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Research Article

# Primary Diagnostic Tools for SARS-Cov-2 Infection (COVID-19): Current Challenge's in Molecular Techniques and Point of Care Testing for Screening

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**ABSTRACT**

Pandemic outbreaks are always a challenge for the health care management to hold the mortality rate when preventive measures are not established. The only solution is to control the spread by conducting massive screening and isolating affected ones from the healthy ones. The research concentration is to develop rapid diagnostics and screening at lower cost. Rapid diagnostic methods are being used and developed worldwide.Multiple studies suggest that RT-PCR, protein testing and CT should be the principal diagnostic instrument for routine testing in patients with COVID-19. But three factors are still indistinct in those diagnostic methods such as rapidity, sensitivity, and specificity. Thus, alternative approaches that provide higher efficiency and lower time requirement are highly appreciated for switching the super spread infectious disease detection.Moderntimes, microfluidics alternatively called lab on a chip or POCT have been widely used in cancer and viral detection, specifically for virus detection in very recent days. The ability of POCT tests to provide short time results,suitable in low resource clinics even at home. So, we saw the importance of miniaturized tools suitable for COVID-19 detection will endure the current expensive methods. Despite the limitations, approved tests are still experiencing good results against a great pandemic. We reviewed and highlighted the point of care testing (PCOT) method is not less than any other screening method by reviewing a wide research setting.

**Keywords:** Diagnostics; Sensitivity and specificity; COVID-19; POCT

## INTRODUCTION

In December 2019, COVID-19 has started to spread human to human and it is shortly called a communicable disease. More patients were let in with actual symptoms such as feverishness, cold, cough, throat infection. At first, infections were diagnosed after the chest computed tomography (CT) reports with different opacities slowly RT-PCR took the opportunity due to the need of mass screening [1]. Later those screened results from both methods revealed negative results because of the unknown origin [2]. A major failure that every country has rapidity and accuracy in types of equipment and a limited number of certified facilities. The currently available molecular test requires several inaccurate steps and hours of sampling this intended to increase the disease severity. Meanwhile, the SARS CoV-2 infection carries the risk of quick spread and hampers widespread testing of all possible interactions [3]. In the field of human epidemiological testing well-equipped laboratories are usually situated far from low-income resource defined areas. Point of care testing technology developed to fit the diagnostic needs of low source systems and small platforms have been prepared to offer speedy and low-cost for screening.It is significant to identify the infected patients and requiring immediate diagnosis will help to handle the patients at an earlier phase of the disease [4]. Rapid widespread of pandemic SARS CoV-2 generates a huge response in clinical alerts for serious illness. The development of new test kits and instruments is in preparation to improve the disease specificity and sensitivity. The importance of reliable and accessible test has become increasingly playing a vital role. Most of the infectious disease present themselves with similar symptoms and further make a co-infection, which will cause delay results with less accuracy. So, there is some potential testing to identify several pathogens using particular tests. Recent research has demonstrated that sensors have sufficient quality to identify the positive/negative results [5]. Although all the technologically advanced diagnostic methods are promising, implementing a PCOT technique encourage the screening participation in remote areas. In the meantime, WHO established a shipping mechanism to accelerate and offset the costs of exporting medical samples from SARS CoV-

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2. Laboratory Assessment Tool (LAT) is specially designed to assess the existing laboratory techniques that implement the COVID-19 test. Here we reviewed the main diagnostic methods with different assay and antibody testing methods. We further addressed the miniaturization of diagnostic kits with reliable accuracy.

## METHODS

We here reviewed important diagnostic methods for COVID-19 and its disadvantages. The search strategies were carried out using a basic science article websites like PubMed, Google scholar and the original articles were retrieved from Sci-hub which includes some important journals followed by the terms called (methods of COVID-19 diagnosis), (types of diagnostic method in SARS-CoV-2 infected patients), (COVID-19 diagnosis), (RT-PCR), (CT) etc. This review explains the original publications done by researchers from the start of pandemic disease to recent outbreaks.

