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Primary Diagnostic Tools for SARS-CoV-2 Infection (COVID-19): Mini Review Based on Current Challenges in Molecular Techniques and Point of Care Testing for Screening

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Pandemic outbreaks are always a challenge for the health care management to control the mortality rate when the preventive measures are not established. The challenge is being faced worldwide with the outbreak of COVID-19 including the developed countries. The only solution is to control the spread by conducting massive screening and isolating affected ones from the healthy ones, which needs the development of screening methods that can reach the maximum population. The next step is to come up with vaccines. The research concentration worldwide is focussed on developing rapid diagnostics and screening at an affordable cost. Multiple studies suggest that Reverse Transcription- Polymerase Chain Reaction (RT-PCR), protein testing and Computed Tomography (CT) should be the principal diagnostic methods for routine testing/ screening in patients with COVID-19. But three factors are still indistinct in those diagnostic

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methods such as rapidity, sensitivity, and specificity. Thus, alternative approaches that provide higher efficiency and rapid are highly appreciated for the super spread infectious disease detection. Presently, microfluidics-based test kits alternatively called as lab-on-a-chip or Point- Of - Care Testing (POCT) have been widely used in cancer and viral detection. The ability of POCT tests to provide short time results, suitable in low resource clinics or even at home should be an attractive alternative. So, the miniaturized tools suitable for COVID-19 detection will replace the current expensive methods. Despite the limitations, the approved tests are still providing good results for this high risk pandemic. Many articles in the recent past highlighted the POCT method is not less than any other screening methods and by reviewing a wide research background, the importance of developing POCTs for screening such pandemic is presented here.

Keywords: Diagnostics; sensitivity and specificity; COVID-19; POCT; pandemic.

1. INTRODUCTION

In December 2019, COVID-19 has started to spread human to human and it is shortly called an emerging infectious disease. More patients were admitted with symptoms such as fever, cold, cough and throat infection. Initially, infections were diagnosed using the chest computed tomography (CT). This method was reported with different opacities and later Reverse Transcrption-Polymerase Chain Reaction (RT-PCR) slowly gained the opportunity due to the demand of mass screening [1]. Subsequently, those screened results from both methods revealed negative results because of the unknown origin [2]. The major challenge that every country has faced are rapidity and accuracy of most of the equipment in addition to limited number of certified facilities. The currently available molecular test requires several steps and hours of sampling, which intended to affect the final test results. Meanwhile, the SARS CoV-2 infection carries the risk of quick spread and hampers widespread testing of all possible interactions in addition to presenting asymptomatic cases [3]. In the field of human epidemiological testing well-equipped laboratories are usually situated far from low-income resource defined areas [4]. Point of care testing technology developed to fit the diagnostic needs of low resource systems and small platforms to offer speedy and low-cost screening. It is important to identify infected patients with the immediate diagnostics schemes which will help to treat the patients at the earliest. Pandemic SARS CoV-2 (acute illness) rapidly generated a huge impact on clinical and non-clinical areas. The evolution of new test kits and instruments aims to ameliorate 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 establish a co-infection, which can cause delayed results with less accuracy. 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 which helps to implement a POCT technique to encourage the screening participation in remote areas. In the meantime, World Health Organization (WHO) established a shipping mechanism to accelerate and offset the costs of exporting medical samples of SARS CoV-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 useful for COVID -19 screening and further presented the miniaturization of diagnostic kits with reliable accuracy.

2. METHODS

We reviewed important diagnostic methods for COVID-19 with their advantages and 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 key terms called (methods of COVID-19 diagnosis), (types of diagnostic method in SARS-CoV-2 infected patients), (COVID-19 diagnosis), (RT-PCR), (CT) etc. Over 100 original research and review articles were reviewed from various sites and 75 of them were shortlisted to prepare this review. The review was conducted to understand the existing schemes for diagnosis and screening and the possibility of mass screening using point of care instruments aiming at controlling the spread of the disease.

2.1 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.

2.2 RT-PCR

Molecular diagnosis is the most appropriate method for detecting infectious agents. This method calls for isolation of SARS-CoV-2 virus nucleic acid from a sample followed by combining reverse transcription of viral RNA and PCR amplification using RT-PCR. An RT-PCR assay was developed in early 2010 to detect Severe Acute Respiratory Syndrome (SARS CoV) type I infection [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 technique for COVID-19 [9]. There are two main steps typically involved in the optimization of the testing method and this nucleic acid technique is performed by utilizing a kit that consists of specific probes and primers. SARS-CoV-2 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 taken over because of its enhanced rapidity, sensitivity and reproducibility [11]. Immediate action was taken by National Medical Product Administration (NMPA) in China, they have been 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 use of human 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 RT-PCR assay can be regarded as the principal method employed to find out the interconnection of the COVID-19. The compelling problem with real time discovery is that it does not give proper developmental character by obtaining 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 were described. 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 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 which 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 because the entire process has done in a closed tube which avoids errors and contamination [17]. Eighty two 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]. Then 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. Hence the role of

nucleic acid testing, which provides confirmative results of the virus becoming a major examination, even the negative results requiring appropriate care due to the false-negative outcomes in every designed assay for SARS Cov-2...Bigtech Labs, Bangalore, a molecular biology based company built a world's first portable micro PCR with different application which contains an optical system to detect a fluorescence signal from sample to reduce false-negative results. This detailed determination of NAT study in the infected SARS samples broadly indicating the lack of sensitivity and specificity

