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TESTING FOR COVID-19 IN INDIA

Relevant for: Developmental Issues | Topic: Health & Sanitation and related issues

Real-time reverse transcription-polymerase chain reaction (RT-PCR)-based tests are recommended as diagnostic tests for COVID-19. The test detects the presence of viral RNA in human samples. PCR is a process where a few copies of DNA are amplified to produce millions of copies. In addition to human samples, the test needs primers, probes, enzymes, nucleotides, enzyme cofactors, and buffer solution. Primers and probes are short stretches of DNA, specific and complementary to regions of the viral genome or target region. Unlike primers, probes are labeled with fluorescent molecules that bind the target region in between where the forward and reverse primers bind. The test uses two primers (forward and reverse) and one probe against each target region of the viral genome. The WHO recommends using at least two target regions for the diagnostic detection of the virus. In the test, first, the viral RNA is converted to DNA. The DNA is then amplified with the help of enzymes, primers, and probes. Each step of the test uses multiple cycles of target melting, primer binding, extension, and probe dissociation. The steps are repeated for 15-40 cycles to produce millions of copies of the viral target DNA, each with its fluorescent signal, which is read by an instrument.

Although RT-PCR tests are quantitative, sensitive, and specific, assay and quality-control measures are essential to get consistent and accurate results. A positive control verifies that the assay is running as intended and identifies authentic negative samples. An internal control checks the process of RNA extraction from the nasal or throat swab and identifies sample-to-sample variation. A negative extraction control verifies the absence of cross-contamination during RNA extraction and RT-PCR reaction set up. Additionally, no amplification control and no template/ negative control are essential in the RT-PCR set up to reduce erroneous test results. Labs around the world are using primers and probes against different SARS-CoV-2 genes/regions in their assays successfully.

The main difference between tests done in a research lab and a diagnostic lab is that the results from the latter are used for clinical decision-making. Therefore, the diagnostic labs need to be certified and follow the standard operating procedures (SOP), maintain directionality of sample flow, and make sure that the lab personnel are properly trained. Also, all diagnostic labs need to maintain documentation on all tests and reporting. RT-PCR results can vary from lab to lab due to many variables. Hence, regulatory agencies mandate the use of SOP, specific lot numbers of reagents and instruments, and software to run diagnostic tests.

The Indian Council of Medical Research (ICMR) has approved 176 labs, including 47 private labs, to conduct the tests. The U.S. Food and Drug Administration (FDA) has approved 20 manufacturers and kits for diagnostic testing for COVID-19. The first test kit that received FDA approval, the Cobas SARS CoV-2 kit from Roche, is also approved in India. The second, the TaqPath COVID-19 Combo Kit from Thermo Fisher, is in the process of getting validated by the ICMR labs. Both these kits are optimised with specific instruments. Additionally, ICMR has validated kits from three Indian manufacturers. Although ICMR has stated that the agency will provide the primers, probes and master mix for RT-PCR to the select 123 government labs, it is unclear whether they are the same primers and probes as per the U.S. CDC protocol or the agency has designed its own. For private labs, ICMR has issued an advisory and recommends the use of any FDA-approved kits with primers and probes. As long as proper certification and training are in place, any accredited lab using the WHO/CDC suggested protocols can yield satisfactory results.

Currently, the import of some critical reagents, especially the probes, makes the tests expensive

to offer in India. India needs to develop the capability to mass-produce kits and all its components within the country. While developing and use of indigenous kits is beneficial in the current outbreak, it cannot take short cuts. While prioritising indigenous kit development, it must ensure that sensitivity, specificity, accuracy are in place before the kits get approved for broader use.

India also needs to invest in developing alternate assays, like serological assays to detect the antibodies IgG and IgM in blood, to test for the infection. Unlike the RT-PCR test, the antibody-based test can help identify individuals who got cleared of the viral infection but were once infected. Antibody tests with similar accuracy and specificity as the RT-PCR test are needed for mass screening. Innovative tests will take interdisciplinary scientific effort and investment. Till then, India can use the existing tests in labs with accreditation and rigorous training to detect each of the COVID-19 cases accurately.

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