

Why is SARS-CoV-2 testing not possible in every medical laboratory?

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Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) spread from Wuhan, China, to the whole world and became a pandemic, spreading to every continent. The number of new cases is now more in Italy, Spain, Germany, France, and the USA than China. Europe is now the new epicenter.^{[1],[2]}

Reporting of new cases largely depends on the testing for SARS-CoV-2. The first quantitative reverse transcriptase-based polymerase chain reaction (RT-PCR) was designed and distributed in January by the World Health Organization (WHO), soon after the virus was identified.^[3] The test protocol is complex and expensive, and is mainly suited for specialized molecular laboratories. The Government of India assigned 52 testing centers at various government medical institutes and hospitals all over India. Later on, seeing the increasing testing load, NABL-accredited molecular biology private laboratories were also given approval for testing.^[4] What makes the testing of this virus so difficult that all the laboratories have not been allowed to test?

The screening and confirmation of CoV-2 requires testing for viral RNA by real-time RT-PCR. The WHO recommends all such testing in Biosafety level-2 (BSL-2).^[5] These labs must be equipped with biosafety cabinets, PCR cabinets, and *in vitro* diagnostic use (IVD) real-time PCR thermocyclers. All these systems must be calibrated and must meet quality standards such as CE, NSF/ANSI 49, TUV, or EN 12469. These instruments incur a huge cost to the laboratory. PCR thermocyclers may itself cost between 15 and 20 lakhs Indian rupees (INR). Certified biosafety and PCR cabinets are expensive too.

Laboratory design is a key element of quality assurance for molecular assay routine testing in clinical laboratories. It requires three physically separate areas for reagent preparation (clean room), sample preparation (extraction room), and amplification room. Unidirectional workflow is required across these rooms. Even with closed systems, sample preparation has to be performed in a Class II biosafety cabinet. Class I safety cabinets do not provide protection for material contained within them. PCR setup cabinets are also required for PCR setup. Other recommended laboratory design elements for amplification assays include separate ventilation systems for pre- and post-amplification areas; maintaining negative pressure in the postamplification area; and dedicated laboratory coats (long sleeved) to specific areas and changing when entering or leaving each area (or disposables used in each area).^[6]

Separate pipettors are required for reagent preparation, specimen preparation, and postamplification analysis. Then, all the pipettes are to be used with aerosol-guarded (filter) pipettor tips to prevent the contamination of pipettor barrels by aerosols. Gloves are to be changed between each step, or more often as needed, and when entering or re-entering separate areas. Surface decontamination daily with 10%–20% bleach, followed by ethanol or clean distilled water, is a must. Monthly swipe tests of exposed surfaces and equipment is required to keep a check on cross contamination. Minor steps such as spinning of tubes to force any liquid down from the sides, before removing caps, are necessary. Tubes have to be uncapped carefully to prevent aerosols and recapped as soon as transfers are completed. Master mix and other nonsample components to the reaction tubes must be added before adding the sample.^[7]

RNA is particularly vulnerable to degradation with contaminating RNases. Samples should be exposed to stabilizing

agents as quickly as possible. Optimum storage is -70°C , preferably in ethanol. Plastic tubes should be sterile, hydrophobic, and not handled with ungloved hands as RNases are present on skin and hair. Because quality at each level is necessary in a molecular lab, a quality control program is a must. Evidence of instrument calibration, maintenance, repairs, and servicing is, therefore, necessary.^[7]

Running a virology molecular laboratory, thus, needs a well-trained and experienced clinical microbiologist or virologist and technicians with experience in molecular biology.

In-house developed extraction methods may show inter-lab variability because of which they cannot be relied on. Only few viral RNA extraction kits have been approved by the US Food and Drug Administration (FDA) and Centers for Disease Control and Prevention for RNA extraction.^[7] Recently, few companies have developed molecular kits which contain ready-to-use master mix, primers, and probes for novel SARS-CoV-2. Some of them have been evaluated by the Indian Council of Medical Research (ICMR), India. My Lab (India) and Altona Diagnostics (Germany) are two such kits which gave 100% true-positive and true-negative results.^[8] However, these kits also need an IVD-approved real-time PCR and a BSL-2 laboratory. Few closed systems have recently been approved by the US FDA for emergency testing, such as Xpert Xpress SARS-CoV-2 by Gene Expert (Cepheid, USA) and Abbott Laboratories (USA) 5-min point-of-care tests (Abbott ID NOW COVID-19) with a portable thermocycler.^[9] However, these assays have been approved in emergency, and their sensitivity and specificity in real scenario is yet to be evaluated. These systems are closed systems, and kits provided by these companies shall only be used on those systems. However, sample processing will still require a Type II A II biosafety cabinet.

Rapid lateral flow-based immunoassays other than molecular assays, such as IgM and IgG antibody based, have also been approved by the ICMR, and these deliver the results in 20–60 min. However, these assays lack sensitivity and specificity in comparison to real-time PCR, which remains the gold standard to diagnose novel SARS-CoV-2 infection.^[10]

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