Enhancing Testing Capacity for COVID-19

A key element of our integrated healthcare response to the pandemic

1. Context

Testing is an essential element in the country's efforts to tackle the spread of COVID-19.

But, 'how much testing is enough testing' is derived from our empirical sense of knowing SARS-CoV-2 virus in last 8 months. The current consensus amongst experts is that if the positivity rate[1] is more than 5%, the testing is inadequate and must be ramped-up. As this may lead to dire consequences especially in densely populated residential areas where the chances of infection blowing up through community transmission are extremely high.

Therefore, along with preparing our frontline and treatment facilities, it is critical to augment testing capacity and escalate tracing efforts. We have been supporting state governments, public health institutions and organisations working in community health towards this since the onset of our response to COVID-19, a brief description of which is given below.

2. Understanding COVID-19 testing

For designing effective responses, it is imperative to understand a few crucial aspects of testing for COVID-19, mainly, the types of test machines and kits approved/available and how do we estimate testing capacity.

Types of tests available/approved

There are two main categories[2] of tests for SARS-CoV-2: 1) diagnostic tests which help detect the current presence of the virus in the sample such as molecular tests or antigen tests and 2) antibody or serology tests which help detect antibodies for the virus in the sample. At present, in India, the following tests/test kits, approved by ICMR, are being used:

a. **Molecular based tests** – detect the genetic material of the virus in the sample, as of now the following tests are being used across the country:

   i. Traditional Reverse Transcription Polymerase Chain Reaction (RT-PCR) which detects the nucleic acid of the virus, by detecting specific genes of the virus in the sample. This is currently the most accurate, considered the gold standard for diagnosis of COVID-19

   ii. Cartridge based Nucleic Acid Amplification Test (CB-NAAT), a modified version of RT-PCR, which was originally developed for tuberculosis, detects the genes of SARS-CoV-2 virus. In terms of sensitivity and specificity, these tests are as good as RT-PCR tests

      1) TrueNat™ is automated, hence is quick, battery-operated and portable, making it useful for inaccessible and/or less-equipped health centres

      2) GeneXpert™ is operationally expensive, but existing systems could be utilised

b. **Antigen tests** – detect for the presence of antigens which are molecules that stimulate immune response to SARS-CoV-2 virus. It depends on the quantity of virus, viral load of the

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[1] *Positivity rate is the ratio of people who test positive for COVID-19 to the total number tested - on reliable tests like a Molecular Test (see sections below)*

[2] *Source: https://www.fda.gov/consumers/consumer-updates/coronavirus-testing-basics; as accessed on 20 Aug. 2020*

sample, hence, if positive it is considered confirmed; but if the result is negative, it will need to be re-confirmed by a molecular test. Currently, in India, we are using Standard Q COVID-19 Ag detection kit (a rapid antigen testing kit) as a point-of-care test for early detection of the virus in combination with RT-PCR/TrueNat™ test.

c. **Antibody/Serology tests** detect the presence of antibodies which help the immune system to fight off the virus in a sample of blood. Antibodies take time to develop, hence, if positive, indicate that the person was previously infected by the virus which can help understand the nature of this illness, its spread and treatment better.

The molecular tests are considered gold standard and hence that is the most desirable way to test. Rapid Antigen Tests, though having faster turnaround times, are much less sensitive and run the risk of classifying infected people as negative. But given the supply constraints and longer turnaround time for molecular test machines, a **cascading model of testing**, where these two tests complement each other, and optimise on time first and then accuracy, could be used. The antigen tests are accurate when the test reports positive; for the ones that report negative in an antigen test, a molecular test can be mandated for confirmation. This will significantly reduce the load on existing molecular based test capacities, enabling faster reporting of positive cases.

Meanwhile, efforts can be directed to increase overall molecular test capacity - RT-PCR or CB-NAAT - for two reasons: 1) their higher criticality and capacity gaps on the ground in the COVID-19 situation and 2) their long-term usefulness since this equipment can help test a host of other diseases such as TB, HIV, Hepatitis etc.

Test Capacity

Test Capacity is a function of several factors working in consonance. The molecular test process itself is broken into two parts – 1) an RNA extraction phase; and 2) an amplification and detection phase. Theoretically, depending on the capacity of the machines (number of wells) these machines can do roughly 96 samples in one cycle. There are protocols being developed (one recently approved by US FDA for emergency use) that may dispense with the RNA extraction step – as the understanding and expertise on these stabilizes, these will help with cycle time.

