Testing Recommendations for COVID-19
Updated on 16th September 2020

I. 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. **This update includes recommendations for Rapid Antigen Based Testing.**

II. 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 oropharyngeal/nasopharyngeal swabs, nasal 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

III. 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 followingRegulatory 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.

IV. Antigen based Rapid Diagnostic Test (Ag - RDT) for SARS-CoV-2:

Antigen based tests are immunoassays, which can detect specific viral antigen in the samples and helpful in determining the acute viral infections such as Influenza, RSV and more recently SARS-CoV-2. Most Ag-RDTs for SARS-CoV-2 use an immune-detection method employing a simple-to-use lateral flow test format commonly used for HIV, malaria and influenza testing. Ag-RDTs devices includes plastic cassette with sample and buffer wells, a nitrocellulose matrix strip, with a test line with bound antibody specific for conjugated target antigen-antibody complexes and a control line with bound antibody specific for conjugated-antibody. For SARS-CoV-2 RDTs the virus' nucleocapsid protein is often the target analyte because of its relative abundance.

In US, antigen tests have been authorized using nasopharyngeal or nasal swab specimens placed directly into the assay's extraction buffer or reagent and there is no age restriction on its use. Antigen based tests are relatively inexpensive and can be used at the point-of-care due to quick turnaround time. Antigen based tests for SARS-CoV-2 are generally less sensitive than assays that detect nucleic acid using reverse transcription polymerase chain reaction (RT-PCR). However, proper interpretation of antigen test results is important for reliable diagnosis and clinical management of patients with suspected COVID-19, or for identification of potentially infected persons for screening purposes.

As such there are limited data to guide the use of rapid antigen tests as screening tests on asymptomatic persons to detect or exclude COVID-19, or to determine whether a previously confirmed case is still infectious. Data on the sensitivity and specificity of currently available Ag-RDTs for SARS-CoV-2 have been derived from studies that vary in design and in the test brands being evaluated. Studies have shown that sensitivity compared to RT-PCR in samples from upper respiratory tract (nasal or nasopharyngeal swabs) appears to be variable, the sensitivity of approved antigen based FDA tests ranges from 84.0% to 97.6 % but specificity is consistently reported to be higher (>97%).[9]

<u>WHO recommendations for COVID-19 Ag-RDTs:</u>

a) To respond to suspected outbreaks of COVID-19 in remote settings, institutions and semi-closed communities where RT-PCR test is not immediately available. It is important to note that positive Ag-RDT results from multiple suspects is highly suggestive of a COVID-19 outbreak and would allow for early implementation of

infection control measures. Efforts to arrange confirmatory testing of all samples giving positive Ag-RDT results (or at least a subset) should be undertaken.

b) In setting with RT-PCR based confirmed COVID-19 outbreaks, Ag-RDTs could be used to screen at-risk individuals and rapidly isolate positive cases (and initiate other contact tracing efforts) and prioritize sample collection from RDT-negative individuals for PCR based testing.
c) To monitor trends in disease incidence in communities, and particularly among essential workers and health workers during outbreaks or in regions of widespread community transmission where the positive predictive value and negative predictive value of an Ag-RDT result is sufficient to enable effective infection control.
d) Where there is widespread community transmission, RDTs may be used for early detection and isolation of positive cases in health facilities, COVID-19 testing centres/sites, care homes, prisons, schools, front-line and health-care workers and for contact tracing.
e) Ag based RDT are not recommended for use in the following situation:
    - In individuals without symptoms unless the person is a contact of a confirmed case
    - Where there are zero or only sporadic cases
    - Appropriate biosafety and infection prevention and control measures (IPC) are lacking
    - Management of the patient does not change based on the result of the test
    - For airport or border screening at points of entry
    - In screening prior to blood donation

General recommendations for the use of SARS-CoV-2 Ag-RDTs:

- SARS-CoV-2 Ag-RDTs that meet the minimum performance requirements of ≥80% sensitivity and ≥97% specificity compared to a NAAT reference assay can be used to diagnose SARS-CoV-2 infection in a range of settings where NAAT is unavailable or where prolonged turnaround times preclude clinical utility.
- To optimize performance, testing with Ag-RDTs should be conducted by trained operators in strict accordance with the manufacturer's instructions and within the first 5-7 days following the onset of symptoms.
- Clinicians and public health personals must be aware about the characteristics of such assays to recognize potentially false negative or false positive results and to guide patient as well as outbreak management.
- Regulatory Considerations such as IVDs with at least one of the following recommendations are applicable for all facilities that undertake serological testing:
    - EU regulatory status such as CE marked
    - FDA approved/notified
    - Asia Regulatory Status and marking

V. 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 generally not recommended for the following reasons;

a. Serological tests are not diagnostic/confirmatory tests with varying degree of sensitivity and specificity
b. 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.
c. Cross-reactivity with other coronaviruses that are normally present in the community and that make the interpretation of results difficult (19).

d. Detection of antibodies after day 7 only indicates previous contact with the virus but does not confirm presence and shedding of the virus.
e. It is advisable to monitor change in antibody titres 2 week apart.
f. Serology based negative tests should be backed up with PCR testing.

Considerations for Serological antibody testing:

In view of the emerging situation of COVID-19 in Pakistan, the previous guidance has been updated with the following comments;

a. Antibody tests may be carried out for individual cases, community and research purposes.
b. For individual cases, serological test can provide evidence of previous exposure to SARS-CoV-2.
c. At community level, serological tests may be used for Sero-epidemiological or sero-prevalence studies to inform public health measures using either rapid testing devices or laboratory based testing.
d. Laboratory based serology tests may be considered as supplementary tests to RT-PCR based testing for private sector laboratories as they are more reliable than the rapid testing devices.
e. Regulatory Considerations such as IVDs with at least one of the following recommendations are applicable for all facilities that undertake serological testing:
    - 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	4hrs	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.
Ag-Rapid Diagnostic Test (RDTs)	15 minutes (approx.)	Several IVDs available	Early sampling for outbreak detection and response

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