TESTING CORONA

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TEST... Test... Test... in the last six months, this word has become trivial. If you sneeze or cough, have a headache or body ache, feel like nausea or congestion, feel sore throat or lose the sense of taste or smell, have fever or diarrhoea symptoms, you may have been attacked by a deadly virus.

If you have any or all of these symptoms, you need to get a test done for COVID-19, irrespective of the fact that some of these symptoms are very common and can occur in many conditions other than COVID-19 like weather change, food poisoning, overburdened with work, depression, anxiety, bad eating habits, allergy due to dust, smoke or pollution, etc.

Since March till date, the situation has worsened. Earlier there were less number of COVID-19 cases in India, but the fear of getting an infection was high. Today it is vice versa. Number of cases has broken all records, but the fear among people has vanished.

We have taken a leap from Lockdown to Unlock. Still, it is difficult to analyse whether we are testing enough COVID-19 cases or the Coronavirus is testing our patience. Since its arrival in our lives, it has tested our medical expertise, our doctors and hospital staff, the medical infrastructure in our country, and the people.

COVID-19 tests are critical to measuring the spread of the disease and determining how to handle the pandemic. The dilemma is no country including India, knows the total number of people infected with COVID-19. All we know is the infection status of those who have been tested. All those who have a lab-confirmed infection are counted as confirmed cases. This means that the counts of confirmed cases depend on how much a country actually tests. Without testing, there is no data.

The National Institute of Virology (NIV), Pune, reported the first case of COVID-19 in India on 30 January 2020. The next two cases were also reported by NIV. Soon after, NIV proceeded to empower 13 Virus Research Diagnostic Laboratories (VRDLs) across the country. Testing protocols were shared, reagents were sent out and via daily video-conferences, NIV was able to equip more VRDLs to join in the battle against COVID-19. Today, 107 VRDLs perform testing contributing to a total of 905 government-run laboratories across the country.

What are the tests for COVID-19?

There are two ways to test for COVID-19: Viral Test and Serology Test.

A viral test is an oral or nasal swab or saliva test that looks for evidence of an active viral infection. Early in the infection, the virus grows in cells at the back of the nose and throat, taking about 5-14 days before symptoms appear. A swab of the nose or throat can be taken and the nucleic acid of the virus can be detected. The virus is detectable several days before symptoms appear and up to eight days after symptoms appear.

However, everything depends on how well the sample was taken and the amount of virus present at the time of swabbing. Once the antibody response begins, the virus is cleared from the body. Once the virus is no longer present in the nose/throat, the test will be negative.

And this is where the samples for viral tests are taken from:

- Lower respiratory tract: sputum, lavage, aspirate
- Upper respiratory tract: nasopharyngeal and oropharyngeal swabs; nasopharyngeal wash/ nasopharyngeal aspirate
- Nasopharyngeal and oropharyngeal swabs

Samples should be taken within 14 days of the person's last documented contact with a COVID-19 case. Viral tests do not indicate whether someone was infected in the past.

Viral test is further of two major types: RT-PCR test and an antigen test.

RT-PCR test (Reverse Transcription Polymerase Chain Reaction test) looks for the presence of a virus's genetic material. RT-PCR is a laboratory technique combining reverse transcription of RNA into DNA and amplification of specific DNA targets using Polymerase Chain Reaction (PCR).

The RT-PCR technology is a fairly expensive method. It requires RNA extracting machines, a laboratory and trained technicians. A minimum of 30 samples are needed to make it economically viable. The cost of chemicals and importing elements required for the test is also high.

The antigen tests that have been approved for COVID-19 diagnosis in India give results in 30 minutes. Antigen tests produce results quickly but may be less sensitive.

These tests look for the 'spike protein' present on the surface of the coronavirus and which facilitates its entry into the human cell. For the antigen test, a nasal swab is collected. For analysis, it is immersed in a solution that deactivates the virus. A few drops of this solution are then put on a test strip. This has to be done within an hour of the immersion of the swab in the solution. The test strips contain artificial antibodies designed to bind to coronavirus proteins. If a person is infected with coronavirus, the test lines will appear on the paper strips within 15 minutes.