The RNA extraction can be done either manually or through an automated system such as RNA extraction machine or a Liquid Handling System (LHS). Manual extraction, which is the norm in most laboratories in India, can result in the end-to-end cycle, from sorting to reporting, taking nearly 5-6 hours. This method also increases the exposure risk of the laboratory staff to the pathogen. Whereas, an automated system with a high throughput enables testing more samples per unit of time while ensuring better sample integrity and staff safety. A typical run cycle of 96 samples using a combination of an automated RNA extraction system with a RT-PCR machine will take around 3-4 hours; more details in the annexure. Of the two automated systems, LHS have a much higher throughput than the automated RNA extraction machine. Therefore, larger public laboratories (such as those in Bengaluru) should use a Liquid Handling System with a RT-PCR.

To summarise, the cycle time, and hence the realised testing capacity, is a function of number of qualified staff, number of shifts, ratio of automated RNA extractors to the RT-PCR machine, type of extraction kit used and so on. Additionally, the capacity is also dependent on external factors such as efficiency of protocols and processes for collection, sorting and tagging of samples, data reporting and management and availability of kits. Therefore, in order to increase and utilise

existing testing capacity optimally, a holistic strategy focusing efforts on improving all or most of the above-mentioned factors would be required.

In this note we are referring to testing of 'un-pooled' samples, i.e., a test for a sample taken from one individual. In some circumstances (where infection spread is low), samples from multiple individuals can be 'pooled', to get more throughput; if one of the 'pooled' sample comes out positive, then each sample within that has to be tested individually.

3. Our approach

Currently, our integrated healthcare response – that include improving processes for awareness, prevention and screening, quarantine, testing and tracing, treatment and containment – are focussed in regions where we have field operations, or our partners have a deep on-ground presence. Within this strategy, our efforts towards increasing testing involves improving the frontline processes and augmenting testing capacity at multiple levels.

Frontline

This includes increasing awareness creation as well as streamlining frontline processes and enhancing frontline health-worker capacity in order to better identify people who are potentially infected, and to collect their samples.

Working with the district, block and village administrations, and partner NGOs, we are helping in enhancing community awareness and reducing fear and stigma around the disease, thus, reducing barriers to getting tested. We are also helping the administrations in training frontline healthcare workers such as ASHA workers, ANMs and Anganwadi Workers on surveillance processes and are supplying them equipment like pulse oximeters and infrared thermometers to help in such surveillance. This is helping increase the pipeline of samples coming in for testing, catching infections early, thereby reducing spread.

Testing capacity

For this, our support has largely been to respective state governments, public institutions and hospitals to ensure that testing is accessible by the most disadvantaged sections of our society. This requires enhancing testing capacity in labs, and decentralising testing where relevant.

In each of these regions, we are working closely with the relevant state and district administration and health department to estimate the testing requirement projected over the next 6-12 months, understand the existing capacity and plan the augmentation needed.
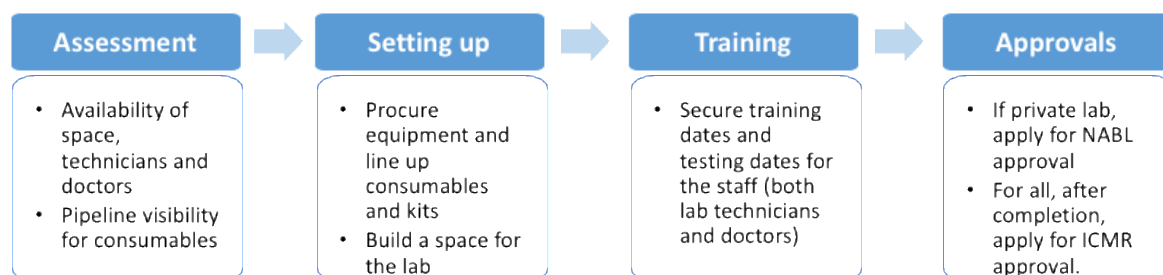

Assessment	Setting up	Training	Approvals
• Availability of space, technicians and doctors • Pipeline visibility for consumables	• Procure equipment and line up consumables and kits • Build a space for the lab	• Secure training dates and testing dates for the staff (both lab technicians and doctors)	• If private lab, apply for NABL approval • For all, after completion, apply for ICMR approval.

Fig. 1: Illustratively, process flow for setting up a TrueNat™ lab

The critical element of our support has been to provide relevant equipment:
a. RT-PCR and TrueNat™ machines to augment number of systems available

b. Automated RNA Extractors or LHS at labs that already have RT-PCR machines, to increase testing capacity of existing RT-PCR systems, including for the ones provided by us

c. Other complimentary equipment such as biosafety cabinets and 'starter kits' where there is supply-chain constraint

We have been successful in establishing a smooth supply-chain for all the equipment, allowing us to achieve intended capacity in less time at relatively low cost and predictable supply.