### Current test methods for COVID-19

Respiratory disease is responsible for over millions of death worldwide [6]. Recently, the SARS CoV-2, a pandemic has emerged as a serious issue and making human life very critical. Visible symptoms are not realized in every patient because of its non-specific features. Each infectious disease consists of single pathogens and it is easily identified through diagnosis. *In vitro* test methods for disease, diagnosis is intended for greater accuracy and supports the patients economically for repeating the test. Infectious diseases can be identified using two different scientific methods as we know one is sociological and the other is called molecular methods. Most of the infectious disease is not visible at the time of infection due to their unresolved pattern. Currently, there are four major tests are used such as CT, RT-PCR, protein testing, point of care testing is widely used for early diagnosis of COVID-19 infection. In this review, we highlighted the important methods that can be run in adjacent analyzers of patients, rather than other centralized laboratory tests in hospitals.

## RT-PCR

A molecular diagnosis is the most appropriate method for detecting infectious agents. Extraction of the SARS-CoV-2 virus nucleic acid from a sample followed by combining reverse transcription of viral RNA and PCR amplification using RT-PCR methods. An RT-PCR assay was developed in early 2010 to detect Severe Acute Respiratory Syndrome (SARS CoV) [7]. During the first outbreak of coronavirus, a variety of conventional methods were developed, including commercially prepared PCR [8]. It is said to be the primary detection testing technique for COVID-19 [9]. There two main steps typically involved in the optimization of the testing method and this nucleic acid technique is done by using a kit that consists of specific probes and primers. Infectious disease is a life-threatening respiratory disease that appears in a non-specific manner. Previous PCR formats have had a decent sensitivity and specificity in viral diagnosis [10]. Also, RT-PCR is widely accepted because of its enhanced rapidity, sensitivity, reproducibility and low risk carry over contamination [11]. Taking immediate action National Medical Product Administration (NMPA) approved for 11 PCR based methods as well as eight antibody testing methods to speed up the diagnostic value [12]. The workflow of the nucleic acid test involved using low respiratory samples, including oral and nasal pharyngeal swabs [13]. Currently, SARS CoV-2 can more reliably detect nasal clots in the sputum after the onset of symptoms. Quantitative imaged RT-PCR assay can be regarded as the principal method employed to find out the interconnection of the COVID-19. Imperative issue from real rime finding doesn't give proper enhancing specificity by eliciting false-positive results [14]. In contrast, producing negative results from respiratory samples due to manipulation and low viral load or may be due to mutations and this type of less load recommendation has not shown to increase the efficiency of real-time results [15]. A challenging field that covers quantitative detection is an optimization of reverse transcription which leads to the low target amplicon generation and selecting one step and two-step assay for reliability [16].

At present Puck B and his colleagues compared seven different commercially available RT-PCR diagnostic kits reporting the efficiency and its limitation. To reforming the issues of viral loads their experiments based on selecting suitable kits provide good diagnostic purposes for the identification of positive samples. Reliable diagnostic molecular techniques will take 5 to 8 hours to report the results while comparing other virus detection methods it has lower possible for contamination as the entire process has done in a closed tube which avoids errors [17]. 82 samples with SARS-CoV-2 infection were taken for N-gene specific RT-PCR examination and absorbed different viral patterns and notably, sputum samples showed higher loads than swab samples from the throat [18]. Apart from different sampling procedures for SARS CoV-2, some researchers have described the protocol for clinical assay evolution. N-gene specific assay sensitivity is proved better than 1bgene specific PCR because of their higher amplification efficiency and this assay proved PK-15 as a control to use along with infected SARS samples [19]. The Chu and his colleagues evaluated an assay using both positive and negative panels of SARS-CoV-2 using their packages of probes and primers because of the similar restrained sequence of MERS. On the other hand, E gene assay was performed using 297 samples with no false-positive results [20]. The collected series of rapid testing of COVID-19 RT-PCR is one of the feasible techniques in research laboratories with its own set of challenges. Therefore the use of nucleic acid testing, which provides confirmative results of the virus becoming a major test yet the negative results of requiring appropriate care due to the false-negative results in every designed assay for SARS Cov-2. This detailed determination of NAT study in the infected SARS samples broadly indicating the lack of sensitivity and specificity.