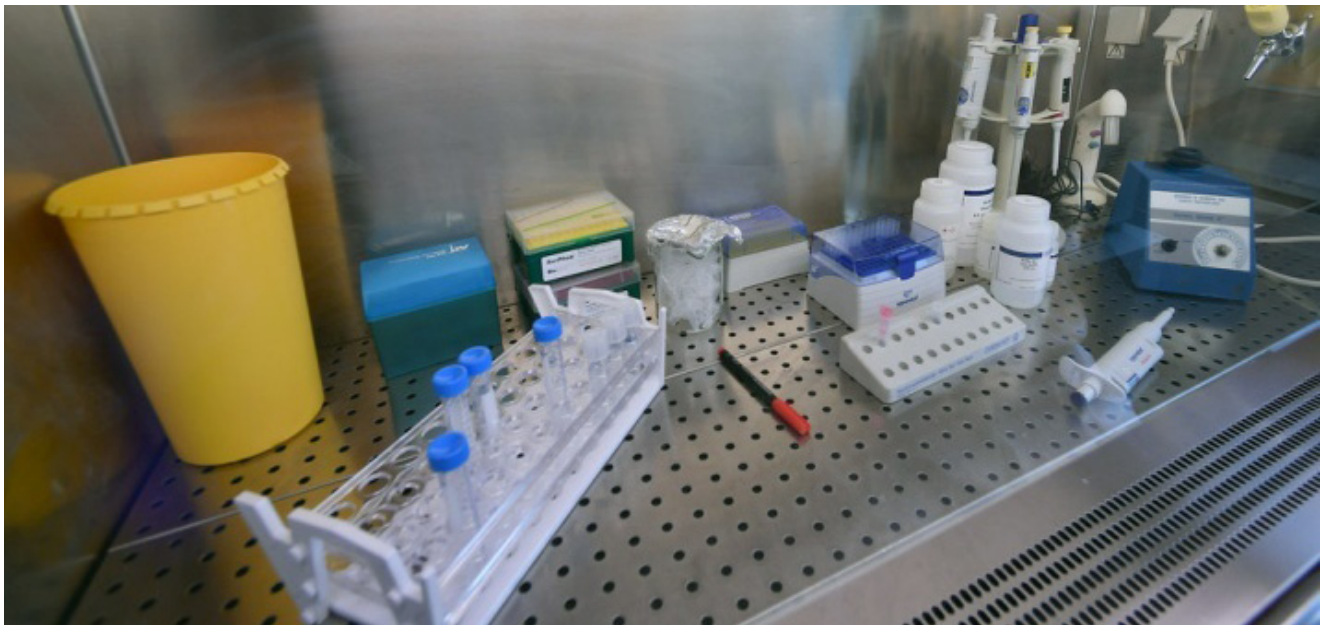
The advantage of using this test is that it reduces the burden of relying on just RT-PCR tests to identify COVID-19 patients. Experts are of the opinion that antigen testing is useful because even if it is less sensitive, it is rapid and the results that are positive will be positive. So, patients who test positive can get into isolation faster.

Professor Priya Abraham, Director at National Institute of Virology advocates, "World over the preferred test for detecting Coronavirus is the real-time PCR assay. In India as well the recommended test is the real-time PCR assay. However, for the purpose of screening in containment zones and hot spots, the antigen test has been used. The main feature of the antigen test is its ease of use and result being ready in 30 minutes."

The major drawback of this test is that if the swab samples lack enough antigen material, it may result in false-negative tests. For this reason, if a person tests negative through antigen testing, they still need to get an RT-PCR test done for confirmation.

Serology test/Blood test/Antibody test

These tests are used to find out the presence of virus in a body. In this method of testing, blood samples are used to find antibodies. This process also detects the quantity of antibodies that are produced by the immune system. It is an indirect method of testing as it cannot find the virus, but can determine if the immune system has encountered it.



Is Coronavirus Testing Similar to other Virus Testing?

The answer is YES. Coronavirus testing is similar to that for other viruses with one difference. Dr Anurag Agrawal Director, CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB) decodes this question, "In many other viruses like Dengue, the virus can be found in the blood. Coronavirus is primarily in the respiratory tract and oral cavity, so early blood test is less likely to work. Antibody blood tests take time to become positive." So, viral tests play an essential role in the testing scenario.

Antibodies can show up between 9-28 days after the infection has set in. By this time, an infected person can spread the disease, if not isolated. This test even looks for evidence of prior infection with the virus. The test provides evidence that someone may have been exposed to the virus in the past, potentially even if they did not have symptoms, by detecting antibodies specific to the virus. The test does not diagnose an active infection or identify who is protected from reinfection.

