Methods of Diagnosis a Preferential Advantage in COVID-19: A Mini Review

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Humanity is going through never seen before health crisis due to the outbreak of novel coronavirus or Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). There are 24.02 million cases and 0.82 million deaths worldwide as of 26th August 2020 due to deadly infection of COVID-19. The disease has been spreading exponentially (R-naught number: 3) and has challenged even the best healthcare infrastructure in the world. With the progression of the disease, the countries shifted the focus from cure to diagnosis and containment to flatten the curve. The review shows that the disease is spreading exponentially while the resources are still limited. We focus upon the probable vectors of the virus, different diagnostic methods with advantages & limitations, and the way forward. This review article covers the different diagnostic methods with more advantages, limitations, and the future sneak-peek into the forthcoming developments for the diagnostic processes such as RT-PCR (Reverse Transcription Polymerase chain reaction).

Keywords: SARS-CoV-2; serology; RT-PCR; COVID-19; CT-scan; X-ray; coronavirus and ultrasound.
1. INTRODUCTION

The coronavirus belong to the family of coronaviridae with four different genera of alpha, beta, gamma and Delta corona virus was first detected in human beings in the 1960's. But, in the last two decades have seen four major virus outbreaks including the 2002 novel SARS-CoV epidemic (Guangdong province, China), the 2009 novel influenza A (H1N1) pandemic (California, United States), recently, the December 2019 SARS-CoV-2 pandemic (Wuhan city, Hubei province, China). The first time, the SARS-CoV-2 name was proposed [1]. The same day, World HEALTH Organization (WHO) called the disease as COVID-19 [2]. The R-naught number of SARS-CoV -2 is three i.e. one patient transfers the infection to 3 normal individuals, if it repeats to 10 cycles; a single infected individual spreads it to 59,000 individuals. As per the WHO report on 27th August 2020, 216 countries have been affected with 24 million cases and 0.82 million deaths [3]. So, the focus must be on containment of the disease, by preventing further transmissions that can be achieved only by early diagnosis and early isolation of infected ones [4]. Even though the SARS and MERS are high in mortality rate (3.4%) than both SARS and MERS combined.

1.1 Vectors of SARS-COV-2

SARS-CoV-2 genome sequencing proved that COVID 19 shows 85% homology with bat-CoV [5]. According to the analysis of Simplot 7genomic study, although high similarity of 89-96.2% is found. No animal reservoir has been confirmed yet, but due to its similarity with SARS, studies show that the receptor is the same for both.

It has a maximum 96% similarity with bat-CoV, indicating its origin from the bat. However, the intermediate host is not clear due to a lack of evidence and data insufficiency. It may have undergone a host jump, but some studies indicate the Pangolin CoV as the intermediate host, based on the similarities. From Blast analysis of around 2,845 known CoVs, 22 contigs matches, it indicates that Pangolin may carry novel genus and can infect all human populations, but individuals over 50 years of age are more prone to develop critical symptoms like hypoxemia, multi-organ failure, etc., ultimately leading to death.

It has a high mutation rate due to poor proofreading of RNA polymerase and recombination between different strains of SARS. Phylogeny implies the mutated to evolve into multiple strains specific to geographical regions. Amplification with MinION based sequencing data can provide a plethora of genetic information, that can help researchers develop more specific primers because in a country like India, USA, etc. multiple strains came from various countries. Thus, travel history will also guide them to sequence multiple strains out of random cases and provide further guidance for diagnosis & disease management. As a positive correlation exists amongst pathogenicity, immunological markers, micro biome, demography, and viral strain, it needs to be further evaluated as a part of complete diagnosis [6].

2. DIAGNOSIS

A rapid and authentic diagnosis kit is the need of the hour, to contain this global pandemic and outbreak management via enabling public health surveillance, as present diagnosis tools are scarce and the public health infrastructure has limited capacity.

2.1 Nucleic Acid Amplification Tests (NAAT)

RT-PCR detects the mRNA by two types of conventional methods that are one step and two step methods which amplifies the DNA fragment with the size upto 10^{-7}. TaqMan probes depend on the 5' nuclease activity of the DNA polymerase used for PCR to hydrolyze an oligonucleotide that is hybridized to the target amplicon. The tested RT-PCRs could detect positive samples with maximal Ct of 35.98.

RT-PCR is the most common choice to detect respiratory viruses with high sensitivity and specificity. The sample collection for NAAT is recommended from upper respiratory tract via naso-/oro-pharyngeal swabs [8]. While lower respiratory samples such as, bronchoscopy, sputum, etc. should be avoided due to its invasive nature. It detects genetic material in a swab that is better for symptomatic patients and used for etiological diagnosis. But, it is often found insufficient due to its limited geographical availability, expense, time, and need for a clinical visit by patients that breach the self-isolation protocol.
It also shows high false-negative rates due to multiple reasons like variable viral loads at various disease stages, calibration error, rapid viral mutation rate, etc contamination with host cells during sample preparation. Some non-specific primers often cause hindrance during amplification leading to false negatives as primers don't intend to amplify host RNA. Despite the false-negative results and debatable congruency, pharyngeal swab samples detected by RT-PCR stay the most preferred choice to determine discharge from hospital [9]. RT-PCR has pre-analytical issues that contribute to errors in the outcome. These issues include inappropriate sample collection, storage, insufficient amount, cross-contamination, handling error, and samples from the patients already undergoing antiretroviral treatment. Sample collection is the most significant part and negligence can jeopardize the results. Thus, to improve accuracy in the results, RT-PCR should be combined with CT-scan (Computed Tomography), clinical and epidemiological findings, and multiple NAATs. And epidemiology could be of great help to isolate patients for disease containment.

