International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Recent Diagnostic Techniques for COVID-19

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Abstract: ***Introduction: The recent outbreaks of human corona virus infections, at the beginning of 21th century have shown the prominent roles of rapid and accurate diagnostic technologies, to contain the emerging and re-emerging pandemics. Despite the development of specific and sensitive point of care tests & serological immunoassays, RT-PCR still remain the gold standard performed on respiratory specimens for diagnosis of COVID-19. Even though excellent techniques are available for the diagnosis of symptomatic patients with COVID-19 in well-equipped laboratories; critical gap still remain in screening asymptomatic people who are in incubation phase of the disease, as well as in the accurate determination of live viral shedding during convalescence to make decisions for ending isolation. Objectives: Recent diagnostic techniques for diagnosing COVID-19. Research question: what the recent diagnostic techniques for COVID-19? Method and materials: In this review of articles, at first, about 92 articles of COVID-19 that were published before July 21, 2020, were studied. After excluding repeated issues, 20 relevant articles were selected for this research. Those articles that were focusing clinical features, treatment & prevention, were excluded. The articles were searched in NCBI, PubMed, Elsevier & Google scholar engines. Meanwhile, recent data about diagnosing COVID-19 were taken from CDC, WHO & Uptodate as well. Results: In this review we found that there is no simple, fast, reliable, specific, sensitive, cost-effective and widely available test for diagnosing COVID-19. Up to now, RT-PCR is the gold standard for definitive diagnosing of COVID-19. Rapid serological tests constitute second diagnostic tests, which are widely available, cost-effective, point of care tests. CT- scan, however if used with RT-PCR, is very accurate in asymptomatic patients even if RT-PCR is negative. The new generation, simple, cost-effective, point of care, specific and sensitive tests are greatly developing for diagnosing COVID-19. Conclusion: To contain SARS-CoV-2 ongoing pandemic and lower the global health pressure, it's important to develop, produce & globally distributed most recent, rapid, widely available, cost-effective& point of care diagnostic test for COVID-19.***

Keywords: COVID-19, SARS-CoV-2, Diagnostic approach, Diagnostic challenges, Serology.

1. Introduction

1.1 Background

The SARS-CoV-2 virus has emerged in Wuhan, China, and poses a global threat to public health around the world. According to the World Health Organization as of July 10, 2020, the virus has infected 120,648,828 people in 216 countries, including 5,50,384 deaths. The most important and appropriate approach to prevent the adverse consequences of viral epidemics, requires the development of surveillances programs and necessary laboratory preparations. During a viral pandemic, diagnostic laboratories (using molecular diagnostic methods) play a crucial role in the rapid and accurate identification and isolation of new microorganisms [1]. In addition, the introduction of molecular diagnostic techniques and rapid serological tests can lead to the rapid identification, isolation and treatment of positive Covid-19 cases [2]. This article discusses the existing molecular and serological diagnostic tests (laboratory-based and point of care diagnostic technologies) for the diagnosis of Covid-19. Advantages and disadvantages of the current diagnostic techniques and the problems in their implementation are also discussed.

1.2 The role of diagnostic techniques in SARS-CoV-2 pandemic

The role of diagnostic tests depends on the type of test available, the resources required to perform the test, and the time required to announce the test result. In other words, the rapid identification of suspected cases is the main goal, to properly observe the use of personal protective equipment (PPE) and to prevent the spread of infection to the hospital and subsequently to the community [3]. Some available point of care tests (POCT), are based on molecular techniques and are suitable for detecting new cases of Covid-19. While other types of these tests are based on serological methods and are more suitable for determining people who have already been infected [4].

1.3 Implementing the current diagnostic tests and its challenges

These tests, with their advantages and disadvantages & challenges are discussed as follow:

a) Pre-analytic errors and errors during analysis of samples

There is undeniable evidence that shows that the stage before analysis of experiments is a major source (46 to 68.2% of the total) errors of medical laboratories [5]. It is estimated that a quarter of all errors before test analysis lead to unnecessary research, inadequate patient care, increased financial burden on the health sector, and ultimately inadequate and slow health care services. Safety and quality of diagnostic tests can be affected greatly by, misdiagnosis of the patient or sample, inappropriate and inadequate preparation of samples, unsuitable conditions for transport and storage of samples (prolonged transfer time or damaged samples), the presence of prohibitive substances in Samples (for example, cellular components due to freezing of blood or unsuitable additives) and finally issues related to sample preparation, including errors in pipetting during manual preparation of samples, cross-contamination and non-compliance of samples.