2.3 CT Scans

A medical imaging procedure is more appropriate for diagnostic and more comprehensive evaluation of internal injuries. Computed tomography was used 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 plays early 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 differentiates 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.

2.4 Protein Testing

Protein (antigen) testing is different from the 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, Centre for Disease Control (CDC) also introduced a serological test using an

infected serum, these tests utilized live virus protein and spike antigen with 99% specificity. 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 to detect 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.

2.5 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 does not 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 tests using 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 [45].

(POC) Point of caring test methods consumes less time, nucleic acid test with (LAMP) Loop-Mediated Isothermal Amplification, amplifies DNA with high specificity also increases the rapidity of diagnosis [46]. 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 [47] 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 [48]. 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 [49]. 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 [50]. 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 [51].Saliva is the preferred bio-fluid specimen for SARS-CoV-2 infection to carry out all types of diagnostics [52]. 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 [53]. 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 [54]. 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 [55]. 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 [56]. In 2017 Du et al. designed an automated sample preparation attached microfluidics utilized air bubbles and magnetic beds to capture Ebola virus [57]. PDMS chip for HCV RNA detection from plasma [58] and PEG methylacetate membrane was integrated into the chip later the virus was concentrated through self-sufficient perfusion [59]. Microfluidics based virus detection doesn't need any related quantification to get results. Eventhough quantitative methods are frequently related by several factors [60]. Digital quantitative methods do not depend on the standard curve to attain a high sensitivity [61]. Currently Yeh et al. developed an *in-situ* detection technique based microfluidic chip to capture the rapid virus [62].

Microfluidic detection with digital quantification is a big challenge in integrated screening method. Viral infections are common but the pandemics 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 lower integrated results from the proposed diagnostics microfluidics are kept for easy configuration for testing, which help to identify future epidemics much more than COVID-19.

3. LIMITATIONS OF CURRENT CLINICAL DIAGNOSTIC METHOD

One of the major issues which we are facing currently in medical diagnosis is false-negative results. This part explains an important issue of nucleic acid detection meanwhile it is a reliable technology for the rapid test [63]. Tahamtan et al. discussed the challenges like false negative results influenced by mutation in probe and primer of the SARS-CoV-2 genome and decreased assay performance due to mismatches between target sequences involved in the real-time detection methods [64]. 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 [65]. 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 Fang et al. identified limitations in their study by correlating lower positive RT-PCR and higher positive CT results [66]. 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 two major tests one test that remains the most important in the diagnosis of COVID-19 is the antibody or protein test. Antibody testing may aid to evaluate the epidemiology of the disease which helps to do the normal activities probably with lower specificity and sensitivity results [67]. 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.

4. DISCUSSION

COVID-19, a highly intense pandemic disease has started to increase globally with the highest death rate in humans (2 million deaths) at the end of Septemeber-2020 (WHO).SARS CoV-2 affects new born to old age people around the world and produced a major impact in both clinical and research areas. This outbreak leads the rapid testing due to its wild characterstics 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 test. 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 in diagnosis. For example, a significant problem with RT-PCR (the gold standard) method has low performance test results. These negative effects might be ascribable to the improper sampling techniques or quality of the kits [68]. 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 was 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 [69]. In protein-based testing, clinicians are recommended not to perform the test solely, in which they suggested that protein test methods are used as a complementary tool for gold-standard tests [70]. However, each technique has its limitations.

Here in this review, we bring in 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 testing is simply called home test which 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 is remains unclear. Promoting high-performance POCT in clinics and also in labs can considerably reduce the expenditure. Taking POCT as a screening tool will help to reduce the time for result output [71]. 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 [72]. 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 [73]. In 2015 Salim et al. designed a microfluidic device which specifically hybridize probe attached with a fluorescence reader for Retinoblastoma in infants (Rb) using miRNA from blood samples. Mixing technique using specific MB- probes can be applicable for detecting different miRNA with specific diseases was suggested [74]. Later in 2017 same technique was established and proved by detecting breast cancer with different grades assessing miRNA 21 as a biomarker [75]. Microfluidics platforms have several approaches over other conventional methods and can be used to improve the existing tools to render low cost by way of reduced assay volumes.

5. CONCLUSION

We hereby conclude that POCT could be a cost-effective screening tool for diseases like SARS-CoV-2 which is a rapidly spreading disease and there is the need for screening large population.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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