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REVIEW ARTICLE

COVID-19 testing and diagnosis: A comparison of current approaches

Rebecca S.Y. WONG*

Faculty of Medicine, SEGi University. No. 9, Jalan Teknologi, Taman Sains Selangor, Kota Damansara, PJU 5, 47810 Petaling Jaya, Selangor, Malaysia.

Abstract

The severe acute respiratory syndrome coronavirus 2 is a novel coronavirus that causes the coronavirus disease 2019 (COVID-19). COVID-19 has been declared a pandemic by the World Health Organisation since March 2020. To date, the number of confirmed COVID-19 cases has exceeded 47 million and more than 1.2 million people have lost their lives to the disease. The disease is spreading at an exponential rate with no signs of slowing down. COVID-19 testing and early diagnosis play a crucial role in not just patient management, but also the prevention of the further spread of the disease. Various diagnostic approaches have been applied to detect SARS-CoV-2 infection. This article will critically review these diagnostic approaches and compare each with the gold-standard, which is viral RNA detection using reverse transcriptase-polymerase chain reaction (RT-PCR).

Keywords: SARS-CoV-2; outbreak; pandemic; COVID-19 testing; diagnosis

INTRODUCTION

The world is currently under the threat of the coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). However, coronaviruses (CoVs) have long existed before the COVID-19 pandemic. CoVs are positive sense, enveloped single-stranded RNA viruses belonging to the coronaviridae family, which can be found in both humans and animals. These viruses can be broadly divided into four genera i.e. alpha-, beta-, gamma- and delta-coronavirus.[1] In the past, the human coronaviruses (HCoVs) have been identified to cause diseases such as the common cold. Other than the more commonly known severe acute respiratory syndrome (SARS)-CoV and Middle East Respiratory Syndrome (MERS)-CoV, four HCoVs have been reported in the published literature, i.e. HCoV-OC43, -229E, -NL63 and -HKU1. The first two have been identified since the 1960s and are a leading cause of mild respiratory diseases after the rhinovirus. The other two, on the other hand, have been reported in the 21st century.[2-4]

More recently in December 2019, a new pathogenic HCoV, known as the 2019 novel coronavirus (2019-nCoV, now renamed as SARS-CoV-2) was recognised in Wuhan, China, causing a serious health issue in the country and globally.[5] As of 3rd November 2020, there have been over 47 million confirmed COVID-19 cases and more than 1.2 million deaths worldwide. The United States of America, India and Brazil are the top three countries with the highest number of confirmed cases, contributing nearly half of the global total confirmed cases.[6] The genomic characterisation in one study revealed that 2019-nCoV is closely related to two SARS-like coronaviruses which are bat derived, namely bat-SL-CoVZC45 and bat-SL-CoVZXC21. However, they share less similarities with SARS-CoV and MER-CoV comparatively. Genetic sequencing revealed >80% identity to SARS-CoV and 50% to MERS-COV but 88% identity to the two bat coronaviruses.[7]

As the mysteries of SARS-CoV-2 continue to unfold, the number of publications on various aspects of the virus has been on the rise ever since its discovery in December 2019. Following the World Health Organisation's announcement of COVID-19 as a pandemic on 11th March 2020[8], to control and prevent the spread of SARS-CoV-2 infection has become one of the top priorities for many countries. Many diagnostic tests are currently available in the market for COVID-19

**Address for correspondence:* Rebecca S.Y. Wong, Faculty of Medicine, SEGi University. No. 9, Jalan Teknologi, Taman Sains Selangor, Kota Damansara, PJU 5, 47810 Petaling Jaya, Selangor, Malaysia. Tel: +603- 6145 1777 (ext. 3616) Fax: +603-6145 2649. E-mail: *rebecca@segi.edu.my; rebeccawongsy@gmail.com*

testing. Early and accurate diagnosis is important as the infected individuals can be isolated and treated promptly and the public can be protected from being infected by these individuals. This review aims to discuss the various approaches used in COVID-19 testing and critically examine the evidence in the published literature. It also gives a brief account of the role of imaging in the diagnosis of COVID-19 while comparisons are made between the gold standard i.e. reverse transcriptase-polymerase chain reaction (RT-PCR) and other methods.

1. SARS-CoV-2

To understand the principles behind the tests used in COVID-19 testing and diagnosis, a basic understanding of the virus causing the disease is essential. Similar to other CoVs, SARS-CoV-2 is also an RNA virus. Phylogenetically, SARS-CoV-2 falls within the Sarbecovirus subgenus and Beta-coronavirus genus. Like the bat-SL-CoVZC45 and bat-SL-CoVZXC21, SARS-CoV-2 has a long branch length. Although SARS-CoV-2 is genetically distinct from SARS-CoV, they share a similar receptor-binding structure.[7] Research has shown that, just like SARS-CoV and COV-NL63, the spike protein of SARS-CoV-2 interacts with receptor Angiotensin Converting Enzyme 2 (ACE-2), to gain entry into the host cells, especially the alveolar epithelial cells, with the help of a serine protease TMPRSS2, for S protein priming.[9]