Dr Shekhar C. Mande, Secretary, Department of Scientific and Industrial Research (DSIR) and Director General, Council of Scientific & Industrial Research (CSIR), admits that "India's testing capacity has increased over the last few months. Still, there is a need to ramp up the testing capacity further given our population and the growing infections and all agencies including ICMR and CSIR labs are working towards it."

He gives a futuristic approach to the testing techniques as well. He foresees, "The desirable development of testing is a quick and cheap diagnostic that can be done similar to testing of blood glucose levels or a pregnancy test which can be done at home. It is likely that in the near future, tests which could be an antibody or nucleic acid-based will be developed that can be done at PHC or local diagnostic centres which will ensure that tests are widely available to all those who need to be tested."

The ICMR had validated the usage of TrueNat/Cartridge Based Nucleic Acid Amplification (CB-NAAT) based tests,

Testing at CSIR-CCMB

commonly used for detecting tuberculosis, for conducting coronavirus tests. For the TrueNat test, the swab is collected and dipped in a viral transmission medium where it gets neutralised. It is then shifted to another liquid in which the cells break and the impurities are removed. Finally, it is transferred into a cartridge where the process of RNA extraction takes place.

TrueNAT is an indigenously developed, portable version of CB-NAAT. TrueNAT is battery operated and portable, while CB-NAAT machines need an uninterrupted power supply and air conditioning, and hence cannot be deployed in containment zones.

These tests are different from the conventional tests in their speed, cost, as well as convenience of application. The machine is portable, and experts find it useful in remote districts from where collecting and sending swabs to big laboratories may not be possible generally. This allows teams to set up mobile testing centres or kiosks in containment zones, instead of having to transport samples to labs.

Storage & Transport of Samples

For some tests, results may be available at the testing site in less than an hour. For other tests, samples must be sent to a laboratory for analysis, which may take 1-2 days once the sample is received by the lab.

So, after the samples are collected, they need to be taken care of till they are tested and analysed. There are specific centres for sample collection and other centres for sample testing. The duration between sample collection and sample testing is very critical. For accurate results, the collected sample has to reach the testing centres within the permitted time frame. For example, if the specimen reaches the laboratory in less than 72 hours, it can be stored and shipped at 4°C. But if the specimen reaches the laboratory in more than 72 hours, it needs to be stored at -80°C and shipped on dry ice or liquid nitrogen.

Now, let's see how the actual testing procedure proceeds. The lab will acquire one of the following samples from you:

- **Swab test:** The lab will take a special cotton swab and collect sample from the inside of the throat or nose.
- **Nasal aspirate:** The lab will inject a saline solution into your nose, then remove the sample with gentle suction.
- **Tracheal aspirate:** A thin, lighted tube called a bronchoscope goes into your lungs, from where the sample is collected.
- **Sputum test:** Sputum is a variation of mucus from your lungs that can be coughed out or sampled from the nose with a swab.
- **Blood test:** The collected sample will be analysed for the virus, either through a blanket test for all variants of the coronavirus or through a specialised gene sequencing test that locates the marker for the novel coronavirus.

What happens to the sample after collection? Dr Rakesh Mishra, Director, CSIR-Centre for Cellular and Molecular Biology (CSIR-CCMB), Hyderabad, explains, "Most of the COVID-19 testing is based on taking RNA out from the virus, converting it into DNA, amplifying the DNA so that we can

What Happens during a COVID-19 Test?

Who can answer this question better than those who have experienced the procedure. Monika Sharma, a housewife, tested positive for COVID-19 along with her two daughters, Riti 12 and Shrestha 7 years. She says, "All the facilities were good, doctors and hospital staff were very co-operative but seeing my babies in the hospital was very distressing. The kids at times got scared seeing the hospital staff in PPE kits. The testing procedure was easy going for us."

About the testing procedure she recalls, "Everyone in the testing lab was in PPE kits, we could not see their faces or make out their gender. They had trays ready with all the necessary equipments."

The kids Riti and Shrestha recount their experience, "At first we were a bit reluctant but when we saw the simple procedure, and the uncle in PPE kit told us all about the simplicity of the test, we agreed. The second and third time, it was not that scary."

