Testing for Severe Acute Respiratory Syndrome Coronavirus 2: Unraveling the Conundrum

Cluster of cases of pneumonia were reported from Wuhan (Hubei, China), which led to the identification of novel coronavirus coronated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] The World Health Organization (WHO) issued the first technical guidance online with advice to all countries on how to detect, test, and manage potential cases of disease named as coronavirus disease-19 (COVID-19) on January 10, 2020.[2]

The WHO then declared it a public health emergency of international concern on January 30, 2020 and a pandemic on March 11, 2020, by which time the infection had already spread to 114 countries.[3] Currently, the pandemic has widely spread to all the continents and 230 countries with a total of approximately 16.3 million infected and 0.65 million dead as on July 26, 2020.[4] India has reported around 1.4 million cases and approximately 32,800 deaths on the same date.[5]

This pandemic due to novel coronavirus has numerous gaps in understanding regarding the agent (characteristics, infectivity, and transmission), host (susceptibility, symptomatology, progression of disease, natural history, and outcome), and environmental factors responsible for transmission and spread. Structurally, SARS-CoV2 is a nonsegmented, enveloped, positive-sense single-strand RNA virus in Orthocoronavirinae subfamily with a diameter of about 65–125 nm with crown-like spikes on the outer surfaces.[6] The genetic sequence of virus was made public by China on January 12, 2020.[7]

Initially, since it is a novel disease, no confirmatory tests were available. However, due to available technology, a plethora of tests have become available within a short span of time. As on July 24, 20, 751 SARS-CoV-2 laboratory tests for the diagnosis of COVID-19 have been listed by the Foundation for Innovative New Diagnostics, which is the WHO Collaborating Centre for Laboratory Strengthening and Diagnostic Technology Evaluation.[8]

These tests may broadly be divided into three types: molecular tests (RT-PCR, TrueNAT, and CBNAAT), antigen testing, and antibody testing. The Centers for Disease Control and Prevention, Atlanta, USA, has classified these as virus tests and nonvirus tests. Another classification is molecular assay or immunoassay. Molecular and antigen testing detect virus directly and antibody is an indirect indicator. The molecular and antigen tests indicate the current infection, whereas antibody may indicate past (IgG) or recent (IgM) infection. Figure 1 shows the time relationship among symptoms, viral load, and positivity of diagnostic tests.[9]

At present, a wide variation exists among countries regarding the testing rates, as seen in Figure 2 depicting the daily COVID-19 tests per thousand population.[10]

The testing of the COVID-19 in India has been led and regulated by the Indian Council of Medical Research (ICMR). The initial testing was limited to the ICMR institutes and designated virus research and diagnostic laboratories; subsequently, the private laboratories were permitted testing. The expansion of laboratories and tests has been remarkable and guidelines have been issued/revised for TrueNAT-beta-CoV and cartridge-based nucleic acid amplification tests on April 14 and 19, respectively. Further, to ramp up the testing facilities, the Government of India will launch three high-throughput COVID-19 facilities on July 27 in Noida, Mumbai, and Kolkata.[11] On June 14, 2020, the range of tests was further increased by including the COVID-19 Ag detection assay. The sensitivity and specificity of the test of Standard Q COVID-19 Ag by SD Biosensor varied from 50.6% to 84% and 99.3% to 100%, respectively. The ICMR recommended its use as point of care diagnostic assay in combination with the gold standard RT-PCR test for various settings (containment zones or health-care settings). However, in view of low sensitivity and consequently high false negatives, the caveat is, "Suspected individuals who test negative for COVID-19 by rapid antigen test should be definitely tested sequentially by RT-PCR" (sic). This was further clarified in their letter dated July 16, 2020. The research is ongoing for an easy and convenient point of care test like spit test as these tests have been used earlier in Zika and Ebola in resource-poor countries.[12]

To this armamentarium, antibody tests were added. Indigenously developed IgG enzyme-linked immunosorbent assay (ELISA) claimed sensitivity of 92.37% and specificity of 97.9%.[13] Advisory for use of rapid antibody blood test was issued on April 4, 2020. Later on, newer additional strategies

