Responses to Audience Questions
6/3/20

How do we handle the false positive results of COVID-19 tests?

According to the the most recent information from the CDC, a positive serology test should be confirmed by a secondary test if used in a clinical setting.

In our small laboratory we find ourselves with limited access to PCR testing. However we are able to do the Ortho IgM and IgG antibody tests. Is there a way we can use the antibody tests to safely screen our pre-surgical patients and be fairly confident they are not infectious without doing a PCR test?

According to the most recent information from the CDC, a positive PCR test used in a clinical setting should be confirmed by a second test (which can be a second serology test using a different platform/target).

What about clinical testing for multisystem inflammatory syndrome in children?

As noted above and in the reference posted above, serology is part of the case definition of MIS-C.

Will there be interpretation of results based on gender and/or age of patients?

At the moment, the instructions and information provided by the manufacturers for FDA EUA-approved assays should be examined to understand if gender and age are important in interpretation. All tests that are approved by EUA are under investigation with the FDA and the CDC to determine the performance of these tests, and gender/age is being examined.

So the treatment with convalescent plasma is really a hit or miss?

At the moment, the use of convalescent plasma is not done assuming any specific antibodies are present, and titers are not performed. It is a shotgun approach which can, as you say, be hit or miss. Please look for new data on these patients as it emerges.

Do we have a sense of the types of laboratories across the country that are bringing up serology testing? Is it mostly immunology/chemistry labs? Or microbiology labs? Or others?

Currently, there are a limited number of serology tests which are on the FDA EUA list. Please see this link: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance. At the bottom, you will see each test. Depending on which part of the laboratory has the platform for these tests, different labs may be involved. Traditionally, this will be either the immunology lab or the microbiology lab. Throughput and limitations in testing may require some labs to run platforms in both labs.

Why don't we join forces nationwide to provide the serological data needed for CDC and state public health epidemiological studies?

Excellent point. The FDA and the CDC are collecting data and requesting that laboratories submit data to them about their existing EUA-approved serology assays as well as any other assay used for serology for COVID-19 so that the value of these tests can be validated and vetted.

Beyond sensitivity and specificity, the predictive value of a positive and negative result are important, particularly when the prevalence remains low.

Excellent point. This link https://bit.ly/2Xp9lX2 provides an explanation of sensitivity, specificity, positive predictive value, and negative predictive value and how prevalence is a MAJOR challenge in COVID-19.

What can you say about labs who are using serology tests to screen patients entering the hospital, and patients that have a negative serology test but have a positive swab result 2 days later?

False negatives (failed test) or true negatives (no antibodies yet developed) can occur early in infection while the patient is asymptomatic with active viral replications.

How will herd immunity help in the control of COVID-19 in our communities?

Herd immunity in modern medicine is a concept that is only discussed with the use of an intervention. Therefore, with COVID-19, we will need an intervention (such as a vaccine). The best approach is testing, contact tracing, and isolation/quarantine. Handwashing and avoiding touching one's face provides excellent personal protection.

What could be the reason for a postive IgM result but a negative PCR result?

IgM serology assays (for any disease) are notoriously non-specific with lots of false positives. Therefore, positive IgM with negative PCR is to be expected.

We have patients in our nursing homes who became symptomatic of COVID-19 and tested positive by PCR, were quarantined, and subsequently became asymptomatic but remained PCR positive for at least a couple weeks. Perhaps PCR is detecting dead virus particles? Would serological testing have any application for determining whether these patients can be moved out of quarantine?

Last week in the Town Hall, this was discussed, and data was mentioned that showed patients can be PCR positive for 5 to 7 weeks. Serology will also likely be positive during this time. However, unless we know that the antibody being detected is protective or indicates no chance of contagion (we don't know either), there is probably not much value or at least difficulty in interpreting the value.

Does the restriction of the target protein to just the Cov-2 spike protein reduce the likelihood of detection of a immune response?