Keeping in mind the multiple drawbacks of RT-PCR, various other NAATs have been developed.

Three variants of RT-PCR depending on various target genes compared with RdRp-P2 assay, RdRp/Hel assay demonstrated higher sensitivity
due to its lowest Limit of Detection (LOD), specificity and also showed no cross-reactivity [10]. The usage of this assay would reduce the false negative percentage of RT-PCR results. This assay also provides high sensitivity for asymptomatic cases [11]. Another less studied tests includes Loop-mediated isothermal amplification (LAMP) and Penn-RAMP. Both are performed in closed tubes and detect nucleic acid either by calorimetric or fluorescence, collecting samples with simple nasal swabs. These are Point of Care (POC) assays, which can be performed with minimal sample processing and are rapid. Penn-RAMP allows multiplexing and 100X more sensitive than both LAMP and RT-PCR. It also requires a low amount of processed sample, reduces false negatives, and is quasi-quantitative. The possible application of Penn-RAMP could be in monitoring of disease progression [12].

LAMP in combination with RT where amplification and transcription happen concomitantly provides a better option. But when cross-checked, RT-PCR showed false-negative results for the samples with a lower concentration of the viral load below the threshold level. Although the overall study shows that RT-LAMP technique is accurate, sturdy and simple in practice. Further enhancement of RT-PCR includes iLACO has more accuracy and consumes less time than previous RT variants. This technique is economical and rapid as well, (utilizes approximately thirty minutes for a sample). RT-LAMP can be used for various blood, urine, saliva, oro, and nasopharyngeal swab samples while maintaining specificity and selectivity and does not need RNA isolation and sample processing, thus saving time and money as well. But in lack of bio-safety lab 3, all experiments were performed not with COVID-19 but with nucleotide oligos corresponding to Genebank thus, can have a higher rate of false positives than RT-PCR.

2.2 Serological Testing

SARS-CoV-2 may possess superantigenic fragments which shows the inflammation and damage in multi organs like heart and lungs which leads to cardiac arrest, cytokine storms which ultimately leads to death of an individual. the S1 subunit of the S protein binds to ACE2 expressed on the host cell surface. Subsequently the conformation of the S protein undergoes a significant structural rearrangement, resulting in shedding of the S1 subunit and transition of the S2 subunit to a highly stable post-fusion conformation, which in turn mediates the fusion of the virus with the host cell membrane and cell entry [13].

Unlike NAAT, it detects immune markers in serum i.e. IgM & IgG (Immunoglobulin M & G). Serological tests can confirm suspected as well as asymptomatic cases. Based on Chemiluminescence Immunoassay such as ELISA (CLIA), the light produced is directly proportional to the number of antibodies (Ab) in serum. Even though, antibody testing could be a better alternative due to rapid results for N and S protein of COVID. Once a person got infected, the IgM titer increase in blood for initial days of infection and falls later. At the same time, IgG titer increases later and remains persistent even after the patient got cured. SARS-CoV-2 is more than 90% in comparison with the molecular diagnosis. Although at beginning days of infection the Ab might not be found indicating false negative result.

Altogether, the serological tests are an effective method to diagnose COVID with a higher positive rate. These tests could be of great help for rapid disease surveillance during the unlock phase, post lockdown restrictions. Along with diagnostics, Immunoglobulin’s could have a therapeutic side, as donating plasma of Patients already cured of COVID can provide passive immunity to immune-compromised patients.

The synthesis of antibodies requires a few days after an individual got infected to elicit its effect. Further to reach a detectable concentration in blood, and in the immune-compromised group who do not produce Ab, it can show false-negative results. It can still be useful for epidemiological or surveillance issues as Ab titer is directly linked to severity of COVID, but not as an alternate of RT-PCR. The virus neutralization studies should be progressed to understand nature of Ab testing.

3. RADIOGRAPHIC TEST/TOOLS

Pulmonary (lung) imaging is done using CT-scan (usually HRCT: High-Resolution Computerized Tomography) and augmented by chest X-rays, which is the chief screening and diagnosis. According to the infection classification concerns its symptoms and imaging. Imaging stages are classified as early, advanced/progressive and severe which correspond with disease symptoms as mild, general/common and severe,
respectively [14]. While in some cases, the mismatch between imaging and symptoms onset may lead to difficulty in diagnosis. Imaging outcome supplements diagnosis when multiple NAAT comes negative, consecutively. A chest X-ray is suitable in the absence of a CT-scan facility. Whenever possible a chest radiography/ X-ray should be done using portable machines. Despite being less sensitive, it is yet helpful in follow-up and early diagnosis of COVID-19. Although CT-scan can detect even small lung lesions early, thus act as the differential diagnosis method for COVID-19 from other respiratory or non-respiratory problems. As the imaging have certain overlapping the final diagnosis should include clinical, epidemiological & laboratory results and etiology [15].