Although analytical errors are the smallest contributors to laboratory errors, there are several potential analytical problems that could significantly jeopardize the quality of testing. These errors include equipment malfunction, non-

adequately validated assays, undetected failure of quality control, active viral recombination, testing carried outside the diagnostic window, poor harmonization of primers or probes, and non-specific rRT-PCR annealing [6].

b) Chest computerized tomography

Chest scan is a routine, non-invasive, rapid and accurate radiological test. The sensitivity of CT scan to detect Covid-19 is high, compared to real time RT-PCR. Recent studies have shown that asymptomatic patients of Covid-19, can show bilateral chest radiographic evidences (ground glass opacities) on CT scan even before real-time RT-PCR is positive. Scientific evidence shows that the best approach to suspected patients of COVID-19 is to perform combination tests including real time RT-PCR, epidemiological evidence (exposure to patients with Covid-19, presence of symptoms and symptoms) and chest CT scan [7].

c) Nucleic acid amplification tests (NAAT)

Studies has shown that molecular methods for definitive diagnosis of Covid-19 are more accurate than CT scans and serological tests. Because molecular methods can target and detect SARS-CoV-2 specific antigens. Currently, molecular diagnostic methods (NAAT) for SARS-CoV-2 include real-time RT-PCR (performed in a laboratory setting) and reverse transcription loop-mediated isothermal amplification (RT-LAMP) performed at a patient care facility. Unfortunately, the current diagnostic tests are time consuming, need professional employees and human resources and lastly, lack of adequate diagnostic kits delays the diagnosis [8].

- ***Manual laboratory based NAAT***: Currently, real time RT-PCR for various clinical specimens including; broncho-alveolar lavage, lavage fluid, fibrous bronchoscopy biopsy, mucus, nasal swab, throat swab, feces and blood, is the gold standards for definitive diagnosis of SARS-CoV-2. Real time RT-PCR has several advantages: It is a specific test that can differentiate SARS-CoV-2 from similar viruses in the early stages of infection, even when the viral load is low. Thus, unlike serological testing, real time RT-PCR is of high diagnostic value for virus detection in the early stages of infection (which aims to prevent the spread of infection) because it can directly detect viral RNA even before antibodies are developed in the patient's serum. On the other hand, test results are shown in a few hours and can easily be performed on a large mass of patients. Low sensitivity and stability, and long time for the collecting & transferring of sample are the disadvantages of this test. Several external factors can also affect the accuracy of this test, including: sampling operation, sample source (upper or lower respiratory tract), sample collection time (before or after the onset of symptoms) and weather serological test are used or not simultaneously. Recent studies have shown that commercial diagnostic kits used in the market for diagnosing SARS-CoV-2 have low diagnostic accuracy (less than 100%) and false negative results in patients during the first week of illness have also been reported [9]. Broad studies carried out on the tests used for diagnosing coronavirus in China show that the results of 41% of cases were false negatives [10]. In addition, real-time RT-PCR testing requires trained individuals to use complex laboratory equipment correctly. On the one hand, these tests are performed in central laboratories (level two and above), on the other hand, they are time consuming and it take several hours to one or two days, to obtain laboratory results. This often leaves a rapidly rising number of potential cases untested and thus opening a gaping hole in SARS-CoV-2 prevention efforts. Finally, the US Food and Drug Administration (FDA) concluded that a negative rRT-PCR test result does not completely rule out SARS-CoV-2 infection and shall not be used as a single element for patient management decisions, and re-testing shall be considered in consultation with public health authorities [43].
- ***Rapid & Point of care NAAT:*** RT-LMAP has made it possible to perform molecular diagnostic tests in a patient-care facility instead of a laboratory setting. This method also increases the number of tests to be performed, shortens the time for announcing test results and paves the way for early detection of positive cases [12]. LAMP (Loop-mediated isothermal amplification) is a fast, accurate, reliable, and inexpensive technology that requires only one thermal cycle to determine the genomic sequence, unlike real-time RT-PCR, which is complex and need sophisticated laboratory thermal cycling equipment. The main advantage of LAMP over real-time RT-PCR is that the amount of DNA it produces is far greater than PCR, and positive results can be seen with the naked eye, as opposed to PCR, which the machine must read the results. In addition, it is simple, inexpensive, and has high performance speed [13].
- **Serological diagnostic tests for Covid-19**: Serological and point of care tests are developing to diagnose positive cases, identify asymptomatic patients and those who are in convalescence period. Serological tests have several advantages over real-time RT-PCR: Serological tests can provide more details by identifying people who have antiviral-specific antibodies in their blood serum. If these tests are positive, they indicate that the person has passed the infection, so they can provide better information about the prevalence of infection in a community. Antiviral-specific antibodies, unlike viral RNA, remain in a person's blood for several weeks to months after the onset of symptoms. When a person's serological results are negative, it means that the person may not have been infected while collecting the sample, but this does not mean that the person will not get sick. Also, the development of antibodies against SARS-CoV-2 does not mean that a person is immune to Covid-19, as many strains of SARS-CoV-2 are not neutralized by antibodies. Given the fact that 20-80% of SARS-CoV-2 positive cases are asymptomatic, in such circumstances the evaluation of the immunity system of individuals in a community by serological tests is valuable. Because serological tests alone are not sufficient to diagnose SARS-CoV-2, the concomitant use of serological tests and molecular diagnostic methods can provide satisfactory results [14].

