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Editorial

Laboratory Diagnosis of COVID 19 Infection

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In December 2019 China reported numerous cases of pneumonia in Wuhan. The pathogen was soon identified as a novel coronavirus and by mid January a number of whole genome sequences of the pathogen were available in the public domain. This virus was reported to be a RNA virus of 30 K bp and with genetic similarity to virus involved in causing Severe Acute Respiratory Syndrome (SARS) in 2003 and so this virus was named SARS CoV 2 and the disease it caused as Covid 19 [1].

With the availability of genetic sequences of SARS CoV 2, multiple protocols for RT PCR were developed. CDC, Atlanta focused on the N gene and identified sequences which were common to Coronaviruses and others which were specific to SARS CoV 2, unfortunately their first prototype RT PCR test did not fair too well when transferred to other public health laboratories. In the mean time, Koch Institute, Berlin identified sequence in E gene for screening followed by RdRp (RNA dependent RNA polymerase) sequences for confirmation. This protocol was adopted by World Health Organization and made available to public health laboratories around the world [2]. Scientists in Hong Kong had found good results using ORF 1ab while Thermofisher made multiple primers and probes for detection of SARS CoV 2 using TaqMan technology and finally devised a one tube, one step multiplex rRT PCR for simultaneous detection of ORF 1ab, N and S genes along with MS 2 (RNA bacteriophage) to serve as a control for RNA extraction and amplification in each reaction as the TaqPath kit [3].

In the meantime, SARS CoV 2 had assumed pandemic proportion and was spreading like a wild fire throughout the world. In the absence of any approved treatment or preventive vaccine, diagnosis followed by isolation for 14 days emerged as the most effective containment strategy. This pressure on laboratory diagnosis by RT PCR made both the manufacturers and the clinical laboratories to not perform full validation and verification of the test and rush the kits into testing clinical samples.

Indian Council of Medical Research (ICMR) was charged with the responsibility of stewardship of India's efforts and from one laboratory performing RT PCR for Covid 19 in January 2020, by July ICMR had created a network of over 1000 laboratories able to perform RT PCR using traditional of Gene Xpert and True NAT, both in the government and private sector. While only NABL accredited labs which had RT PCR for RNA in their scope of accreditation were permitted, no such restriction or precondition was applied to laboratories in the government set up and many sites were seeing a PCR machine for the first time [4].

Since a positive report was followed by isolation for 14 days and contact tracing of the contacts, soon accusations of false positives by private (accredited) laboratories followed by negative reports by government laboratories started emerging. In Covid 19 RT PCR, since the sequence chosen were novel and not present as commensals in anyone, chance of a false positive was only possible after mix up or gross contamination of reagents, while possibility of false negative was far more plausible. The suggestion that laboratories share the classical tracing showing amplification of specific genes in their positive report along with CT values, were not acceptable to overworked workers in the laboratory.

Reports from China had already indicated that the incubation period varied from 1 to 14 days with mean of 5 days post exposure and typical symptoms were non productive cough, fever and diffi-

culty in breathing. Recently loss of smell and taste have been found to occur a couple of days before fever or cough and this appears to be the first and sensitive clinical indicator of Covid 19 infection. Over 80% of infections were mild but in some breathlessness and hypoxia forced persons to be admitted in ICU and death occurred in less than 10%. RT PCR test started becoming positive a day or so before appearance of symptoms and maximum detection in symptomatic patients was in the first week, the frequency falling thereafter, but could persist for weeks and in some cases a negative report could be followed the next day by a positive report [5].

The virus infects Vero cells as it binds to ACE2 receptors via its Spike protein and enters the cells and subsequently kills it, leading to cytopathogenic effect on a cell monolayer. Scientists in Canada performed both RT PCR and cell culture simultaneously on respiratory samples obtained from suspected Covid 19 patients and reported that virus could infect cells upto 8 days after appearing of symptoms, though RT PCR could be positive for weeks in these patients. At the time when viral was infectious to the cell cultures CT values for E gene were below 24. Authors suggest that RT PCR positivity after that was due to amplification of remnants of viral RNA, rather than due to the presence of infectious virus [6]. Unfortunately, due to the high biosafety concerns, ICMR has not permitted clinical laboratories in India to culture the virus and only reference laboratories with BCL III facilities should do so.

As pneumonia was the presenting diagnosis, respiratory secretions were tested by RT PCR, but it was reported that the presence of the virus in different samples from the same patient at the same time was different with BAL being the most productive, followed by sputum [7]. Subsequently a study from Yale university indicated that the titre of virus was highest in saliva and this was consistent while the presence and titre in nasopharyngeal swab was both lower and inconsistent [8]. Unfortunately, both the scientists and decision makers have not been able to exploit this observations. There have recently been reports that dry swabs were suitable for RT PCR without sample transportation in VTM, thus decreasing the turn around time for RT PCR reports [9,10].