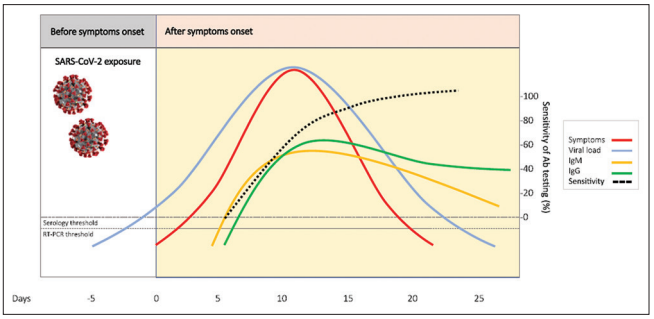

Figure 1: The time relationship between viral load, symptoms, and positivity on diagnostic test[9] (reproduced with permission)

were issued by the ICMR on June 23, 2020, wherein it was mentioned that IgG-based ELISA and chemiluminescent immunoassay assays be used only for surveillance and not diagnosis. The ICMR has also encouraged other manufacturers/developers to come forward for validation. However, there is a mismatch between the list of kits approved by the ICMR and the Central Drugs Standard Control Organisation, Drug Controller General of India.[14] Testing strategies too have evolved along with the pandemic as the initial testing restricted to risk-based approach and clinical symptoms due to limited capacity and availability of kits in the country and later expanding to on-demand testing. Capacity has been increased by approving 1000 COVID-19 testing laboratories (730 public and 270 private sectors) (RT-PCR laboratories 557, TrueNAT laboratories 363, and CBNAAT laboratories 80). The number of COVID-19 cases, tests conducted, and positivity is shown in Figure 3.

How much testing is sufficient for surveillance? The WHO in its definition of comprehensive surveillance has defined minimum testing of suspect cases to be in the order of 1/1000 population/week.[15] This translates to 142.86 (~143) tests/million/day. The number of tests done per day in India along with the number of new cases detected is given in Figure 3. The total number for India works out to be 195,338 tests/day. Epidemiological criteria for control of epidemic of less than 5% of samples testing positive for COVID-19 at least for the previous 2 weeks should be read with conjunction with the number of total cases and tests conducted. The WHO has also suggested a positive rate of around 3%–12% as general benchmark of adequate testing. This can also be interpreted as to how many tests are conducted by a country to find one COVID-19 case and translated to at least ten tests per confirmed case as a benchmark of adequate testing.[16] These guidelines are based on the analysis of data received from various countries. The guidelines are meant for minimal number and not for optimal number of tests, which should be decided by local epidemiological assessment and will be dynamic in nature.

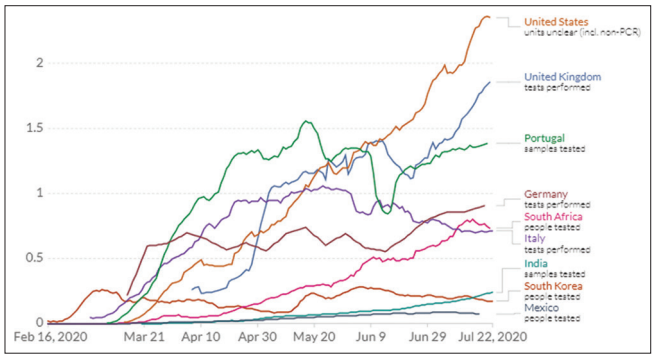

Figure 2: Daily COVID-19 test per thousand people (figures are given as a rolling 7-day average)[10]

What Algorithm is Best for Diagnosis?

RT-PCR remains the gold standard for diagnosis as on date. Although as per available literature, RT-PCR is capable of picking up a single RNA strand of virus, yet a false negativity rate of 41% has been reported, depending upon the technique and training of swab collector, timing of the swab, site of swab collection, expertise in handling swab, extraction in laboratory, etc.[9,17] The technical expertise, equipment, and time needed for results make it unsuitable for point of care diagnosis. Antigen tests have proclivity for use as point of care diagnosis. However, owing to their moderate sensitivity and specificity, the antigen-based test remains far from ideal to be used independently as point of care diagnosis and needs the crutches of RT-PCR. Thus, there is a scope and requirement for improvement in the point of care diagnosis and newer techniques and modalities like spit test may develop in the future.

