FEATURE ARTICLE

Learning From a Pandemic: Developing Capacity to Monitor COVID-19

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AS India moves towards easing restrictions through the continuing COVID-19 pandemic, it is time to consolidate our experience over the summer and implement improved strategies for managing SARS-CoV-2 diagnosis and surveillance. As a premier research organization, the Council of Scientific and Industrial Research (CSIR) has been at the forefront in helping deal with this healthcare crisis on all fronts and has initiated multiple efforts to mitigate the impact of the pandemic in India.

CSIR-Centre for Cellular & Molecular Biology (CSIR-CCMB), Hyderabad, has been involved in dealing with COVID-19 from the very beginning, even before it was designated as an ICMR approved testing centre for COVID-19. Over the last three months, one-sixth of the total samples of Telangana have been tested at CSIR-CCMB and we are currently performing around 400 tests per day. We have also sequenced and analyzed the virus samples to analyze the prevalent clades in the country (Banu *et al.*, *bioRxiv,* May 31, 2020. DOI: https://doi.org/10.1101/2020.05.31.126136 2020). Here we highlight our journey over the last few months and focus on our learnings for diagnosis and testing in India.

A Multi-pronged Approach

Training of personnel and defining SOPs for testing

The first step in our process began with laying down clear in-house guidelines to handle the virus as well as enhance capacity by developing a trained workforce. In April 2020, the infectious biology researchers at CCMB developed a series of standard operating protocols (SOPs) and trained a set of students on working with the live virus in BSL-3 labs.

The team then trained doctors, staff and students from government hospitals in Hyderabad and Warangal in the state of Telangana, as well as research scholars from CSIR-IICT, University of Hyderabad and CDFD – 47 of them in total. Specialized training videos were created and circulated among other research institutes beyond Hyderabad as well. The SOPs were optimized, stated out clearly, and distributed to other testing centers across the country to set up their facilities (https://www.ccmb.res.in/Covid_sop).

Patient samples for testing (RT-qPCR based)

With our pre-set protocols and precautions in place, patient samples from hospitals were accepted for testing following government approval on 30 March 2020. The work was mainly carried out by CCMB's student volunteers and a few dedicated scientists for guidance and logistics. They remain the backbone of the programme as Telangana ramps up testing and we bring in more trained staff and hire technicians for increased Covid testing over the next six months.

Training for COVID-19 testing

Diagnosis of COVID-19 infection currently involves identifying the presence of viral RNA in patient samples as one of the top approaches. Real time quantitative reverse transcription polymerase chain reaction (RT-qPCR) of RNA extracted from nasal swabs is the gold standard for SARS-CoV-2 detection (Corman a*et al.*, *Eurosurveillance* 25.3 (2020): 2000045; Choudhary *et al. Indian Journal of Medical Research* 151.2 (2020): 251; and Jung *et al.*, *BioRxiv* 2020).

Polymerase Chain Reaction (PCR) allows identification of specific DNA sequences in the sample. The SARS-CoV-2 is, however, an RNA virus, and doesn't have DNA. Reverse transcriptase is an enzyme that converts RNA into DNA. For COVID-19 testing, viral RNA is first converted into DNA using reverse transcriptase and then subjected to PCR to allow for selective amplification if stretches of viral DNA are present. Using specific primers, the sequences of 2-3 viral genes can be targeted for real-time detection of the PCR amplification products.

Though adopted worldwide as the gold standard, RT-qPCR is an expensive, time-consuming process requiring highly trained personnel. This limits the number of tests that can be performed. With the increasing need of testing, CCMB developed several strategies to circumvent the limitations of the original RT-qPCR protocol and increase capacity and efficiency while lowering the cost and manpower requirements of the testing process.

Developing improved testing strategies

Pooling of samples: Pooling of samples is one way of increasing the capacity and reducing the cost. Pooling of samples involves testing them in batches, and when a pooled sample tests positive, then individual samples in that pool are further assessed. To increase the number of individuals that can be tested, CSIR-CCMB had early on developed an SOP to pool patient samples prior to processing for RT-qPCR (http://www.ccmb.res.in/Covid_samplepool), for 5 patients/batch as per ICMR advisories (https://www.icmr.gov.in/). To increase the sensitivity of the pooled testing protocol, the collection of samples was advised in 1 ml and not 3 ml VTM (Viral Transport Medium).