The strategy used for containment of infection is Test, Trace and Treat (isolate) an infected person in which speed and short turn around time (TAT) can play a very significant role. Unfortunately, most laboratories found themselves overwhelmed and a test that takes 4 to 6 hours to perform was being reported back in days, leading to even the courts of justice to intervene.

One solution to long TAT was to detect viral antigens in respiratory samples since lateral flow method would allow results to be available in clinically relevant time for isolation of the person. Unfortunately, while in RT PCR we can amplify the starting RNA sequence many fold and thus increase sensitivity of detection, in rapid antigen detection, we have to make do with the available viral load which is lysed and fragmented to release the antigens. So while specificity for detection of SARS CoV 2 was not compromised, sensitivity was much less than RT PCR leading to many false negative results and need to follow up a symptomatic person who tests negative with RT PCR.

LAMP and Crisper, both nucleic acid amplification strategies but with much shorter TAT and naked eye read out of results may make diagnosis of SARS CoV 2 easier and faster but commercial kits are yet to be introduced [11,12].

After a week or two of infection, antibodies appear in infected persons. Higher titres have been reported in persons who suffered severe infections and titre and duration of antibody presence in persons with either mild infection or asymptomatic were both lower and lasted for shorter duration. Isotypes of antibodies also appear to be a little bit different and either IgM and IgG appear together or one follows the other. Higher IgA than IgM has also been reported by some scientists. The Nucleocapsid protein (NCP) of the virus appears to be most immune-dominant and detection of Pan antibodies to NCP may be the best test to identify if a person has been exposed to the virus. Spike protein is slightly less immunogenic but antibodies to Spike protein, especially the Receptor Binding Domain, would offer neutralisation of spike protein and may be protective. Though lateral flow rapid antibody tests are available, in the field they performed poorly and ICMR has recommended that either ELISA or CLIA should be the method used for detection of antibodies. To rule out false positive reactions CDC Atlanta has recommended that an orthogonal strategy should be used for serology in Covid 19 infections. Screening for presence of any type of antibodies against NCP would indicate if a person has been previously exposed to SARS CoV 2 and all positive samples should be retested for the presence of anti Spike antibodies, which would determine if they are subsequently protected [13].

Determination of Neutralising antibodies would offer the best evidence and this has now been made possible by the availability of a genetically engineered pseudovirus which expresses Spike protein but does not have SARS CoV 2 RNA, making it possible for use in clinical laboratories without BSL III facilities [14].

Evidence is emerging that not all antibodies are necessarily protective in nature and may participate in setting up a cytokine storm or be associated with immune mediated damage to the organs [15].

Recent data indicates that activation and expansion of innate and adaptive lymphocytes may play a major role in Covid 19. Recovery was associated with formation of T cell memory. Understanding T cell response in context of clinical severity might serve as foundation to overcome the lack of effective anti viral immune response in severely affected covid 19 patients and can offer prognostic value as biomarker for disease outcome and control [16].

Bibliography

1. WHO. Naming the coronavirus disease (Covid-19) and the virus that causes it. Technical Guidance (2020).
2. Corman V., *et al*. "Diagnostic detection of Wuhan coronavirus 2019 by Real time RT PCR". *Eurosurveillance* 25(2020): 2000045
3. Appliedbiosystems. TaqPath Covid-19 Combo kit Catalogue No. A 47814.
4. ICMR Press Release: How India ramped up Covid 19 testing capacity (2020).
5. Gua I., *et al*. "Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19)". *Clinical Infection Disease* (2020): 1-8.
6. Bullard J., *et al*. "Predicting infectious SARS-CoV-2 from diagnostic samples". *Clinical Infection Disease* (2020).
7. Wang W., *et al*. "Detection of SARS-CoV-2 in Different Types of Clinical Specimens". *JAMA* 323.18 (2020):1843-1844.
8. Wyllie AL., *et al*. "Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs". *medRxiv*.
9. Srivatsan S., *et al*. "Preliminary support for a "dry swab, extraction free" protocol for SARS-CoV-2 testing RT-qPCR". *bioRxiv*.
10. Kiran U., *et al*. "Improved and Simplified Diagnosis of Covid-19 using TE Extraction from Dry Swabs". *bioRxiv*.
11. Österdahl MF., *et al*. "Detecting SARS-CoV-2 at point of care: Preliminary data comparing Loop-mediated isothermal amplification (LAMP) to PCR". *medRxiv*.
12. Zhang F., *et al*. "A protocol for detection of COVID-19 using CRISPR diagnostics". *Nature Protocols* (2019).
13. Westgard JO., *et al*. "Covid-19 serology testing strategy: confirm positive results a two test prediction value calculator to assess effectiveness of confirming positive results". (2020).
14. Tan CW., *et al*. "A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction". *Nature Biotechnology* (2020).
15. Dorward DA., *et al*. "Tissue-specific tolerance in fatal Covid-19". *medRxiv.*
16. Odak I., *et al*. "Reappearance of effector T cells is associated with recovery from Covid-19". *EBioMedicine* (2020).