Laboratory Testing Recommendations for COVID-19

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Approach to available Laboratory Assays:

SARS-CoV-2 is rapidly emerging globally and accurate diagnosis of COVID-19 infection is an important requirement for management and control of infection. There is also widespread interest and demand for implementation of rapid reliable lab testing capacity in Pakistan. This document is intended as guidance towards use of various laboratory test based on current knowledge of SARS-CoV-2 virus and available diagnostic tests.

Molecular Methods:

a. **SARS-CoV-2 diagnosis and sample handling requires biosafety level 2 conditions and use of a Class II Biosafety cabinet for specimen processing for PCR based testing [1]**

b. Routine confirmation of COVID-19 cases is based on detection of COVID-19 virus nucleic acid (RNA) by real time RT-PCR assays. RNA can be extracted from samples such as oropharngeal/ nasopharangeal swabs, nasals swabs/secretions, broncho-alveolar lavage fluid/ washings or sputum, using any standard extraction protocols or kits. In general, the sample lysis step in RNA extraction inactivates any live virus. Thus, lysed samples are generally considered non-infectious. Sputum samples require liquefaction prior to molecular extraction [2] while tissue samples require lysis and homogenization.

c. Molecular detection protocols are available at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance[3]

Implementation and interpretation:

a. Most authorities recommend that laboratory confirmation of cases should be based on detection of two different genetic targets (E-gene followed by RdRP);

b. Once COVID-19 virus circulation is established and widespread in a given area/country, it is not necessary to run the PCR for both genes. Thus, confirmation through the detection of a single genetic target can be implemented, if the curves and other quality assurance parameters are optimal. Either E or RdRP genes can be used for the diagnosis; nevertheless, the E gene PCR has demonstrated slightly higher sensitivity, so we recommend prioritizing the E gene as the selected target.

Causes of False Negative & False Positive Results:

False Negatives	Suggested Measures	False Positives	Suggested Measures
Poor sample quality, handling, transportation and/or storage	Qualitative detection of a human housekeeping gene [e.g.,RNasePin CDC assay{4}]	Sample contamination during handling	- Clear SOPs for PPE changes between samples - Decontamination procedures
Poor/failed sample extraction	Use of extraction control	Low specificity kits	- Use validated assays/kits
Time of sample collection; very early or very late during infection	Sample collection in optimal period	Cross contamination of samples	- Use dedicated areas to prepare PCR Master mix Always include appropriate **positive** and negative controls

Considerations for Point-of-Care (POC) Tests:

a. Biosafety requirement for sample handling in appropriate containment (Biosafety Cabinet) must be fulfilled
b. Use of Molecular (PCR) based assays only
c. IVD with the following Regulatory Status:
    - EU regulatory status such as CE marked
    - FDA approved/notified
    - Asia Regulatory Status

There are a number of POCT testing equipment that are currently being validated at the National Institute of Health Pakistan based on comparison against established/validated methods, and may be deployed to the field in consideration of applicable biosafety considerations once validated.

Rapid diagnostic tests (RDTs):

There are no rapid diagnostic tests (immuno-chromatography or colloidal gold detection) that have been authorized by competent regulatory authorities and/or have been formally validated. In general, these types of tests have low sensitivity. Therefore, their positive predictive value is good (they can be used to rule in the cases), but their negative predictive value is low (they should not be used to rule out cases).

Also, the limitations described above for serological tests and antigenic detection apply to RDTs.

Serological methods:

Several assays (both ELISA and rapid diagnostic tests) are available for the detection of IgM / IgG antibodies and are marketed for the detection of COVID-19 virus infections [5,6]. However, their use alone for diagnostic purposes is NOT recommended;

a. During the first 6-7 days from the onset of symptoms, less than 40% of patients have detectable level of antibodies (IgG and IgM). Thus, serological tests should not be used to rule out a case during the first days of illness.
b. Cross-reactivity with other corona viruses that are normally present in the community and that make the interpretation of results difficult (19).
c. Detection of antibodies after day 7 only indicates previous contact with the virus but does not confirm presence and shedding of the virus.
d. It is advisable to monitor change in antibody titres 2 week apart.
e. Serology based negative tests should be backed up with PCR testing.

Considerations for Serological antibody testing:

a. Sero-epidemiological studies.
b. Population based disease prevalence studies to inform public health measures.
c. Research purpose.
d. Regulatory Considerations such as IVDs with at least one of the following recommendations:
    - EU regulatory status such as CE marked
    - FDA approved/notified
    - Asia Regulatory Status

Annex 1: Summary of the laboratory testing methods for COVID-19 (SARS-CoV2)

Test	Turn- around time	Availability of IVD	Application to public health diagnostic facilities
Real-time PCR or molecular assays for the viral nucleic acid	6-8hrs or Less (Some POCT tests can shorten turnaround times. Xpert COVID(Cepheid) upto 45 minute/test or ABBOTT ID NOW System; 5 – 13 minutes)	Several IVDs commercially available and use should be guided by laboratory validation of manufacturer claims	Pharynx and oral swabs specimens collected during 3-10 days since the disease onset. Positive results from multiple specimens are diagnostic. Specimen quality exerts a major impact on the results
LAMP & LAMP-based Covid-19 Near-Patient Assay	1 hr	https://www.rapidmicrobiology.com/news/lamp-based-covid-19-near-patient-assay-provides-results-in-one-hour. Several IVDs commercially available and use should be guided by laboratory validation of manufacturer claims	Similar to PCR tests, however this test does not require a thermal cycler. However, there remain concerns about biosafety and specimen handling as well as potential to scale-up
ELISA & fluorescence detection for IgG & IgM	4 hrs	Yes (Research Use only)	The method may be applied to mid and late stage diagnosis but is most useful in epidemiological studies / seroprevalence studies and research.

References:

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