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COVID 19 pandemic testing time – Crisis or opportunity in disguise for India?

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ABSTRACT

The current SARS-CoV-2 infection or the COVID 19 pandemic has taken the world by storm, where the best health care systems in the world seem to be overwhelmed and still this virus is eluding us as we are compelled to explore the preventive and/or therapeutic interventions to control the disease outbreak as well as to prevent deaths. In parallel to clinical services, laboratories have been overwhelmed with task of keeping up with ever increasing demand for testing. Real time PCR detection of COVID19 is the gold standard method, however, has certain shortcomings in terms of availability of infrastructure, reagents, consumables, and technical expertise. All these have paved the way for the alternative testing algorithms and strategies. Countries like United States and Italy have struggled with these issues. India has been criticized for not testing enough and not adopting the right policy, but has been managing the disease within its resource limited health care system to a fair extent.

The present review provides the Indian perspective of COVID 19 testing, the journey from not testing enough in the past to a vast expanse and depth of testing in present time.

Overview of COVID 19 pandemic

Corona Virus causing global pandemic was unknown to the world till December 2019, when a cluster of patients with pneumonia like symptoms were reported in Wuhan, China. The causative pathogen was subsequently identified as severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2), a newly described betacoronavirus is the seventh known corona virus to infect humans [1], responsible for the Corona Virus Disease (COVID19).

Globally, as of August 2, 2020, there have been 17,660,523 confirmed cases of COVID-19, including 680,894 deaths, reported to WHO [2]. This infection has spread to more than 216 countries, areas, or territories (https://covid19.who.int/).

The reasons for the pandemic spread include high transmissibility of the virus, the apparent absence of any cross-protective immunity from related viral infections; and delayed public health response measures [1]. Although SARS-CoV-2 infection seems to occur with mild, influenza-like symptoms in the vast majority of subjects, in 10%–15% of COVID-19 patients (especially the older and those with important co-morbidities), the disease may progress into a severe form of pneumonia, which may then evolve toward acute respiratory distress syndrome with a mortality of 2%–5% [3].

In the last few months, the world has witnessed the struggle of best health care systems such as Italy and United States in COVID 19 patient care management. The primary goal of epidemic containment is to reduce disease transmission by reducing the number of susceptible persons in the population or the basic reproductive number (R0). This number is modulated by such factors as the duration of viral shedding, the infectiousness of the organism, and the contact matrix between infected and susceptible persons [4]. Given the lack of effective vaccines or treatment, the only currently available lever to reduce SARS-CoV-2 transmission is to identify and isolate people who are contagious.

Although excellent tools exist for the diagnosis of symptomatic patients in well-equipped laboratories, important gaps remain in screening asymptomatic persons in the incubation phase. Also for the accurate determination of live viral shedding among patients in the convalescence phase to implement deisolation decisions is an issue

This brings us to address the most pertinent issue of how laboratories in India have handled this pandemic and their perspective. Always the first course of action is the right diagnosis of the disease. It is impossible to test everyone using sophisticated tests viz. Real time Polymerase chain reaction (RTPCR) in the second most populous country in the world like India. Mass screening of the

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population was done using available options like thermal scanners, recording the travel history, and vigorous contact tracing. In a pandemic situation while racing against clock, providing the right and timely diagnosis poses the next set of challenges.

The testing methods

What to test?

Virus isolate is the gold standard for establishment and standardization of assay performance. Since SARS-CoV-2 virus isolate was not available earlier, based on the genetic sequence of SARS-CoV-2 and closely related SARS-CoV (2002-2003), the World Health Organization (WHO) shared protocols (E, N, RdRp, and S genes) for screening and confirmation of probable cases, and RT PCR was considered as the gold standard for rapid nucleic acid amplification of the SARS-COV-2 [5].

Adopting diagnostic testing guidelines

The WHO has published testing guidelines that are being periodically updated. (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technicalguidance/laboratory-guidance). According to these guidelines, clinical and epidemiological factors linked to an assessment of the likelihood of infection should guide the decision to test but should be adapted to the local situation and national policy for testing. Nucleic acid amplification testing (NAAT) for detecting COVID19 is recommended and is considered as the gold standard for diagnosis. The test should be performed on acute or symptomatic cases. The testing should also be considered for asymptomatic or mildly symptomatic contacts of the COVID 19 positive patients.