Improvements to current protocol: With experience, multiple modifications in the sampling and testing procedures were developed to make it faster, safer and more economical to test a large number of people. These measures can help scale-up and simplify the testing process.

By eliminating the usage of Viral Transport Medium (VTM) to collect patient samples, we developed a method to perform the RT-PCR test directly from dry nasopharyngeal swabs (Kiran *et al.*, *BioRxiv*. 1 June 2020). This tested protocol shows that dry naso/oropharyngeal swabs collected from patients and transported as it is in tubes to the testing centre have the same efficacy and accuracy as the samples collected in VTM. This protocol further simplifies testing by performing the RT-PCR directly on Tris-EDTA (TE buffer) extracts of the dry swab sample, without performing an RNA extraction step.

If required, RNA extraction step can be included in the dry swab method which increases the sensitivity of the test by 20-30% compared to the current accepted gold standard. The benefit of CCMB's modified technique is that not only is it fast and convenient but also at par with the traditional method in terms of accuracy and, therefore, can be used for improved screening efficiency.

Further, VTM (which is generally imported, and hence, an expensive and rare commodity at the moment) is not required at all in our method. Sample handling, transport and shipping, therefore, becomes easier and safer due to the use of dry swabs. Samples in VTM are also under risk of leakage and mixing with other samples during transport.

Using dry swabs for testing in the lab

Finally, the optimized protocol offers 40% cost saving in reagents (including RNA extraction reagents cost), 75% of the human resource, 50% of testing time, and is safer for sample handling.

Nested PCR technique instead of RT-qPCR: As described above, RT-qPCR measures amplification of a short segment of a viral gene in the course of a PCR reaction following reverse transcription of viral RNA. Performing the RT-qPCR test requires a real-time thermal cycler which is an expensive instrument. Most major research laboratories are equipped with only a limited number and smaller laboratories may have none which has placed constraints on the number of tests as well as places for conducting them. Further, the need for fluorescent oligonucleotide probes to detect the RT-qPCR signal adds to the cost of the tests.

CSIR-CCMB has developed a test that does not require real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR). Instead, it uses a reverse transcription nested PCR endpoint assay (RT-nPCR) which shows comparable performance to the standard RT-qPCR test. The RT-nPCR protocol comprises a multiplex primary RT-PCR for amplification of four SARS-CoV-2 amplicons and a control human RPP30 amplicon followed by a secondary nested PCR for individual amplicons and visualization of amplified DNA by simple agarose gel electrophoresis.

The test demands care in sample handling due to its higher sensitivity. It holds promise for confirming the samples which Rt-qPCR shows negative. The RT-nPCR protocol can also work with the pooled testing and direct amplification without RNA isolation protocols described above. Pooled testing with RT-nPCR can be undertaken for community surveillance (1:5 or 1:10).

Automation of RNA extraction method: The current protocols for SARS-CoV-2 detection in patient samples depend on RT-PCR that detects viral genomic RNA, and this involves the laborious RNA isolation step. This bottleneck can be eased by using automated RNA extraction machines that can prepare up to 96 samples in 1½ hrs. Due to high sample load at CCMB currently, we have also started KingFisher Flex, ThermoFisher Scientific's automated benchtop system. This enables rapid RNA isolation at a pace almost three times faster than manual extraction.

NGS-based testing, ramped up surveillance

The time, expense to conduct RT-PCR tests, and availability of expertise and facilities create a barrier to high throughput scale-up of testing at a population level. To overcome this issue, we have proposed the use of NGS technology for increasing testing manifold (in partnership with Syngene International, Bangalore).

NGS is a high throughput sequencing technology that enables the generation of hundreds of millions to billions of DNA sequence reads in a single run at a reasonable cost. This technology can be combined with sample barcoding to process and sequence multitudes of patient samples simultaneously. Briefly, the collected swab samples are subjected to viral RNA isolation and single-stranded RNA is converted to cDNA as in the normal protocol. Next, the viral genome is amplified using barcoded primers. Multiple samples, each having a unique barcode, are pooled and libraries are prepared for high

throughput sequencing. Downstream bioinformatic analysis is performed to test for the presence of the virus among all the pooled samples.

