COVID-19 ECHO Session #9 _May 08, 2020: DIAGNOSTICS in COVID-19 PANDEMIC RESPONSE: Knowledge, gaps and updates on performance of serology tests

SN	Questions	Answer/ Response / Comments
Sample management		
1.	Ag RDT Lateral Flow: what type of specimen has been used?	The same specimen as for molecular tests
Reagents and consumables		
2.	Are there any RDTs being manufactured within Africa	Ghana developed Ab RDT which is currently under validation by FDA.
3.	Please provide update on the RDTs under development in Senegal? Any performance data?	Performance data not yet available
4.	Aren't there antigen tests that you could evaluate?	There are several antigen tests under evaluation
5.	Please can you share the list of available and internationally recommended serological kits for covid-19	The WHO recommends the use of immunodiagnostic (i.e. antigen and antibody) tests only in research settings. These tests are available on FIND website. In the USA, some serology tests have FDA emergency use authorization.
Safety Measures		
6.	What is the biosafety level or precaution for conducting the serology test	BSL-2 or higher
7.	When collecting blood sample for the analysis should the use of N95 facemask, gowns etc. be compulsory & should analysis be performed in a BSC?	Full PPE should be used when approaching any suspected COVID-19 patient. See WHO and CDC guidelines: https://www.who.int/docs/default-source/coronaviruse/laboratory-biosafety-novel-coronavirus-version-1-1.pdf?sfvrsn=912a9847_2 https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html
8.	Do you have to be in full PPE to collect specimens from primary contacts?	Full PPE is recommended for collecting samples to mitigate against the risk of contracting the virus from a positive patient
9.	In which type of laboratory do blood samples for clinical chemistry of confirmed COVID-19 positive patients analyzed, in a hospital central laboratory or by POCT devices in the isolation ward? Is it advisable for theses samples to be analyzed in a laboratory without a functional Biosafety cabinet?	BSL-2 is needed for all samples. If as POC RDT, steps need to be taken to ensure inactivation. Inactivation methods need to be verified to be effective. https://www.cdc.gov/coronavirus/2019-ncov/downloads/OASH-COVID-19-guidance-testing-platforms.pdf
Diagnosis		

SN	Questions	Answer/ Response / Comments
10.	Most of the COVID tests so far have <98% specificity and sensitivity and most even lower. Given the need to rollout exposure screening. Do you see the utility in an algorithm where you verify negatives from symptomatic individuals? In other words applying both RDT and clinical criteria	Clinical criteria will always be needed. Algorithms will also be developed.
11.	Which standard can be used for selecting an assay? Do you go for sensitivity and specificity only, or you should also consider PPV and NPV regardless of the prevalence	Need to consider the trade-offs between accuracy, accessibility and affordability. PPV and NPV are based on prevalence.
12.	Are there any additional lab tests associated with the diagnosis of covid-19 in addition to the serology and PCR tests?	Some hematological tests such as clotting tests, CRP and lymphocyte counts are used as ancillary tests. Radiology is also used (Chest X-ray).
13.	Is there a recombinant protein which target all coronaviruses?	No data on that yet.
14.	How close are we towards using saliva for COVID-19 using PCR?	Saliva is one of the samples that can used. However, the chance of getting the virus is much better for nasopharyngeal swabs. In a hospital setting, the best sample to use is deep lung samples obtained through bronchoalveolar lavage or intubation.
15.	We detected 102 COVID-19 positive cases in Nepal with no single fatality case and clinically majority were asymptomatic. Given contact tracing is essential for COVID-19 detection in community, is sample pooling reliable?	There is only 1 study that shows pooling samples works for PCR testing: Lancet Infect Dis 2020, Published Online, April 28, 2020; https://doi.org/10.1016/S1473-3099(20)30362-5
16.	Which is the best monoclonal antibodies to use when developing antigen test RDT for covid-19?	No consensus on that yet.
17.	How useful is screening of employees as a way of keeping the workplace safe?	There is no good way to do that: if you monitor temperature, not everyone who has COVID has fever. You can use If PCR is used, it is costly and how often would you need to screen? If you use serology test, when the prevalence of infection is low, you will get more false positives than true positives.
Method validation /verification and evaluation of kits		
18.	In clinic evaluation what is the gold standard of reference?	The gold standard now is molecular testing such as RT-PCR
19.	Is the gold standard qPCR test?	yes
20.	For COVID19 test, do we need to validate it?	Check the presentation on the difference between validation and verification

