use. In order to be approved, the manufacturers of at-home tests will have to show that they are comparable to medically available tests, and many of the tests will still require sending a sample taken at home to a lab for analysis.

Q: How long after a person is infected does IgM test show positive (i.e., convert)?

- Typically right after symptoms appear, so about 5-6 days from becoming infected (assuming an incubation period of 5 days)

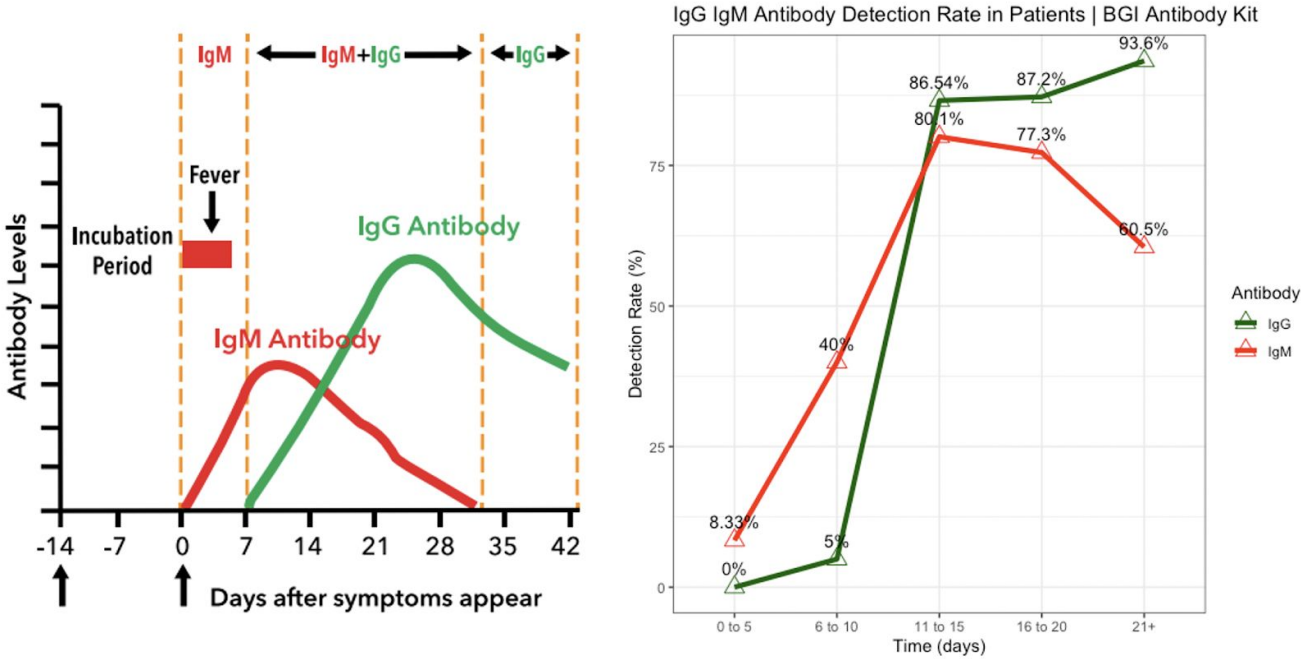

Figure 1. Side-by-side of Biomedonics detection and BGI test kit for antibodies

Q: What is the turnaround time for rapid antibody tests?

- ~10-15 minutes

Q: What does it mean if a person is PCR negative but IgG positive?

- If both tests are accurate, it likely means that the person was exposed to the virus and has recovered, but one can still have a positive PCR test and be "recovered" if the viral load is low enough and there are enough IgG antibodies. PCR is very capable of measuring even trace amounts of viral protein, so it's important to interpret PCR positivity in the context of clinical data.

Q: How accurate are these tests?