The infrastructure

The nonpropagative work of NAAT should be performed in BSL 2 facility and propagative work- isolation of RNA from the viral transport medium (VTM), should be done in a Biosafety level (BSL) 3 facility.

A controlled ventilation system maintains inward directional airflow into the laboratory room [6]. Air must be High Efficiency Particulate Air (HEPA) filtered, if reconditioned and recirculated within the laboratory. When exhaust air from the laboratory is discharged to the outdoors, it must be dispersed away from occupied buildings and air intakes. This air should be discharged through HEPA filters.

The extraction and assay should be performed in designated Class II Biosafety cabinets with restricted access. The staff performing these assays should be trained in handling of infectious agents and should always wear appropriate personal protective equipment [6,7]

Guidance for the use of personal protective equipment and infection prevention and control was also provided by the WHO.

The laboratories performing these tests should be accredited for extraction of nucleic acids.

Samples include oropharyngeal and nasopharyngeal swab collected and transported in VTM from the patients presenting with symptoms of COVID19 infection in the fever Out Patients Department (OPD).

Sampling strategy

The availability of diagnostic test versus the ability to do virus isolation has influenced decision to do away with the viral culture as it is more time consuming and requires more advanced set up; however, the correlation between RNA shedding and infectivity requires further investigation [8].

Repeat sampling or a lower respiratory tract sample may facilitate the diagnosis of more severe cases and may be important if a patient has a clinical picture of viral pneumonia, and/or radiographical findings (chest computed tomography or magnetic resonance imaging scan) consistent with COVID-19 pneumonia or a potential exposure history. Consideration should also be given to the optimal specimen to exclude other respiratory pathogens [8,9].

COVID 19 detection assay- The gold standard

The major challenge in offering the diagnostic services is to choose which is the right kit, right platform and which method is the most robust and cost effective. Several RT-PCR protocols for detection of SARS-CoV-2 RNA have been recommended by the WHO. The most widely used 3 different testing approaches viz. The United States Centers for Disease Control and Prevention (CDC) protocol utilizes primers that target the viral N gene, while the China CDC uses primers matching both the N gene and the ORF1ab region, and Charité Germany primers target the RdRp and E genes [10,11]. Multiple commercial master mixes exist that enable sensitive one-step RT-PCR. The US CDC uses a one-step real time RT-PCR (rRT-PCR) assay, which provides quantitative information on viral loads, to detect the presence of SARS-CoV-2 [12].

Pre-analytical and analytical parameters affecting NAAT

The pre-analytical phase is the major source of errors in laboratory testing. The aim of swabbing is to sample the upper respiratory tract and sometimes the swab collection may not be tolerated well by patient leading to sample failure. Failure to comply with the recommended procedures (eg, use of wrong swabs, inappropriate absorption of diagnostic material, insertion into inadequate VTM, contamination, and so forth) may be a significant cause of diagnostic errors, as clearly reported for other viral diseases [3] According to recent evidence, the diagnostic accuracy of many of the currently available RT-PCR tests for detecting SARS-CoV-2 may be lower than optimal (ie, $<$100%).

The fact that RT-PCR testing may be initially negative in patients with SARS-CoV-2 infection, especially in those who will later develop overt COVID-19, is not really surprising considering the probable kinetics of SARS-CoV-2 infection. Reliable evidence suggests that the incubation period of SARS-CoV-2 is around 6 days (interquartile range [IQR], 2–11 days), and that the median period between symptom onset and hospital admission is 7 days (IQR, 4–8 days), whilst the median period of symptom duration is around 13 days (IQR, 5–24 days) [13], slightly longer in patients with severe disease [3]. The accuracy of RT-PCR can be substantially plagued by lack of harmonization (of primers and probes) [14] as well as by a variety of technical and analytical errors.