### CT scans

A medical imaging procedure is more appropriate for diagnostic and more comprehensive evaluation of internal injuries. Computed tomography was started to use in China because of the sensitivity issues recorded from NAT for SARS-CoV-2 [21]. Also, CT is one of the major diagnostic tools which playsearly detection for COVID-2019 [22]. This comprises a key finding of bilateral involvement and local distribution [23] and these characteristics are observed using small cohorts [24]. Early changes in CT were examined in asymptomatic COVID-19 patients, supporting a true model with the symptoms [25]. Using CT scan for SARS infection in humans depending on the different stages of the disease CT scores distinguishes accordingly. For example, patients with SARS-CoV-2 undergone chest CT at 4-day intervals with 4 stages of the lung showed both decreased and increased CT scores [26], and the severity of the disease was recorded. In addition to areas where the lower lobes are most affected some studies have shown that ulcers are placed in the dorsal part of the lungs [27]. Meanwhile, the subtotal glass opacities of 50 infected patients were investigated in

typical CT manifestation while the sensitivity was higher compared to the sensitivity with RT-PCR [28]. The inadequacy of CT analysis in various fields showing CT cannot be used specifically for COVID-19 detection, although it has a high diagnostic sensitivity [29]. Later CT have been extensively compared with RT-PCR for better results, where 167 patients with negative RT-PCR results proved to be CT positive [30]. This finding from CT may be more susceptible to novel coronavirus also repeated testing is to be considered with more interval time. Since SARS-CoV-2 emerging in late 2003, now this new strain of SARS may not allow differentiating the exact imaging features of COVID-19 [31].

Nevertheless, various traditional modalities have been evolving lately with unique drawbacks. Here the evidence shows that CT is a significant method for diagnosing SARS CoV-2 infection even in asymptomatic individuals and may be considered as a screening tool in conjunction with RT-PCR. Notably, the asymptomatic abrasions are progressed in the first to second week after the onset of symptoms [32]. As discussed previously CT is not specific to COVID-19 or any other viral infection having high cost also RT-PCR will not identify the pre-infection for asymptomatic lesions [33]. Disease diagnosis using molecular and imaging techniques is preferable according to the medical environment, but both techniques are challenging in COVID-19 diagnosis. Utilizing of RT-PCR and CT techniques are needed to clarify with direct insight. Further research should allow space for proper diagnosis with exceptional value, especially for communicable diseases.

**Protein testing**

Protein (antigen) testing is different from above-discussed test methods this requires a protein from the viral coat from infected samples. Those portions of the viral protein line should be exclusively developed from the laboratory using cell lines that entered into an Immunoassay to detect antibodies [34]. Antibody test against Receptor Binding Protein (RBD) and Nucleoprotein (NP) was tested using urine, rectal and saliva samples of SARS-CoV-2 validate viral kinetics and control policies for the infectious disease [35]. A complete form of serological test can detect antibodies again certain infectious diseases with major cross-reactivity leads to false results, but some newly developed systems able to break this prevalence. For example, Elecsys Anti-SARS-CoV-2 detects antibodies for the novel coronavirus with 98% specificity and no cross-reactivity (ROCHE). Immunoassay estimates the immunogenic protein of a coronavirus which is the highly expressed viral proteins during an infection such as S and N proteins [36,37]. Antibody profiles of suspected individuals with undetectable levels against SARS-CoV-2 after 20 days may be a real negative event since, the occurrence of IgM and IgG antibodies [38]. Serum and plasma samples were employed to define the optical time points of antibodies, as well as monoclonal antibodies, were generated using peripheral blood B cells [39].

During this pandemic, CDC also introduced a serological test using an infected serum, these tests utilized live virus protein and spike antigen with 99% specificity [CDC]. Combined IgM and IgG ELIZA and GICA tests were performed using suitable antibody and plasma samples resulted in 87.3% and 82.4% sensitivity also it is proved to be a fast diagnostic test with a large number of samples [40]. Although antibody tests provide clear results with major drawbacks, antibody tests take several days todetect after the exposure of foreign substances [41]. The former phase of disease findings is still doubtful even with a big field of testing methods. Due to its false-positive results, it becomes less suitable for the SARS-CoV-2 diagnosis. Cross-reactivity can be a range of immunomodulators because it severely affects the specificity and sensitivity of the test [42]. This problem has been accosted in the RT-PCR technique with suitable solutions.

**Point of care testing**

In February 2020, authorization of medical devices was approved by the Department of Health and Human Services under the 564 FDC act. Point of care testing is a simple and easy way for detection. This method doesn't call for any sampling procedure or centralized laboratories [43]. A sample from the respiratory tract detects viral proteins and this protein will bind to specific antibodies attached to a paper tower enclosed in a plastic envelope generating a visible signal within 30 minutes if it is present in sufficient quantity. On the other hand, RapiPREP COVID-19 (LAMP based) test for SARS infected salivary swab samples using fluorescent dye has shown equivalent accuracy for PCR methods [44]. Another POC test includes smartphone-based testsusing specific nucleic acids with 1µl of sample volume it is also based on LAMP which helps to gather a fluorescent image can able to determine positive and negative results [44].