The approved serology tests on the FDA EUA list (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance) use either the spike, nucleocapsid, or both (you can see the specifics in the list). Broader targets are likely better but do increase the risk for false positives.

Do you think serology tests will allow the authorities to test as many people as possible (from the public health perspective)?

One doesn't need to necessary test "as many as you can." What has to be done is a random sampling of a population that represents a geographic area to determine the epidemiological prevalence. The key is to perform true random sampling. This is what will inform public health.

What is the usefulness of serology in MIS-C?

Please see this article https://emergency.cdc.gov/han/2020/han00432.asp from the CDC on MIS-C. Serology is listed as one test that can be used in the case definition.

There seems to be a bit of a disagreement between the experts here. One said that a positive serology test most likely predicts exposure while the other brought up the PPV in a test with even a good specificity in a population with low prevalence (less than 3%). Could they resolve this?

See this link https://bit.ly/2Xp9lX2, which helps to clarify this issue.

So what's the solution for those that want/need to be comfortable going back to work or out in public, and the intense pressure to go out? If based on health care workers at Stanford, is it only face mask protection until we get the vaccine? And if so, how do we convince all those that are not wearing face masks?

The best way to protect oneself is with hand washing, avoiding touching one's face, isolation if you have symptoms or exposure, and decontaminating work spaces (if shared). In the long term, face masks are not likely to provide protection and more likely to provide a false sense of security when used outside of a healthcare setting because of improper use, poor filtration, prolonged use, and air constancy. A vaccine will certainly change the dynamic and the tools we have available. Following epidemiological data and protecting oneself personally will be the key to returning to work, which is fueled by good decision making by leadership and testing.

What can you say about rapid test kits used for IgG/IgM mass testing for COVID-19?

The question is what is the goal of "mass testing." Random sampling using a high quality serology test with a secondary confirmatory test is sufficient for epidemioogical purposes to survey the geographic and population distibutions. Providing serology to everyone, because of the low prevalence and low PPV, would be difficult to implement and unclear on role.

If we do not have a trusted system for testing, how can we trust the vaccine?

There are currently tests that perform very well and are not in question. The testing systems, algorithms for testing, and use and interpretation of tests is more of a challenge. The process of producing a vaccine is completely unrelated to developing a test; however, having a good, reliable serology tests related directly to the vaccine candidate will be required.

Comment on the use of a second serology test for confirmation of an initial serology test.

The CDC and the FDA recommend a second serology tests that uses a different platform and a different target to confirm a positive that is to be used in a clinical setting. The preferred second test is RT-PCR. However, if RT-PCR is not available, the second serology test can be used.

In low prevalence populations where you see more false positives, do you recommend performing two different serology tests to help identify false positives?

The current recommendation from the CDC is that if you are using serology in a clinical setting, a postive test should be confirmed by a second test using a different platform or modality.

How do the assays differ in terms of the target viral proteins?

The currently approved FDA EUA tests listed at this link https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance have, if you scroll down, the targets for each assays listed. They use either spike, nucleocapsid, or both. Other parameters of these tests are also listed.

What is the implication of a FDA-approved serology test failing validation at the facility level?

The FDA has an active program of receiving information from laboratories about the performance of tests. If you want to report failures to the FDA, their website has contact information. To date, they have pulled 15 assays OFF the list because of failed performance and/or voluntary withdrawing. Please consider contributing your data to the FDA to help with this activity.

If the virus can be detected in urine, why can't we test urine instead of those painful nasal swabs?

The FDA has several urine tests in consideration, but none have been added to the EUA lists currently.

So should labs performing the EUA platforms have 2 different platforms set up and do both?

The FDA and the CDC recommend a "second test" if using serology as a primary tool in clinical settings. The second test can be another serology or RT-PCR (preferred).

What is the rationale for using GeneXpert machine for TB testing for COVID-19 in Africa?

The GeneXpert machine is an agnostic platform to the cartridges used. These machines will run ANY cartridge produced by Cepheid. As there is large market penetration of GeneXpert devices already, use of these COVID cartridges will allow for rapid scale up of testing in African countries.