d) Manual ELISA

Different types of ELISA kits are designed to detect neutralizing antibodies (IgM / IgG / IgA) against SARS-CoV-2. Various ELISA kits are also available for coronavirus antigens (SP and NP), but these kits are used for

research purposes and have limited values for clinical diagnostic purposes. Despite the current problems, serological tests using the ELISA test still play a major role in the diagnosis and control of the current pandemic. Therefore, the development of ELISA hand kits as a complementary test for real-time RT-PCR and the removal of some of its shortcomings and limitations in the future, is still a top priority [15].

e) Automated serology

The growing demand for diagnostic tests on the population of communities imposes a large clinical and economic burden on diagnostic laboratories. The usage and implementation of serological diagnostic tests has increased the quality assurance and reduced the time for the samples to return, as well as reduced the false negative and false positive results. Automated techniques are now common in most serological tests. Conventional serological tests, which are more acceptable than automated tests, are deployed in a laboratory setting to identify individuals' immune status. These tests will be very useful later when the outbreak reaches its peak. The majority of manual ELISA kits available for SARS-CoV-2 use a 96-well microplates as solid phase & standard calorie metric method to receive the signal. While in automated ELISA, solid phase materials are different, for example, instead of microplates, polystyrene or metal-based nanoparticles (magnetic beads) are used. In automated method, highly sensitive systems such as chemiluminescence technology are used. In April 2020, a fully automated serological test for SARS-COV-2 antibodies was launched. This test was developed to obtain specific IgG antibodies against S1 and S2 domains of spikes proteins of SARS-CoV-2. This increases the specificity of this test and prevents the interaction of different types of coronaviruses and thus avoid false positive results [16].

f) Rapid serological test

Rapid diagnostic tests are designed to evaluate asymptomatic patients who are in convalescence period. These tests are small and portable and are based on qualitative measurements with either negative or positive results. Some rapid serological tests have used lateral flow techniques. For example, Surescreen Diagnostic COVID-19 IgG / IgM rapid test cassette and Biomedomics rapid IgM-IgG combined antibody test for COVID-19. Others have used time-resolved fluorescence immunoassays. For example, Goldsite Diagnostics Inc. SARS-CoV-2 IgG / IgM kit. All rapid serological tests can detect antibodies from different samples like, blood, plasma or serum. The procedure for all of these tests is the same. for example, taking blood from a patient's finger, adding it to the kit, and then adding buffer solution to it. The results of these tests take 10-15 minutes [16].

g) Tissue culture & Neutralizing test with Actual or Pseudo Virus

Virus neutralization assay (VNA) is one of the most specific tests to study the reaction of antibodies to the virus and prevent their proliferation. This test detects only those antibodies that prevent the virus from multiplying, not all antibody reactions. Because common antigens among viral groups can be the same, only some of these antigens are targeted by neutralizing antibodies [17]. VNA testing is performed in four steps: diluting the serum, incubating the serum with the virus, inoculating the cell culture, and identifying and detection. Although VNA testing is highly specific, it is extremely complex, time consuming, and requires skilled staff to perform the test.