The early diagnosis in lung area CT-scan is limited with RT-PCR facilities. CT-scan is 97% sensitive in diagnosing SARS-CoV-2 while the sensitivity of RT-PCR varies from 60-70% [16] and varies highly across countries (65-96%) [17]. But the radiology department could prove to be a potential hub in spreading nosocomial infection that must be taken care off. Artificial intelligence might be useful in mass screening. CT-scan staging must be taken into consideration to examine progression as CT-scan results vary with age, immunity, drug intake, etc. Final diagnosis should be based on PCR and etiology eventually as CT-scan shows non-specific findings and overlapped results with other diseases [18]. But CT-scan could help as an auxiliary method for early diagnosis that could be confirmed using NAAT. In comparison with NAAT, CT-scan is rapid with high reliability as clinical symptoms are usually in sync with pulmonary lesions. Therefore, CT-scan has no alternative to its role as a preliminary screening of COVID-19. Unlike viral cases of pneumonia, COVID-19 is rare in children than adults. Even though, it affects children the pediatric comorbidities are extremely low in number.

Ultrasound (US) is non-invasive and can diagnose COVID-19 effectively. US data training deep convolutional neural network worked to develop open web service i.e. Point of care ultrasound imaging (POCUS). It enables rapid assessment and serves as a guide for further testings i.e. serological or NAAT etc. Since Ultrasound shows more details than CT-scan & X-ray, it gets preference at resource-limited settings. It is well established to monitor lung infections and could be a better alternative to X-ray as a prefatory screening method. Ultrasounds can detect physical lesions in correlation with Histopathology even before hypoxemia and is portable thus can be good option for follow ups. It is simple, repeatable, portable with high ease of disinfection, and non-invasive.

CT-scan can be a great tool in epidemiological context i.e. to flatten the curve but is expensive. The curve could be flattened by early diagnosis and early isolation of infected patients. But X-ray has low sensitivity and CT-scan have low specificity. Thus, we need more assays to complement their results for monitoring patients.

3.1 Additional Assays

Certain common indexes such as reduced leukocyte (WBC) count, increase in CRP (C - reactive protein) concentration, lymphocytopenia, etc. may indicate COVID-19 infection. Microfluidic devices could also be a better POC device due to small size, less sample requirement, and provide rapid results. Electrochemical sensors and Raman spectroscopy are still at naïve stages of development. Forward-Looking Infrared Radar (FLIR) could help in thermal detection. A test paper-based technique is rapid & accurate in comparison to RT-PCR (1 hour) utilizes Cas13a ribonuclease to detect RNA. There are no doubt those quantitative assays like serology has more advantages over qualitative. But a lot more is yet to discover in serology for COVID, IgM appears on the tenth day of infection but disappear soon, IgG appears after the twentieth day onwards and persists even after infection. But in the case of COVID-19, one out of the two is neutralizing, is yet to be ascertained. Some individuals never develop antibodies leading a false negative test, indicating the downside of serological assays. Another assay is; CLIA, a biomarker-based assay, having a high output of samples that could be analyzed; to detect IgG, IgM, and CRP. It needs to be worked upon in COVID-19 context as it can provide a rapid, cost-effective and convenient alternative. Fluorescence Immuno Chromatographic assay detects N-protein in nasopharyngeal swabs and urine within 10 minutes. It is rapid and can diagnose COVID even before symptoms.

4. CONCLUSION

The repercussions of SARS-CoV-2 on the global health economy are widespread. Although various diagnostic tools are available, no single technique is fully reliable, due to the fallacious
nature of these techniques. The only full-proof strategy to quarantine and self-isolation, and early diagnostic methods can achieve it. Early diagnosis can help in proactive isolation and better treatment leading to the containment of the disease. The technique with high diagnostic value at the early onset of disease will reduce the recovery time and would help in pandemic containment at a global level. Currently, RT-PCR is the golden diagnosis method that could be reduced highly by decreasing the time-lapse between primary symptom onset and test results i.e. around 20 days. As RT-PCR has low positives, it cannot fulfill an emergency need at a global outbreak. Thus for early diagnosis, isolation, proactive treatment, screening, and follow-up CT-scan would be a potent tool. The diagnosis with combined results of PCR and CT-scan provides a better alternative. In the absence of NAAT, imaging is a better choice for screening, and in resource-limited areas, CT-scan for diagnosis can be a better solution. Radiological tests would help in all spheres of diagnosis, screening, and monitoring.

**DISCLAIMER**

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

**CONSENT**

It is not applicable.

**ETHICAL APPROVAL**

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**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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