The ICMR's guidelines dated June 23 regarding the use of antigen-based tests suggested that all negative tests on antigen-based test should be subsequently tested with RT-PCR. This could lead to operational problems and low yield, especially in community settings. To illustrate this further, in a setting of prevalence of 5%, if one is testing 200

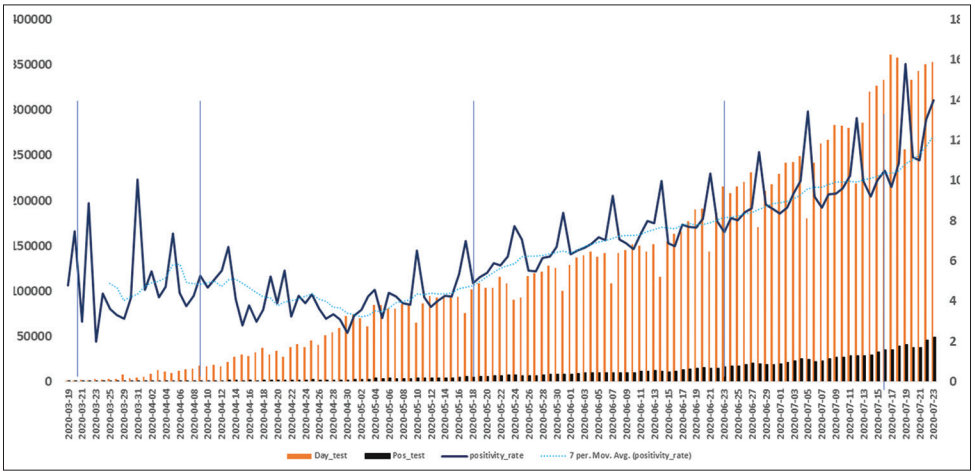

Figure 3: Testing status of India with positivity rate. Blue vertical lines: Date of new testing strategy by the Indian Council of Medical Research

persons and assuming sensitivity of antigen-based test as 50% and specificity as 99%, one has to retest nearly all (193, 96.5%) persons again with RT-PCR to get additional five tests (assuming sensitivity of 100% for RT-PCR). However, this was later modified so that only symptomatic negative antigen test be tested with RT-PCR before labeling the test results negative.

However, antigen-based tests might have a role in emergency setup where timeliness of the results is required. In a community/quarantine setting, due to its moderate sensitivity and low prevalence, its use to identify positive cases is not recommended for its low yield and high probability of missing the COVID-19 positive cases.

Among diseased people, the antigen test has correlated well with viral load, and its use for triage of patients is recommended by some authors.[9] However, the same needs to be validated in further studies.

Sero-surveys

Sero-surveys by antibody testing are important for identifying the extent of the spread in the community, for understanding natural history of disease, and risk stratification.[18] The findings of sero-surveys reflect the COVID-19 epidemiology of approximately 2 weeks ago. However, with latest trends in the number of new cases, it might provide good indication regarding the direction of the epidemic. As of now, there is no clarity on the duration of persistence of these antibodies as well as the level of protection offered as per their levels. Hence, serial sero-surveys in longitudinal studies are required to fill the knowledge gaps, especially for risk stratification, protection, etc., Various antibody kits are available in the market (card-based rapid, ELISA based) and users need to be aware of the approvals, validity, and other characteristics of each kit before using any of these. The currently neglected area of research is the role of cell-mediated immunity in COVID-19 which may be crucial in understanding the natural history and management of cases.

Way Forward

There is a rapid development in diagnostic methods over a period of few months. Various new ways of identification of virus either by modifying the existing ones or by newer techniques (gene editing) to identify the virus directly or indirectly are in pipeline.[19] Evidence-based clear guidelines regarding various tests are required; some of the tests may be used in combination (serial or parallel testing) to provide best results with increased efficiency. Research and innovation is the key for the implementation of COVID-19 tests, interpretation, and upscaling of the testing capacity, as till date, test, track, and treat remains our best bet to mitigate and control this novel virus pandemic.

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