CSIR-CCMB proposes to utilise this massive capability with a logistic approach to collect and process thousands of samples in a batch for detection of SARS-CoV-2. This is an improvisation of the Broad Institute method utilizing compressed barcode space; the combination of the dual barcoded library preparation approach along with parallel processing of samples will make diagnosis more reasonable and scalable.

The strategy is to collect and process about 1000 samples at multiple collection centres. This will involve preparation of RNA followed by single-step simple PCR in pre-supplied barcoded plates. Such processed products can then be pooled in one tube and submitted to NGS facility. Each NGS facility can process 10,000-50,000 samples within two days, using a second barcoding step prior to sequencing. When performed at scale, the NGS method can cost an order of magnitude lower than the current PCR based methods.

The technology is also being tested for RNA extraction-free approach using dry swab collection method. That will not only bring down the cost under ₹ 200 per test but the throughput will also be closer to 50,000 tests/cycle.

Validation of new diagnostic kits

CSIR-CCMB has been identified as an ICMR Centre of Excellence for validation of non-US FDA and non-European CE/IVD approved COVID-19 qRT-PCR diagnostics, in keeping with the overall aim to promote Indian academia, industry and commercial entities for developing novel solutions for Covid-19 prevention, control and treatment. Since mid-April, we have been active in testing kits indigenously developed by companies in India and giving expert recommendations for their improvement.

Taking cognizance of the fact that access to clinical samples from COVID-19 patients is an essential requirement for developing test kits and R&D capacity, the Govt of India also notified a network of National COVID-19 Biorepositories in May for collecting, storing and maintaining clinical samples (oropharyngeal/nasopharyngeal swabs, bronchoalveolar lavage, sputum, blood, urine and stool). As part of the biorepository network, CSIR-CCMB is also authorized to share the samples with academia, industry and commercial entities involved in the development of diagnostics, therapeutics, vaccines, etc., after due scientific assessment, in addition to using the clinical samples for R&D purposes within our own institution.

We have since been involved in storage, distribution and reagent sharing to facilitate R&D for COVID-19 testing in terms of solutions for small kits, viral transport medium and other specific reagents based on our own research and expertise. These measures have helped provide multiple new additions in the nation's armory against the viral pandemic.

Working towards indigenization of diagnostics and surveillance tools

The Atal Incubation Centre-CCMB and Common Research and Technology Development Hub at CCMB have been supporting MSMEs/startups working in diagnostics, therapeutics for COVID-19 as well as sanitization strategies right from January 2020.

AIC-CCMB launched the CSIR-CCMB COVID-19 Fast Track Challenge in association with C-CAMP Bangalore's COVID-19 Innovations Deployment Accelerator (CIDA) in April with an objective to identify and support startups in the greater Hyderabad region who had ready-to-deploy solutions. Under the CIDA initiative, CCMB has pledged to provide resources in the early deployment and commercialization of these innovations. By joining this initiative, our startups and researchers have been able to leverage the combined support of partners like C-CAMP, UNHIE, Social Alpha, XYNTEO India2022, MedTech Connect, India Health Fund, PATH, and Action COVID-19 Team (ACT).

In addition, we invited ideas from research scholars at CSIR-CCMB and supported their efforts in developing newer methods of diagnostics and surveillance. This includes development of a platform for testing antigenicity of the virus and testing potential drugs, and sensitive detection of the virus.

A Perspective for the Future

COVID-19 has thrown up unforeseen challenges for all of humanity. Academia has responded to this unprecedented situation by setting up training, testing and therapeutic R&D against the SARS-CoV-2 virus. It goes without saying that the country requires and expects a quick response from its scientific community, but it is worth reflecting that systems set in place in this troubled time offer the potential for long term gains.

Indigenization efforts, collaboration and partnership with industry, goal-oriented rapid funding measures are just a few of the positive changes being brought about by the pandemic. If we can learn a lesson to be self-sufficient in healthcare R&D and diagnostics, maintain an open dialogue between scientific and government policymakers, and recognize the role academic institutions must play in the hand-holding industry for maintaining quality in endogenous facilities, we will emerge stronger and better prepared for the future.

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