SN	Questions	Answer/ Response / Comments
21.	What type of positive specimens (Value of Cts) should be used in verification of reproducibility, repeatability?	Specimens with medium and high Ct values
22. .	When we have a lot of RDTs in the country to evaluate, with a low prevalence 1 or 2 %, how many samples (positive and negative) do we need?	Serology RDTs are not recommended for low prevalence settings as you will end up with more false positives than true positives.
23.	Why include malaria positive samples in the evaluation study? Is it to rule out cross reactivity? How about cross reactivity with Polio?	As COVID patients present with fever, panels to check possible cross reactivity should include common causes of fever in the community.
24.	Is there evaluation data available for COVID-19 serological tests that are currently in the market?	FIND has the data on their web site.
25.	Are there any antigen tests in evaluation by FIND? Any results yet?	No results yet. Results will be updated.
26.	Do we have any POCT already Pre-qualified by WHO?	Not yet
27.	Does FIND evaluation automatically endorse WHO pre-qualification?	No
28.	Is there another way to calculate the sensitivity and specificity without using a gold standard? What assay will you recommend as gold standard for COVID-19.	Sensitivity and specificity are based on a gold or reference standard. The performance of each type of assay should be compared to its own gold standard.
29.	How can counties that have less than 100 positive samples be able to do evaluation since they need a total of 150 positive and 150 negative results?	Each country should not try to duplicate the evaluations already done by Africa CDC and/or FIND.
30.	When doing the Validation and verification processes did particular companies use known positive samples from patients or are there commercially available positive control with known specificity and sensitivity?	Positive and negative controls are not always available from the manufacturers. Quality control materials for some kits may be available from organizations such as Quality Control for Molecular Diagnostics (QCMD), an independent International External Quality Assessment (EQA) / Proficiency Testing.
Result interpretation/ Reporting		
31.	What is ROC for quantitative assay?	
32.	Are you aware of cross reactivity with dengue?? Since dengue is very common in some of our countries, can this represent a challenge to interpret results?	Not aware of any cross-reactivity with dengue.
33.	Comment on the cross reactivity of SARS-CoV-2 IgM and IgG antibodies with antibodies produced against other commonly circulating coronaviruses?	Evidence suggests limited cross-reactivity with the S1. S1 seems to be more specific to SARS-CoV-2.
Sensitivity/ Specificity/ Detection limit		

SN	Questions	Answer/ Response / Comments
34.	What is clinical sensitivity and Specificity.	Please refer to the slides.
35.	Experienced a scenario in the field where sensitivity & specificity of the test kit was 95% but different were obtained with different sample type e.g.: whole blood, serum or plasma. What would be the reason for diminishing sensitivity overtime, for some of the test kits evaluated?	Sensitivity of a test may be slightly lower with whole blood than serum or plasma but there is no reason for loss of sensitivity over time.
36.	Would it be wise to do parallel testing using two different assays so that a high sensitivity and specificity is achieved???	2-test algorithms using either parallel or sequential testing can be used to increase the final accuracy of testing.
Prevalence / PPV/ NPV		
37.	Currently, the prevalence of COVID-19 is unknown, and this can only be established via wide scale testing or availability of reliable data to develop models. So how do we decide on utility of these kits	The approx. prevalence can be estimated by considering the risk of exposure or infection. Performing testing in symptomatic patients, contacts of confirmed cases are recommended. In settings where PPEs are not readily available, frontline health care workers may also be considered at high risk of exposure.
38.	With new diseases such as covid19 with unknown e prevalence, can focus be placed on the sensitivity and specificity for diagnostic accuracy?	Both accuracy and predictive values are important. There is no point in generating false positive or negative results that lead to the wrong public health measures being put into place.
39.	With most countries rushing to adopt the use or RDTs for Covid-19 with unknown prevalence of the disease, are the not effects to watch out for?	Please watch the presentation.
40.	The prevalence of ovid-19 is currently unknown for most country, and the infection dynamics keeps changing. What is basis for determining PPV and NPV values in such an evolving situation?!	True. This will be a challenge. It will be good to consider targeted groups where the prevalence maybe clearly high.
41.	Why does sensitivity and specificity depend on the prevalence of an infection?	They do not. NPV and PPV depend on the prevalence.
42.	Can the use of serological assays be optional in Africa due to the low prevalence in most countries, (less than 1%) since the PPV will be below 30%?	Serology testing is not useful in a low prevalence setting such as in the general population. It's important to consider targeted groups like health care and front line workers and also those arriving at borders and airports with fever.
Cross cutting		
43.	In an emergency like COVID-19, can we take <95 % confidence interval for potential test to be used?	Depend what the test is used for and the expected prevalence of infection.

SN	Questions	Answer/ Response / Comments
44.	Timely diagnosis and scaling-up testing in light of COVID -19 is critical globally. What is the likelihood of having a reliable serology test with antigen detection capacity?	These tests are being evaluated
45.	Is there some data on durability of antibody responses to SARS-CoV-2? How long after onset of symptoms can RDTs or ELISAs be used for Sero-surveys?	Variable duration at the moment. IgG may last for weeks. Serology tests may optimally be used 7 days after onset of symptoms
46.	What is your opinion of immunity passports?	Immunity passports are unlikely now. More data are needed on the durability of IgG response and the correlation with protective immunity.
47.	In the airport scenario that we saw earlier. Is Ok to hospitalize positive cases. What about those testing negative?	If there has been a confirmed case on the plane, then even those who test negative should be quarantine for 2 weeks and followed up. If they show symptoms they should be tested.
48.	Since this is a respiratory disease attacking the mucosa of the respiratory system, is there any data on mucosal antibody response available?	No data available yet on this.
49.	A patient who is tested negative for COVID 19 RT-PCR but have medical history and may develop symptoms may be quarantined and repeat testing recommended.	The rule is to quarantine all those who are exposed for at least 7 days. Test with PCR and if they are negative, they are good to go.

Resources

1. Cohen et al. STARD 2015 Guidelines for Reporting Diagnostic Accuracy Studies: Explanation and Elaboration. BMJ Open 2016 Nov 14;6(11):e012799. doi: 10.1136/bmjopen-2016-012799

   https://pubmed.ncbi.nlm.nih.gov/28137831/

   https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4623764/
2. Some persons have been observed to recover more than once from the COVID-19 infection. For instance Dybala (https://www.thecable.ng/finally-dybala-recovers-from-covid-19-after-testing-positive-four-times)