Approaches to improve the accuracy of Covid-19 diagnostic tests

As SARS-CoV-2 is highly contagious, to avoid false positive results that can adversely affect epidemiological efforts for containing the ongoing pandemic, the following measures can increase the effectiveness of current diagnostic methods:

a) During the implementation of NAAT (Nucleic Acid Amplification Test), samples should be taken from the desired sources. Preliminary research has shown that the nose and throat are the most accurate sites to take a swab sample (18). Which study is preferable between the two is a different study. The Centers for Disease Control (CDC) recommends nasal swabs for NAAT tests [18].
b) The Test results should be confirmed using various diagnostic techniques and false negative results should be reduced. The development of these combined diagnostic techniques (serological tests and NAAT) is helpful in achieving high quality results and containing the pandemic of SARS-CoV-2.
c) Multiple combined diagnostic techniques should include tests that are performed during the course of the illness, from the time the patient enters the hospital and at weekly intervals [20].

COVID Diagnostics Technologies/Techniques under Development

Diagnostic tests that have not yet been widely used to diagnose Covid-19 and are under the developments are as follows:

a) **CRIPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats):** This test is one of the most sensitive, specific, fast, and simple PCR test, by which nuclear acids are detected [21]. This molecular method can target and detect up to 10 copies of SARS-CoV-2 nucleic acids in samples without using any special equipment. Therefore, this method is considered suitable for use in local centers and hospitals [21].
b) **Gold Nanoparticles**: this is one of the new technologies in the field of medicine and diagnostics. This molecular method is simple, fast, and sensitive which facilitate quantitative detection with excellent multiplexing capabilities. Gold nanoparticles were greatly envisioned as state-of-the-art technologies for rapid viral detection. But so far no evidence of their use has been reported for detection of SARS-CoV-2 [22].
c) **SERS (Surface-Enhanced Raman Scattering):** SERS, which uses fluoresce in, has emerged as a powerful molecular analysis method. This method is one of the most sensitive techniques used to obtain multiple components in a mixture or sample. This technique can still be used as a point of care test. However, no studies have been reported on the use of this technology to detect SARS-CoV-2 [23].

2. Results

As we discussed in the introduction, there is not a single, sensitive, specific, cost-effective, widely distributed & point of care diagnostics test. To contain the ongoing SARS-CoV-2 pandemic, a comprehensive strategy including extensive surveillance program, early case finding & diagnosis, isolation of patients to prevent transmission, clinical treatment, researches & development of vaccines, is urgently needed. The role of diagnostic tests for the diagnosis of Covid-19 depends on the type of test available, the resources required to perform the test, and the time required to announce the test results. The results of this research are summarized in the following table:

Available diagnostic tests for COVID-19				
Technique	Principle of working	Advantages	Disadvantages	Results / timing
Next generation sequencing (NGS)	Whole genome sequencing	Very sensitive & specific Provide detailed information	Expensive, laboratory based, need sophisticated lab equipment & trained staff	1-2 days
Real time PCR	Detect nucleic acids using specific primer & probe	Rapid, sensitive, specific, gold standard	Expensive, laboratory based, delayed results	4-5 hours up to 1-2 days
Loop-mediated isothermal amplification (LAMP)	Gene sequencing	Very accurate, needs one thermal-cycle, the results can be read by naked eyes instead of machine	High rate of false positive/negative results due to cross-contamination & contamination during transmission	1-3 hour
Rapid serological test (traditional)	Based on detection antigen-antibody (IgM/IgG)	Sensitive & specific	High rate of false positive & negative results	4-6 hours
Point of care serological tests (POCT)	Based on detection antigen-antibody (IgM/IgG)	Rapid, Sensitive & specific	High rate of false positive & negative results	15-30 minutes
Chest CT-san	Radiographic evidence (ground glass opacities)	Very accurate	Absence of radiographic evidence if delayed, lack of comparisons between other viral radiographic evidence	Minutes
Virus Isolation	Viral culture	100 specific	Very low sensitivity	5-15 days