How accurate is the SARS-CoV-2 serology testing?

The performance of the tests as reported to the FDA for the EUA can be found here: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance. The FDA and the CDC are actively collecting data on all of these tests to determine their value in a variety of populations and prevalence.

Nucleocapsid vs. RBD (spike protein)? Are both of these neutralizing antibodies?

The presence and identification of neutralizing antibodies is still being researched, but we will query the panelists.

If someone who thinks they had COVID-like symptoms gets the antibody test and it is positive, have they most likely had COVID in the past?

There is a higher probability than not. However, the CDC currently recommends that antibody tests used in a clinical setting should be confirmed with a second modality (RT-PCR or serology on a different platform).

Please discuss differences between antibodies to nucleocapsid vs. RBD (spike protein)?

This article from ASM summarizes these different targets: https://jcm.asm.org/content/58/6/e00461-20. We will posit this question to our panelists. Thanks!

We have businesses wanting to know what we have available for testing - to have staff go back to work.

There is no clear national or state guideline and certainly none based on rigorous evidence that any current testing can be used in such a way as to guarantee a 100% safe work environment. Space cleaning, hand washing, hand sanitizer, reasonable social distancing, and avoiding close contact are all additional methods to keep a work environment safe. Having policies about individuals with exposures or symptoms to stay home and/or work from home is advisable. Employers must consider the risk exposure of their employees' commute, which is often within the public domain, as well as work space.

Is there any evidence whether IgM or pan or IgM/IgG is better?

There are more false positives with IgM, both generally and for SARS-CoV-2. But there is variability among IgG assays as well. If the test is being done to detect past exposure with high specificity, then IgG or possibly high avidity total antibody is likely better. If you want to know immunity status, then serology is not currently a good test to assess that.

Is your lab treating positive serology as a critical value?

No, reporting serology as a critical value does not appear to be a standard of care or practice.

In the scenario of an asymptomatic patient with no recollection of disease testing positive, could it be a false positive?

Yes, it is likely a false positive and the result of low prevalence. The CDC suggests that for clinical use, a second test should be used (PCR or other serology platform) to confirm a positive serology with low probability/suspicion.

Can you talk about if the antibody response disappears, and when?

Non-peer reviewed studies from France have shown that neutralizing antibodies become undetectable in hospitalized individuals after about 50-60 days. But the implication to immunity is unknown. Maybe we react sufficiently with few in memory. Maybe the innate immune system plays a big role and antibodies matter less.

Are there any benefits for choosing a platform that tests for IgG vs. panIg? Does anyone have any helpful information about saliva testing?

Roche claims their panIg, which is selective for high avidity antibodies, is at least equivalent to specific IgG assays, but we and others are currently comparing internally, so there will likely be more data on that soon. But the implications for immunity of the various tests is going to be much harder to determine and much more what people typically want to know. The answer to that is we do not know.

There have been a number of articles in popular press covering serology testing for SARS-CoV-2. Some seem to draw broad and generic conclusions referring to "immune passports" for people who test positive for antibodies and making a case that positive people should be the ones returning to jobs. This is creating several bioethical questions. What is your advice to popular press with regards to serology coverage?

There is insufficient data for drawing any conclusions about immunity from serologic tests in this disease. Long-term studies correlating re-infection with past serology results in a variety of platforms would be needed. There are adverse consequences of misutilizing the data clinically, socially, and economically. Advice might be to focus on what information might be needed to be able to draw appropriate conclusions from test result patterns and guide behavior, which is currently not serology. Testing patterns for presence of virus will be valuable. Detection of reinfection would have impact. Serology could play a secondary role in the long term.

Can you please explain from a public health perspective why a positive serology test that has cross-reactivity with other coronaviruses should be used for a prevelance perspective?

From my perspective, certainly tests without other coronavirus cross-reactivity are much preferred, and our experience in validation suggests there are several assays that satisfy that need.