It is also important to note that negative RT-PCR test result does not completely rule out SARS-CoV-2 infection and shall not be used as single determining factor for patient management decisions and retesting shall be considered in consultation with public health authorities. Despite the urge to provide high throughput and short turnaround time for diagnosing SARS-CoV-2 infection, extensive validation of RT-PCR assay is compellingly needed to enable the adoption of the most appropriate public health measures on individual and population bases [15]. If an asymptomatic patient was infected with SARS-CoV-2 but has since recovered, PCR would not identify this prior infection, and control measures would not be enforced. Likewise, dead viral RNA may be detected in an otherwise recovering patient.

Evolution of COVID19 testing in India

As on March 31, 2020, the daily testing in India was close to approximately 5,500 tests across public and private laboratories

as per Indian Council of Medical Research (ICMR) database. Delays in testing had led to large disease cluster forming, unchecked progression of severe cases and a threat to overburdening of the health system with critically ill patients [16].

The immediate demand to offer these assays to diagnose patients and screen the mass population was a great challenge, as there were many governments run laboratories, private laboratories, and academic institutions involved. The lack of accredited laboratories for performing the COVID tests as per the WHO recommendations was the rate limiting step for most of the centers across the country, as infrastructure for handling such infectious materials was not readily available. Even when the funds were provided, setting up all these in a short period was quite a challenging task.

The next important challenge faced globally was availability and access to reagents. In the first few weeks of the pandemic, required reagents were already in short supply, and researchers and testing centers reported issues acquiring almost every necessary reagent from commercial suppliers—from nasopharyngeal swabs to lysis buffer to RNA extraction kits [17,18]. ICMR and Ministry of Health and Family Welfare (MoHFW) intervened timely to scale-up qRT-PCR-based testing capacity per day across public laboratories in India. Several laboratories resorted to amalgamation and repurposing of resources to cover the need for testing with microbiologist joining hands with molecular pathologist and/or basic researchers. The interventions ranged from optimizing the existing capacity of manual qRT-PCR instruments through multiple shifts and reduction in laboratory-level manual RNA extraction effort; deploying additional manual and automated machines from other public institutes and research organizations and procuring automated high-throughput instruments [5,19].

The strategies for optimization of testing

Government and private laboratories

The testing facilities with recommended infrastructure were approved by ICMR for performing the COVID 19 detection test. This was broadly categorized into 2 sectors: Government institutes and Private laboratories. The testing guidelines were laid down by ICMR and all the testing centers were needed to report the positive cases and total cases tested at local, state, and central level. This ensured uniformity of reporting and real time data collection. A total of 1,276 laboratories are authorized to perform the tests of which 892 are government laboratories and 384 are private laboratories as on July 22, 2020 [20].

Scaling up the testing capacity

The scaling up of testing capacity was the imperative requirement from public health point of view. During first week of March nearly 5,500 tests were being conducted and with a population of 130 crores in India, this number certainly was nowhere closer in assessing the phase of the infectious wave in the country.

Redeployment of RT-PCR systems

The qRTPCR from multidisciplinary Research Units and noncommunicable diseases diagnostic facilities were redeployed and allocated for COVID-19 testing.

Optimized working capacity

The existing RT-PCR systems were made to run from form 9 to 16 hours and then gradually moving it to 24 hours without any down time.

High throughput platforms capable of conducting up to 1,400 tests/day/machine which were used in combination with Emergency Use Authorization detection kits for COVD 19. The automated systems were also redeployed from National Aids Control Organization (NACO) and National Tuberculosis Elimination Program. Also point of care NAAT platform for National Tuberculosis Elimination Program was used for this purpose.

Introduction of combination testing kits

The Emergency Use Authorization or ICMR approved testing kits which can be used for screening and confirmation in one run on the same machine. This saved a lot of time spent in performing screening and confirmation tests separately.

Additionally, all private and government medical colleges were urged to create the state-of-the-art virology laboratories and support the country's fight against COVID-19 [19].

This multipronged strategy helped in improving the testing capacity and by July 22, 2020 nearly 14.724,546 people were tested with the daily testing capacity escalating to 343,243 tests per day [21].