Nupur Prakash, Manager at an SBI branch, who tested COVID-19 positive on 17th May and was admitted to a Government hospital, quarantined from family, could not believe the test results as she was asymptomatic. Nupur remembers, "The test procedure was very normal, but obviously when the swab is taken from the throat or nose, it is a bit irritating and the trauma of being tested positive is threatening."

Nupur had to stay in the hospital for more time as seven COVID-19 tests continuously tested positive. After the eighth test was negative she could return home with another 14 days of quarantine at home. Nupur has now joined back at work. But, she still wonders how she was testing positive again and again without showing any symptoms.

Seventy-year-old Umesh Chandra Prakash, who had mild symptoms like pain in chest and legs, describes the test as being normal with all the precautionary measures taken by the laboratory staff. He was appreciative of the special care and attention given to the old.

Testing & kit validation at CSIR-CCMB

visualize it. Taking the RNA out from a virus is a tricky thing because it requires specific biosafety standards. And such facilities are available in basic research institutes. Besides, such techniques are routinely used for other work in these institutes."

The procedure is complicated and lengthy, says Dr Mishra and emphasises that the most important asset that the institutes designated for testing samples bring with themselves is the pool of experiences researchers, acquainted with advanced technologies, and who can keep up with the wide literature in these unprecedented times. "It does require reorientation and reorganisation at the institutional level," he says. "But it is also the right time for basic research institutes to rise to the next level to address the COVID-19 pandemic. After all, basic researchers are trained to address unsolved problems."

Sometimes there can be test errors. For example, a test can be false positive which means the test is positive, but the virus is not present, or false negative, which means the test is negative, but the virus is present.

Dr Anurag Agrawal, Director, Institute of Genomics and Integrative Biology (IGIB) explains, "Negative tests do not always mean that infection is not there. This problem is less with RNA tests because they can be amplified. Later, one can detect an infection by the immune response to the virus. This happens about a week after infection."

Sometimes, negative results might be obtained from an infected individual for various reasons:

- Poor quality of the specimen
- Specimen collected late or very early in the illness
- Specimen not handled and shipped appropriately
- Technical reasons inherent in the test, e.g. virus mutation or PCR inhibition

If a negative result is obtained from patients with a high index of suspicion for infection, new specimens from the lower respiratory tract are collected and tested. Experts, however, stress that regardless of whether you test positive or negative you should continue to take steps to protect yourself and others.

Innovations in Testing

Dr Anurag Agrawal helped list the following innovations at different steps of testing:

- **Sample collection:** In many countries, saliva is now shown to be as good as swab for testing. This opens up the possibility of self-collection kits that patients can use. Problems in India may be due to *paan, supari*, etc.
- **Sample processing:** It is now shown by many labs and also confirmed at the Council of Scientific and Industrial Research (CSIR) that it is possible to skip RNA extraction from samples. Such methods can make testing faster and cheaper. This is not possible with the current sample collection method in viral transport media so the entire system needs to be changed.
- **Testing:**
  - *Nucleic acid:* There are now methods such as COVID-seq where Next Generation Sequencing (NGS) can be used to test thousands and even tens of thousands of samples daily, using nucleotide barcodes to identify positive samples.
  - *Oxford Nanopore MinIon* is a small device about the size of a large stapler that can go from sample to sequence-based diagnosis for a few hundred samples in about 6 hours. The advantage is a low capital investment of only 2 lakhs and greater resistance to mutation-related false negatives than rtPCR.
  - *Microfluidic PCR platform* (Fluidigm Biomark) with an assay for SARS-CoV-2 is already in use in many leading global laboratories as a Lab-developed Test (LDT). Advantages of this assay over conventional qPCR assays are very high-throughput with minimal manual labour. With full automation, it can reach a throughput of up to 6000 samples/day.

There are also smaller and faster PCR machines. And there are tests that don't require PCR machines at all like CRISPR-based paper diagnostics – SHERLOCK, DETECTR, FELUDA. These workflows and tests are now becoming available in India.

India is mostly using swabs collected in Viral Transport Media (VTM), followed by rtPCR. Most countries follow similar protocols. But some nations are using a mix of methods including very high throughput systems as well as very rapid systems like Reverse Transcriptase-Loop Mediated Isothermal Amplification (RT-LAMP), and trying saliva, antigen tests, etc. All these tests are available in India as well.

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