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Letter to Editor

Microbiology Section

COVID-19: A Laboratorian's Perspective

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Dear Sir,

Coronavirus Disease 2019 (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is diagnosed by real-time Reverse Transcription-Polymerase Chain Reaction (rtRT-PCR), which detects the viral RNA. Rapid collection and testing of appropriate specimens from patients meeting the suspect case definition for COVID-19 is a priority for clinical management and outbreak control and should be guided by a laboratory expert [1]. While hospitals are striving hard to manage the increased caseload, laboratory professionals are also facing hurdles in the background.

Minimum Criteria for Government and Private Laboratories to Commence COVID-19 Testing [2]

For rtRT-PCR

- Biosafety Level 2 (BSL-2) level laboratory: This facility should be equipped with a Molecular Biology setup for virological diagnosis.
- Biosafety cabinet: The laboratory should have a type 2A/2B/III biosafety cabinet.
- Centrifuge: A cold centrifuge should be present in the laboratory for RNA extraction.
- Thermal cycler: This is absolutely essential for carrying out rRT-PCR.
- Staff Requirements:
- Medical microbiologists: One or more with experience in Molecular Virology.
- Technicians: Atleast 4-6 with relevant experience in Molecular Virology.
- Institutional biomedical waste management policy: An effective and functional policy for management of biological waste products in the institution should be in place.
- National Accreditation Board for Testing and Calibration Laboratories (NABL) accreditation: All applicants from Private Medical Colleges must submit a copy of the NABL accreditation certificate and scope of accreditation for carrying out rRT-PCR for RNA viruses.

Cartridge Based Nucleic Acid Amplification Test (CB-NAAT) [3]

- Government laboratories: These laboratories must meet the above criteria before initiating testing using CB-NAAT.
- Private laboratories: These laboratories must have NABL accreditation for molecular detection of RNA viruses (either by rtRT-PCR or CB-NAAT) before initiating testing using CB-NAAT.

What are the Samples to be Collected for Viral Detection? [4]

The following samples are to be collected:

- Upper respiratory tract samples: These include nasopharyngeal/oropharyngeal swabs and nasal swab.
- Lower respiratory tract samples: These include sputum and Bronchoalveolar Lavage (BAL).

What are the available tests? [4]

The main tests that are currently available include the following:

- Whole genome sequencing: Sequencing was used primarily in the early days of the outbreak for initial identification of this novel virus and is largely a tool for viral discovery.
- Molecular methods: These include the following:

a. **RT-PCR:** This test targets the structural proteins, namely, membrane (M), nucleocapsid (N), envelope (E) and spike (S). Species- specific genes for SARS-CoV-2 are also targeted. These include RNA dependent RNA polymerase (RdRp), Haemagglutinin Esterase (HE), and open reading frames (*ORF 1a* and *ORF 1b*) genes. The Centers for Disease Control and Prevention (CDC), Atlanta, recommends demonstration of two nucleocapsid targets N1 and N2, whereas the World Health Organisation (WHO) recommends first line screening for E gene followed by confirmation by detecting RdRp gene. A cycle threshold value (Ct-value) less than 40 is defined as a positive test, while a Ct-value of 40 or more is defined as a negative test [4].

b. **True Nat:** This is a chip-based RT-PCR test for detection of the E gene of β-coronaviruses. It is employed as a first line screening test. The E gene is common to all Sarbecoviruses. Hence, the testing laboratory must be mapped with an existing laboratory registered with the Indian Council of Medical Research (ICMR) for RT-PCR [5].

c. **CB-NAAT:** The Xpert Xpress SARS-CoV-2 test is a self-enclosed, cartridge based, molecular invitro diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on nucleic acid amplification technology targeting the E and N2 genes [6].

Serological Methods

The antibody responses are non specific and do not play a role in active case management. Rapid tests to detect IgM and IgG antibodies are of poor sensitivity and are currently being used in India only for surveillance purposes [7]. Serological diagnosis may be employed for seroprevalence and epidemiological studies, identification of individuals with prior COVID-19 infection to serve as convalescent plasma donors, and to monitor immune response during vaccine trials [8]. Standard Q COVID-19 Antigen detection assay available in India has high specificity and low sensitivity and ICMR recommends it to be used as a point of care diagnostic assay in combination with RT-PCR in containment zones and in healthcare facilities [9].

Validation Studies and Method Verification [10]

The United States Food and Drug Administration (USFDA) recommends testing of a minimum number of 30 positive and 30 negative samples for validation studies under the Emergency Use Authorisation (EUA). A 95% positive and negative agreement is considered to be acceptable clinical performance.

Logistical Issues in COVID-19 Testing [11]

There are several logistical issues associated with testing for COVID-19. These are briefly highlighted below:

a. **Shortage of testing kits:** Despite being approved by the ICMR for COVID-19 testing, there have been difficulties due to shortage of testing kits. Even after many days of obtaining approval, laboratories did not receive the testing kits and viral transport media due to the lockdown and suspension of international flights, leading to a great backlog in clearing the samples that awaited results, which in turn, increased the Turn-Around-Time (TAT).

b. **Equipment and medical supplies:** Laboratories approved for testing competed with the providers due to limitation of supplies. Procuring other supplies, such as disinfectants and cleaning agents, and restocking them once the supplies were exhausted also posed a challenge.

c. **Shortage of Personal Protective Equipment (PPE):** PPE is mandatory for testing personnel. Procuring PPE for all the frontline hospital workers has been a challenge due to lack of a robust supply chain.

d. **Personnel competency and training:** The suspension of public transport made it difficult to allocate staff for testing as the system was forced to function with skeletal manpower. Despite the difficulties, online training made it possible to initiate the functioning in a smooth manner.

e. **Quality control:** There have been constraints in procuring reference materials due to delay or cancellation of shipments as a result of suspension of transportation by land and air.

It has been and still is a testing time for laboratories across the globe during the ongoing pandemic in which millions have been confirmed to be affected so far and the true number assumed to be much higher. Governments have rightly waived the regulatory requirements and permitted the use of kits under the EUA. The onus lies on the laboratory professionals to make an informed decision on the choice of the methodology, quality assurance, and management of logistical issues in order to provide the correct report in a timely manner. Let us work together to combat this contumacious coronavirus by comprehensive strategies and contain its spread to save the community!

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