(POC) Point of caring test methods consumes time, nucleic acid test with (LAMP) Loop-Mediated Isothermal Amplification, amplifies DNA with high specificity also increases the rapidity of diagnosis [44]. Smartphones become widely accessible technology worldwide, it can leverage for this role as it has connectivity, computational power, and hardware to facilitate the epidemiological database [45] and integrate large response during a COVID-19 outbreak. Most of the POC devices are one time accessible with single-purpose cartridges. The Abbot ID kit requires 2 minutes of sample preparation time. The antibody POC test detects the infectious disease using IgM and IgG antibodies [46]. The test kits are namely called Assay Genie rapid POC kit, Gold site diagnostics kit, and VivaDiag COVID-19 IgG-IgM test (gold immunoassays). The Antibody POC test was compared to PCR assay for reference standard usage and IgM and IgG rapid test evolution is huge in the diagnostic accuracy [47]. One important revolution in PCOT method is using a microfluidics platform. Microfluidics are designed to perform screening by inexpensive method utilizes small sample volumes with high sensitivity [48]. Microfluidcs based smartphone sensor was developed by Laksanasopin et., in 2015. This sensor detects antibodies against sexually transmitted infectious disease by sequentially moving reagents prestored on a cassette.That shows 87% clinical sensitivity and specificity and further, he reported, these technologies can be adopted for our current pandemic detection [49]. Saliva is the preferred bio-fluid specimen for SARS-CoV-2 infection to carry out all types of diagnostics [50]. For COVID-19 infection, saliva sample has some advantages because of a non-invasive collection procedure will further reduce the nonsociomial spread of communicable diseases [51]. Clinical biomarkers such as small RNA, messenger RNA, including cytokines like IL-8, IL-1b and TNF-α are already recognized as an oral fluid sample [52]. It is told apart as that saliva can be a good specimen for SARS-CoV-2 infection.Several researchers from the past have reported microfluidics based detection of important viruses like HIV, Zika, Hepatitis B, Influenza [53]. Another important detection method based on the RPA technology, which, utilize microfluidics that integrates 3 PCR steps into a single chip weighs 3 kg and RTisochip proposed in china can able to detect 6 common respiratory viruses

within one hour which effectively identifies COVID-19 [54]. In 2017 Du et al. Designed an automated sample preparation attached microfluidics utilized air bubbles and magnetic beds to capture Ebola virus [55]. PDMS chip for HCV RNA detection from plasma [56] and PEG methylacetate membrane was integrated into the chip later the virus was concentrated through self-sufficient perfusion [57]. Microfluidics based virus detection doesn't need any related quantification to get results. Eventhough quantitative methods are frequently related by several factors [58]. Digital quantitative methods do not depend on the standard curve to attain a high sensitivity [59]. Currently Yeh et al. Developed an in situ detection technique based microfluidic chip to capture the rapid virus [60]. Microfluidic detection with digital quantification is a big challenge in integrated screening method. Viral infections are common but thepandemics of large scale infection are rare. In terms of good health, we think the way is too constricted to help out much with simple COVID-19 detection, but researchers are working as much as possible to break the SARS-CoV-2 chain. Researchers are rendering their attention to discover a complete cure and settle the situation back. However, testing is a key role to avoid deaths and it also can be avoided in the limited capacity of health systems. Because of lowerintegrated results from the proposed diagnostics microfluidics are kept for easy configuration for testing, which help to identify future epidemics much more than COVID-19