The setting up or enhancing of testing capacity is the lynchpin for effective monitoring, surveillance and containment at the front end. So, in addition to assessing requirements and providing test equipment, we are working closely with the respective departments and institutions in many of these regions to help set up processes and systems as per ICMR guidelines, including training and infrastructure for safe swab collection and cold-chain transport to test centres. And then to develop and implement test and contact tracing protocols for surveillance and monitoring - both proactive and reactive.

It is useful to note that the testing capacity that we are helping set up has use for multiple pathogens and will, therefore, continue to be extremely useful for the health system even after Covid-19 is contained.

4. Effect on the ground

Our support for testing has extended to 71 locations across 10 states – Andhra Pradesh, Chhattisgarh, Jharkhand, Madhya Pradesh, Maharashtra, Karnataka, Himachal Pradesh, Rajasthan, Tamil Nadu and Uttarakhand.
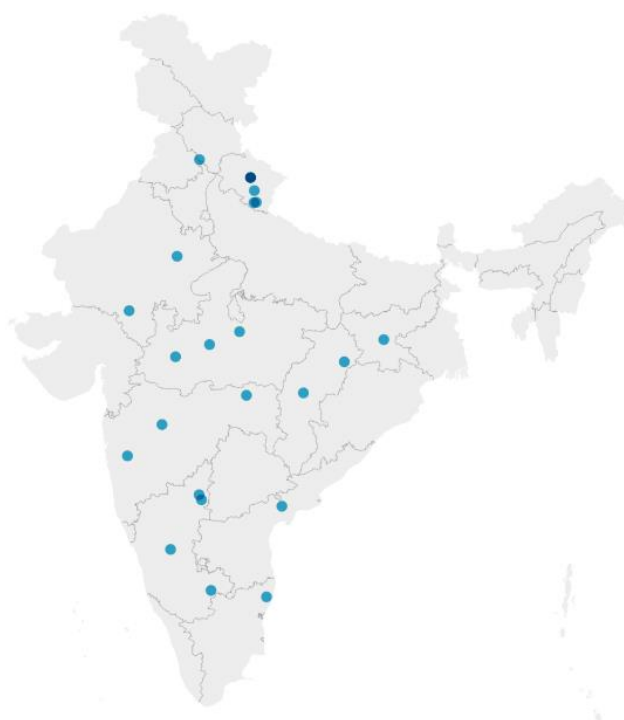

Fig. 2: Regions where we are supporting an integrated healthcare response to COVID-19
Note - Blue dots are only indicative of regions where equipment is installed which could be distributed across locations within that region. For example, Jharkhand is considered one region, but equipment is installed across 5 districts

We have **added capacity** for about 80,000 tests per day across the country with 75 new RT-PCR machines, so far. Across many of these installations, matching automated RNA Extraction

machines have been provided[3]. Because of a variety of constraints (e.g. number of lab staff) at many locations, capacity utilisation would be less than rated capacity[4]. But this will be improved over time – as planned by the host institutions.

For instance, at **Raigarh, Chhattisgarh,** we have supported setting up of a laboratory at Government Medical College (GMC) from scratch – providing all such equipment which will be helpful in conducting tests such as RT-PCR machine, RNA extractor machine, sample mixers, refrigerator centrifuge, thermomixer, refrigerators (-86 degree, -40 degree), storage box, dry bath, rack, nuclease-free water plant, refrigerator, bio-safety cabinet and PCR workstation hood. This facility is critical as this district is one of the largest in the state, bordering Odisha, housing several major industrial projects. There is a huge migrant population thus increasing its susceptibility to a high spread of infection. This facility caters to around 40 lakh people in the region and is currently running at full capacity in 3 shifts. In just 3 months, the lab has successfully conducted over 65,000 tests reaching a high of nearly 2000 tests in a day. We are further supporting the district administration in planning its testing at scale; for example, identifying and prioritising testing of people with co-morbidities; or dedicating days for regular testing of people whose job entails high interaction with the community – such as, Monday for all auto drivers, Tuesday for all fruit/vegetable vendors and so on.

> *"Foundation's support to GMC is appreciable. The various equipment helps us in increasing the testing capacity. We are serving 6 to 8 districts from this lab, so such testing capacity is very much required."*
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> *~Dr. M. N. Luka, Dean GMC Raigarh*
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> *"The quality of equipment is very good, accurate and we received them on time. The team of Foundation has also coordinated well to reach all the equipment on time; it normally takes more time."*
>
> *~Dr. Rakesh Kumar, Asst. professor and in-charge, Microbiology lab, GMC Raigarh*

Our support has also helped enhance testing numbers by **unlocking existing capacity**, by enabling higher throughput of samples. We have provided 27 automated RNA extraction machines across locations and 5 liquid handling systems at Bengaluru. Together, these have increased effective capacity by 20,000 tests per day across these locations.