- The test accuracy depends on the timing of the test and antibody tested but is reported to be most accurate from 7-10 days after symptom onset for IgM and from 21-28 days after symptom onset for IgG. Cellex reports a positive percent agreement with RT-PCR of 93.8% (95% CI: 88.06-97.26%) and a negative percent agreement of 96.4% (95% CI: 92.26-97.78%). BD's new test, developed by Biomedics, is not

under EUA but has a reported sensitivity (true positive rate) of 88.66% and a specificity (true negative rate) of 90.63%. The sensitivity and specificity for IgM are about 88% and 91%, respectively and the sensitivity and specificity for IgG are about 88-97% and 91-100%.

- In our experience with scientists using commercial kits for research purposes, and therefore doing their own validation, such kits to tend to have LOWER sensitivity than reported and comparable, if not HIGHER, specificity. In english: The kits are not great at identifying positive cases, but are excellent at identifying negative cases.

Q: Where can these tests currently be administered?

- Currently, tests are administered for diagnostic use in laboratories or by healthcare workers at the point of care.

Q: Are the tests specific for SARS-CoV-2 or do they identify antibodies for coronaviruses in general?

- Companies reporting on the cross-reactivity of their tests reveal promising results: the company BTNX claims no cross-reactivity with any unrelated infections tested, including other coronaviruses,[1] and BGI has reported a cross-reactivity rate of ~0.5-1% for its colloidal gold antibody kit.
- Based on the external validation studies we have seen for two companies, there is no meaningful cross-reactivity with other coronaviruses (How does one test for this? By running tests on banked blood from >9 months ago when no one had SARS-CoV-2, but presumably, some people still had recent exposure to other CVs)

BGI IgM/IgG rapid assay kit (Colloidal Gold)

Q: How accurate is this test?

- BGI does not give information on how they tested - what they report to be - a 99% specificity and a sensitivity of 85%. Notably, the kit does not cross-react with other coronaviruses (a reported ~0.5-1% cross-reactivity rate) and BGI claims it has 20% higher sensitivity compared to point-of-care and rapid-PCR tests[2]. However, the qualitative results of whether antibodies are present/not in a given blood sample does not indicate the quantity of antibody, if present at all. Quantitative evaluation of how much antibody is present in serum will be an important metric for evaluating one's immunity.

[1] Most notably, coronavirus strains HKU1, NL63, OC43, and 229E, and SARS, and MERS
[2] Both cross-reactivity and sensitivity comparison reported in email correspondence

Q: How does the test work and what could results indicate?

- The test contains specific SARS-CoV-2 antigen marked with colloidal gold. If a minimum concentration of antibodies is present, they react to form a detectable compound. IgM positive indicates a recent infection (detectable around day 7 of infection or day 3 after onset of symptoms); IgG positive indicates a previous infection and such antibodies take longer to detect (increasing from ~7 days after symptom onset and continuing to increase as the infection begins to subside)[3] (Figure 2);

Q: What are the possible results I would visually see on the assay?

- Depending on where in the time course of viral infection (if there are antibodies present), there are three possible results for antibodies: positive IgM only, positive IgG only, and positive for both IgM and IgG. The test result could also be negative for both antibodies. It is important to note that if no control line appears, the test is invalid and should be redone (Figure 2).

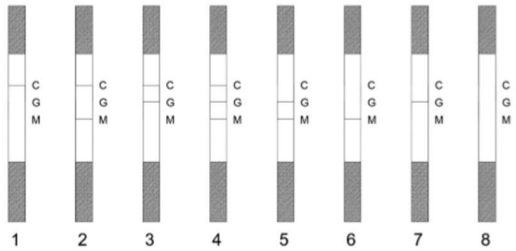

Figure 2. Interpretation of test results: 1) negative for both antibodies, 2) IgM positive, 3) IgG positive, 4) positive for both IgM and IgG, 6-8) no control (C) line, so test invalid and should be redone

[3] IgG appears about 7 days after and peaks about 21-28 days after symptoms appear (according to BioMedomics)