**Limitations of current clinical diagnostic method**

One of the main issues which we are facing currently in medical diagnosis is false-negative results. This part explains an important issue of nucleic acid detection, which is a reliable technology for the rapid test [61]. Tahamtan et al., discussed various challenges involved in the real-time detection methods [62]. Using primers in different genes during RT-PCR can affect due to the variations found in viral sequences [63]. Sampling procedures are largely contributed in the case of false-negative results and this important issue were reported in many cases of SARS CoV-2 infection [64]. One of the important assessments for detecting COVID-19 is RT-PCR, which is widely applied in research fields, but it is not being validated without limitations. Another important test method is CT which is also meant for the early detection method of COVID-19 affected patients. A study conducted on Feb 2020 by Zhang et al., identified limitations in their study by correlating lower positive RT-PCR and higher positive CT results [65]. This proves that CT tests are valid partially than RT-PCR. When it comes to effectiveness of diagnostic tools, there may be compromise as there is no better solution to the urgent need. Due to misdiagnosis, many patients suffer with or without actual illness. Bringing this into account sample collection and loading methods to be improved to obtain high accuracy solutions. In chest CT the time proceedings for results of patients were reported to be very high and some major limitation of CT in hospitals having fewer CT instruments. Aside from these 2 major tests one test that remains the most important in the diagnosis of COVID-19 is the antibody or protein test. Therefore, the current protein testing cannot provide a proper diagnosis in the estimated time also there is some noted significant lag period as targeting viral antibodies usually appears between 7-14 days after the onset of the disease [66]. Hence, the accuracy and timeliness for COVID-19 infection are unclear because of the misdiagnosis or complexity of individuals. Limitations of various diagnostic methods to be adopted into consideration and those misleading's of diagnostic value should be ameliorated by increasing the test kit's value. New implementation for rapid test tools is needed with minimum accuracy by designing a cost-effective tool also high-quality measurement is essential in each type of test method.

## DISCUSSION

COVID-19, a highly intense pandemic disease has begun to increase globally with the average death rate in humans.SARS CoV-2 affects newborn to old age people around the Earth and made a major shock in both clinical and research fields.This outbreak leads the rapid testing due to its wild features and testing starts to lead off in every clinical lab.The biggest loophole in the clinical examination is misdiagnosis which needs to be concentrated, this follows the important molecular-based laboratory exam. Every observed molecular test showed individual performance based on their accuracy. RT-PCR results were found to be more substantial than the chest CT and the rest protein testing studies had shown to be less appropriate.Although studies conducted by diverse sorts of research having fewer errors that let in the sensitivity and specificity of the disease. For example, a significant problem with RT-PCR (the gold standard) method has low performance test resultsThese negative effects might be ascribable to the improper sampling techniques or quality of the kits [67]. The actual sensitivity of CT for SARS-CoV-2 infection may have less accuracy also the quality (methods) of the test methods is still unclear. After CT and RT-PCR were compared using 601 patients, in that only 59% had positive PCR results while 88% had chest CT positive, i.e., 75% sensitivity of RT-PCR and 97% of chest CT [68]. In protein-based testing, clinicians are recommended not to perform the test solely that is suggested that protein test methods are used as a complementary tool for gold-standard tests [69]. However, each technique has its limitations. Here in this review, we notified some important issues in molecular tests. Firstly, the low RT-PCR performance and shortage of kits with lack of tool availability in rural areas. Secondly, time management in decisions affects sick individuals taking their supplements. Thirdly, misdiagnosis which affects the individual who does not experience the disease.And lastly, the monetary value of lab tests affects less economically stable people (unaffordable). So, a key part of achieving our health goal with low budgets is to promote preventive health care that will benefit to reduce the number of persons involved in the laboratory operation. Diagnosis is one of the major components of health care advisories also an emerging emphasis on common (Screening) methods to preclude the onset of any sort of major disease. Point of care testingis simply called home test is simply cost-effective in the screening of diseases. Now, as we know the cost of four recommended tests which is less affordable to take up every infected patient isremains unclear. Promoting high-performance POCT in clinics and also in labs can considerably reduce the expenditure. Taking PCOT as a screening tool will help to reduce the time for result output. Thus far many adopted techniques were built up with high performance equal to molecular-based viewing. This includes microfluidics miniaturized sensors have likely to meet the most challenging factors in global health care for technical requirements. Microfluidics includes smartphone-based LAMP assays, silicon chip assay, PCR assay, on-chip amplification, and fluorescent technology assays which assure the sensitivity of early detection for both communicable and non-communicable diseases [70,71]. Microfluidics has several approaches over other conventional methods and can be used to improve the existing tools to render low cost. We take these recommendations by reviewing various articles from different countries and hereby conclude that PCOT can be a good and cost-

effective screening tool also it is likely to reduce the integration of false positive and false negative results in SARS-CoV-2 infected patients.

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