5. Going forward

We will continue to support State governments and public institutions in augmenting their testing infrastructure; consistently strive for improving efficiency in processes for collection, sorting and tagging of samples; nimbly adapting to newer proven protocols of sample collection, testing protocols and reporting as well as better data reporting and management.

To complement the same, we will continue to work with our institutional partners such as National Centre for Biological Sciences, Bengaluru, to find better pooled testing algorithms and strategies to scale up this entire effort.

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[3] *The data on overall RT-PCR capacity in the country is difficult to assess – realistically, we can only look at the actual number of RT-PCR tests being done per day – but unfortunately even that data has also become unreliable in past 4-6 weeks as significant percentage of the test are being done using Rapid Antigen Tests (which has 16-50% false negatives). This is driven by the inadequate RT-PCR capacity, and hence not desirable.*

[4] *Capacity calculation assumes full utilization of 24 hours per day on the rated capacity of testing machine and the time per run provided by the Original Equipment Manufacturer (OEM). This is rare. In our experience aside from large cities, the machines run for 12-14 hours. Capacity stated above includes some installations that will be up by end Aug. 2020.*

6. Annexure – One run-cycle of a RT-PCR test

Using an automated RNA extractor and RT-PCR machine for a batch of 96 samples

#	Steps	Activity	Who and where it is performed	Time taken	Min. time
1.	Sample Collection	Specimen is collected through throat and nasal swabs, inserted into a virus-transposing medium and sent to the lab	Collection is manual, using swabs; outside lab	--	--
2.	Extracting RNA	Separate the RNA, done using a centrifugal process, could be manual or automated depending on the sample load	Manual using manual RNA extraction kits or automated using Liquid Handling System or automated RNA extraction machine; in lab	Manual – NA Automated - 70 mins min. per batch of 96 samples on RNA extractor (as per OEM)	70 mins
3.	Setting up plate for PCR testing	Set up 96 plate from master sample (from RNA extractor) using multi-channel pipette accessory – to be done carefully to avoid contamination	Typically, this is manual setup work prior to PCR testing run	30 - 40 mins depending on skill of technicians	30 mins
4.	Conversion to DNA	RT enzyme is used to convert the RNA into DNA, i.e., going from one strand to two. To set up reaction need a 'master mixture' of nucleotides and taq DNA polymerase.	Automatic run time per batch is 45 mins -75 mins depends on PCR reagent kit used; there are 85 PCR testing kits approved by ICMR, each gives a different PCR testing run time (run time is same for 24/96 sample batch)	45 mins is least time and 75 is maximum time as on date, depending on PCR reagent kit used	45 mins
5.	Amplifying the DNA	This 'master mixture' is then added to the PCR machine, which alternately raises and decreases the temperature for 15-20 seconds. In one cycle, temperature is raised to separate the DNA strand and cool it down - usually, raised to between 55°C and 60°C, and to 72°C, all within 1 to 1.5 minutes, at which the new strand will be synthesized. For one batch, this cycle has to be repeated 35-40 times, taking approx. 1 hour.			
6.	Results	PCR machine is ready to detect. A fluorescent dye or 'probe' is added to the test tube. A special light-measuring instrument in the PCR machine reads these fluorescence patterns to determine which samples have the virus in them and which don't.			
7.	Ensuring accuracy	Controls are introduced to ensure that the test is accurate. There is a positive control, a known COVID-19 positive sample and a negative control, a known negative sample; and an internal control, which is usually a "housekeeping gene" or gene required for the maintenance of basic cellular function.	Manual verification, takes around 15 mins per batch	15 mins	15 mins
8.	Report	Verifying the result to swab sample and entering data on ICMR portal and preparing test report for each sample	60 min- 120 mins for each batch as entry is sample wise; done manually	60 mins minimum	60 mins
	Minimum time to complete one full cycle from extraction to RT PCR result and report for one batch				**220 mins**

#	The testing rate is dependent on the following:	Remarks
1.	Skills and availability of technician for sample preparation and set up of micro pipettes	Typically, 96 samples will need 3-4 technicians working simultaneously per hour
2.	Type of extraction and PCR kit has a bearing on run time of machine – time range of extraction kits is up to 80 mins, similarly for PCR testing over 85 makes of testing kits are approved with time range up to 120 mins	Type of kit used has bearing on number of test result output from testing lab
3.	Use of multichannel pipette glassware helps in faster setting up	Many labs lack this which slows down testing output rate