Commercial companies are already offering this test without much clinical oversight- essentially on demand. Are they shaping our discourse through serology?

It is true that the availability of serology tests far preceded a clear role in stopping the pandemic, as there was a lack of a national testing strategy or clear guidelines (beyond epidemiology) for the value. However, the FDA has been vigilant about rigourously evaluating these tests through the EUA process and has, in fact, removed 15 tests (mostly serology) from the list because they failed to perform. For all the serology tests on remaining on the FDA list, study by the FDA, the CDC, and the companies with data submission by laboratories using the tests is underway to validate and vet these tests. The argument still remains, "How do we use them?"

For Dr. Torres' question regarding performance of the same kit in different populations and everyone's concern about performance BETWEEN kits, can we consider known samples sent from a national source, like a proficiency? I would appreciate seeing the results from such a comparative study.

The CDC is coordinating a nationwide effort with other agencies and academic and commercial labs to do comparison studies. I expect that data will start becoming available as more and more centers do more and more serology. That might be a reason not to restrict serology testing too severely at this point, as long as we do not engage in clinical misuse.

Is a patient without symptoms but who has the virus considered contagious ? And does he develop antibodies? And should we do PCR to confirm he is negative for the disease? What is the difference between the patient that develops symptoms and the infected patient without any symptoms in the results of serology?

A large French study that is not yet peer-reviewed reports that some asymptomatic individuals develop antibodies, but less than half of the detected antibodies neutralize viral infectivity in in vitro assays. There is some (limited) evidence that fewer symptoms results in lower antibody levels and so asymptomatic and less symptomatic individuals are less likely to result in positive serology test. The relative sensitivity of the various assays in this setting is unknown (and would be great to know).

How feasible is it to confirm a reactive result with a neutralization assay?

Neutralization assays are cumbersome and technically challenging. A rapid commercial assay which is easy to perform, robust, low-cost, high-throughput, and properly validated would be valuable, but I do not know of any.

My lab is doing serology testing that is qualitative. How effective is this test moving forward as far understanding immunity or building immunity. That is how much antibody is needed to provide a minimum immunity (titer levels) and is a qualitative in this sense of any use moving forward?

Most assays, including the instrument-based ones, are meant to be qualitative. Since the utility of all assays for immunity detection is unknown, it is not a given that the qualitative tests are inferior. Further down the line, quantitation may become more relevant, as might antigen substrate and Ig type.

I'm a PA that has been in the urgent care setting - one of the clinics I was at is currently using serologic IgM/IgG testing (lateral flow) for symptomatic patients. Can we discuss the utility in the acute care setting? Would another approach be more beneficial in this setting?

A key point is that serology should not be used for diagnosis in the acute care setting. If it is your only option, a secondary tests such as a second serology platform or RT-PCR is recommended to confirm positives.

Isn't IgA more relevant?

In respiratory infections, IgA is a critical infectious response. Titers generally get very high with such infections and avidity tends to be very high as well. But specificity may decrease. It is also unclear if what is seen in blood correlates to what is present in mucosal areas or how the type of antibody relates to immunity.

What would be the significance of testing IgA for this? I've seen some companies/platforms test for this.

Not at all; avoid IgA unless you do celiac testing.

Does anyone have the citation for the paper Dr. Kadkhoda is referring to?

Kadkhoda K. COVID-19: are neutralizing antibodies neutralizing enough? Transfusion. 2020. doi:10.1111/trf.15897 & Li L et al. Effect of Convalescent Plasma Therapy on Time to Clinical Improvement in Patients with Severe and Life-threatening COVID-19. JAMA. 2020. doi:10.1001/jama.2020.10044

In MIS-C only 20% have PCR positivity and serology is 95% positive?

We don't much yet, but if tested early, in fact over 50% are RNA positive; use a highly sensitive assay like the CDC one AND test AS EARLY AS POSSIBLE; this combination is key.