Other ancillary diagnostic tests

Point of care testing (Rapid tests)

These tests are qualitative or semiquantitative in vitro diagnostics involving small or single quantities, non-automated procedures. These are usually antigen tests or antibody tests. The European Centre for Disease Prevention and Control (ECDC) [22] is working in close cooperation with the European Commission, member state authorities, FIND and WHO on validating rapid tests and will make the results available as soon as possible on the FIND website.

However, rapid antibody tests are not indicated for diagnoses of clinical cases by themselves [8]. It was suggested that the combination of IgM ELISA and/or total antibodies versus SARS-CoV-2 plus qRT-PCR can increase the sensitivity of diagnosis in the second week of illness, as the sensitivity of qRT-PCR, especially on upper respiratory tract specimens, declines significantly during the immunological phase of illness [8,23,24]. IgG against SARS-CoV-2 will have an important role to determine if someone is immune against SARS-COV-2. This could help to identify health care workers who can safely treat patients, identify serum donors, and determine the true infection fatality rate of the COVID-19 pandemic.

Low-complexity, rapid (results within 1 hour) molecular diagnostic tests for respiratory viral infections that are CLIA waived (FDA approved for use outside the laboratory by nonlaboratory personnel) include cartridge-based assays on platforms that include the Abbott ID NOW (Abbott Laboratories), Bio Fire Film Array (bioMérieux), CobasLiat (Roche Diagnostics), and GeneXpert (Cepheid) [25].

Simple antigen-based tests, if sensitive enough, might be useful in lower-resource and home settings to inform quarantine and spatial distancing measures for patients without severe illness and their contacts. Novel technologies, such as Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)-based diagnostics are being used to develop rapid, simple, low-cost, portable, temperature-stable assays for deployment in the field in nontraditional and resource-limited settings, such as airports and border crossings [26].

COVID 19 testing- continuous evolving entity

In response to the rapidly emerging COVID-19 pandemic, countries have used different testing approaches depending on testing capacity, public health resources, and the spread of the virus in the community[10]. To expand access to testing, the health care authorities (ICMR and MoHFW, India) [27] formulated policies to allow laboratories to use their validated assays in a more timely manner.

There have been compounding factors which are responsible for differences and variability of results such as – lack of an established reference standard, use of differing sample collection and preparation methods, and an incomplete understanding of viral dynamics across the time course of infection hamper rigorous assessment of the diagnostic accuracy of the many newly introduced SARS–CoV-2 assays [28].

Up to 30% of the patients clinically suspected of Covid-19 may have initial or repeat RT-PCR negative results before positive test conversion, most notably when upper respiratory tract specimens are processed. False-negative RT-PCR results may hamper the clinical management of patients and hinder the adoption of epidemiological measures to control the pandemic. A number of preanalytical and analytical factors may impact on the diagnostic efficiency of RT-PCR, including the type of specimen and time to specimen processing, testing, quality of samples, the timing of sample collection after symptoms onset, or the intrinsic performance of the assay (i.e, limit of detection) [29,30].

India must also pay much greater attention to the health sector and recognise the importance of having robust public sector capacity, especially in primary care and at the district level. India's public health-care system is chronically underfunded (at just 1·28% of GDP), leaving primary care fragile and vulnerable [31].

To conclude the SARS-CoV-2 crisis has helped us understand the importance of cooperation, communication, and collaboration among national and international agencies (eg, WHO, CDC, ICMR) and the laboratories at academic medical centres.

Every crisis is an opportunity in disguise. The current testing time has provided us such an opportunity to upgrade the existing healthcare system to prevent, test, and treat infectious diseases in India.

Author contributions

Omshree Shetty: Conceptualization, Writing- Original draft preparation, Mamta Gurav: Data curation, draft editing. Prachi Bapat: Reviewing, editing draft, Nupur Karnik:Resources, Gauri Wagh:Resources, Trupti Pai: editing draft, Sangeeta Desai: Supervision, reviewing and Editing.

Conflicts of interest

None.

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