3. Discussion

To contain the ongoing pandemic, we need a rapid diagnosis of SARS-COV-2. To reach the accurate diagnosis, a history of travel from endemic areas of the disease, clinical signs and symptoms, and laboratory diagnostic tests, are essential. The primary goal to contain Covid-19 pandemic is to reduce the transmission of infection among the population by reducing the number of susceptible individuals or reducing the basic reproductive number (R0). R0 can be modified by several factors, including the persistence of the virus, the pathogenicity of the microorganism, and contact-materials between susceptible and infected individuals [24]. Since vaccine and effective treatment against Covid-19 are not yet available, the only way to reduce the transmission of SARS-CoV-2 as much as possible is to quickly identify and isolate patients. Only some of Asian countries, including South Korea, have successfully conducted unprecedented national tests, performing 300,000 tests during the first nine weeks of Covid-19 outbreak, to prevent the spread of infection to that country [25]. In the same way, Singapore implemented different protective measures including a broader case definition, aggressive contact tracing, and strict patient isolation [10]. Most importantly, to identify asymptomatic patients who did not meet the case definition, a Singapore-wide screening program on patients with pneumonia, influenza-like illnesses, severely ill patients in ICU, and deaths with a possible infectious cause was performed [11]. Similar approaches were taken in Taiwan and Hong Kong as well. These countries broke the infection transmission chain by conducting widespread tests, early detection of cases & isolation of patients and were able to successfully control the spread of COVID-19 to these countries [25]. Given the fact that 20-80% of SARS-CoV-2 positive cases are asymptomatic, in such circumstances the evaluation of the immunity system of individuals in a community by serological tests is valuable [26]. Because serological tests alone are not sufficient to diagnose SARS-CoV-2, the concomitant use of serological tests and molecular diagnostic methods can provide satisfactory results [14]. The role of diagnostic tests for the detection of SARS-CoV-2 depends on the type of test available, the resources required to perform the test, and the time required to announce the test results: **Next-generation genomic sequencing**; Based on the determination of the genomic complement sequence, the pathogen is stable, highly descriptive and sensitive, and provides complementary information. The test results take one to two days, but require technical personnel, the price is high and the equipment Requires advanced and complex laboratory [27] **Real-time PCR**, using specific primers and probes, detect nucleic acids. This method is rapid with high sensitivity, and requires a small amount of DNA to perform the test. It has great results in diagnosing viral diseases. But the disposable and sophisticated laboratory equipment is very expensive and the test results take longer time [28]. **Loop-mediated isothermal amplification (LAMP)**; In contrast, RT-PCR requires only one thermal cycle to determine the genomic sequence. This test is very accurate, the amount of DNA it produces is far greater than RT-PCR, and the results can be seen with the naked eye instead of the machine. Test results take one to three hours. But false positive results in it relative to contamination [29]. **Traditional rapid serological tests**; It is based on detection of antigen-antibody (IgM / IgG) this technique is laboratory based. It is considered as a sensitive and specific test. Test results take about 4 to 6 hours. These tests become positive at least three days after the onset of symptoms and have no diagnostic value before that. Its false positive results are also high [16]. **Point of care rapid serological tests;** It is based

on detection of antigen-antibody (IgM / IgG. These tests can be performed near the patient's bedside and in the patient care area. The results take 15-30 minutes. This test should be done three to four days after the onset of symptoms, because they are negative before that. In addition, its false positive results are high [27]. **CT-scan;** Chest CT scan of asymptomatic patients shows bilateral radiographic evidence (ground glass opacities) even before RT-PCR is positive. Its efficiency is enhanced when performed in combination with RT-PCR. However, CT scans cannot differentiate radiographic findings of other viral infection causing pneumonia [18]. **Virus isolation;** the virus is cultured and isolated in a laboratory environment. This method is 100% specific and is the gold standard of diagnosis. The results take 5-15 days. However, its sensitivity is very low, because the isolation of the virus is not 100% certain [30].

4. Conclusion

To prevent the SARS-CoV-2 pandemic and reduce the pressures on health systems around the world, the capacity of diagnostic tests should be improved. Rapid diagnostic tests that are fast, reliable, cost-effective, available, sensitive, specific & point of care, must be urgently developed, produced and widely distributed all over the world.

5. Suggestions

Considering the discussion and the final result, the following points are suggested:

1) To contain the SARS-CoV-2 pandemic, prevent infection & avoid slowing the identification process, new diagnostic strategies must be devolved.
2) Similar viral pandemics will occur in the near future that will not be rapidly detected by current specific molecular diagnostic methods, as developing a specific molecular diagnostic method for a particular pathogen will take weeks and months. In such cases, the development of rapid serological diagnostic tests and triage of patients to differentiate viral & bacterial infections; Maintaining health care resources (capacity confirmatory tests), improving operational efficiency (triage and congestion prevention), and strengthening the Ministry of Public Health's efforts to control pandemics (case identification and quarantine).

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