Zhu *et al*. isolated SARS-CoV-2 from bronchoalveolar lavage samples obtained from infected patients and studied the structure of the virus inoculated on human airway epithelial cells. Light microscopy revealed cytopathic effects in the human airway epithelial cells, with a lack of cilium beating after 96 hours of inoculation. Under the electron microscope, SARS-CoV-2 exhibited morphological features that were consistent with the coronaviridae family. The virus appeared spherical while showing some pleomorphism. The size of the virus measured approximately 60-140 nm in diameter. Due to the presence of distinctive spikes (measuring about 9-12 nm), the virions had an appearance resembling a solar corona. Transmission electron microscopy revealed the presence of viral particles both outside the human airway epithelial cells and within cytoplasmic inclusion bodies of these cells.[5]

2. COVID-19 testing and diagnosis

Early detection and diagnosis are important when it comes to patient management, disease containment, as well as prevention of further spread in an outbreak, especially for a highly infectious disease. COVID-19 testing can be broadly divided into two approaches. The first approach identifies the presence of the virus itself such as via the detection of its RNA or antigen. The second approach involves the detection of antibodies produced as a result of the infection. The former mainly identifies those infected during the acute phase of infection, while the latter identifies those who have developed antibodies against the virus. However, it is noteworthy that antibodies may not show up for weeks in asymptomatic or mild cases.

2.1 Viral RNA detection

Advances in molecular biology have allowed rapid developments in nucleic acid detection methods that have revolutionised viral detection. Techniques such as real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) and reverse transcription-loop mediated isothermal amplification (RT-LAMP) have been applied to detect the presence of viral RNA in patient samples. Currently, RT-PCR, known for its high sensitivity and specificity, is the gold standard used in the diagnosis of COVID-19. However, the use of RT-PCR requires specialised machines and expensive reagents, as well as skilled technicians, and the test can take up to hours. The common targets of RT-PCR include various genomic regions of SARS-CoV-2 such as the open reading frame (ORF), RNA-dependent RNA polymerase (RdRP), nucleocapsid, spike protein and envelope genes.[10,11]

For RT-PCR, specimens from the upper respiratory tract (e.g. swabs from the nasopharynx or oropharynx, tracheal aspirate or bronchoalveolar lavage [BAL]) may be used.[12] However, the use of bronchoscopy to obtain the specimen is usually avoided as the generated aerosols pose a high risk for the healthcare professionals. Hence, it is mainly considered if the patient is intubated or when specimens of other sources are negative, subject to meeting certain clinical and safety criteria for cases with uncertain diagnosis.[13] It is worth mentioning that SARS-CoV-2 viral RNA not only exists in specimens obtained from the respiratory tract, it can also be found in the urine, blood and anal swabs as reported by Peng *et al*.[14] Interestingly,

the presence of viral RNA did not indicate the presence of symptoms. For example, urinary tract symptoms were absent despite the presence of viral RNA in urine samples.

Although RT-PCR has been routinely used in detecting coronaviruses, including SARS-CoV-2, RT-LAMP has been reported to be a faster way of detecting viral RNA and the whole LAMP reaction can be as short as 30 minutes as demonstrated in one study.[15] Compared to RT-PCR this method does not require repeated cycles of sample heating and cooling during the nucleic acid amplification process. Therefore, a thermal cycler is not needed for this technique. In the published literature, many RT-LAMP assays have been developed for rapid and cost-efficient detection of SARS-CoV-2. Studies have demonstrated that LAMP can detect SARS-CoV-2 RNA at levels that are significantly low and is more sensitive compared to conventional RT-PCR.[10,11] Key differences between RT-PCR and RT-LAMP are summarised in Table 1.

2.2 Antigen detection

In comparison with viral RNA detection, there is not as much information on antigen detection for SARS-CoV-2. An antigen can be referred to as a substance derived from a pathogen that is capable of triggering an immune response. Such substances can be proteins and in the case of coronaviruses, usually, these proteins are derived from the surface spikes of the virus. In the United States, the first antigen test for SARS-CoV-2 was approved by the Food and Drug Administration (FDA) under emergency use authorisation (EUA) in May 2020 The test was intended to detect SARS-CoV-2 nucleocapsid protein antigen present in specimens taken from nasopharyngeal and nasal swabs, which is usually detectable during the acute phase of the infection.[16] One advantage of antigen testing over nucleic acid testing is that it can be done within a shorter time. As the antigen is specific to the virus, a positive result is highly accurate but a negative result cannot rule out infection. Therefore, in cases of negative results, confirmation using a PCR test is necessary before treatment.

On the other hand, researchers in China reported the use of an antigen test using fluorescence immunochromatography, for the detection of SARS-CoV-2 nucleocapsid protein present in samples of urine and nasopharyngeal swabs. Antigen testing was done parallel with nucleic acid testing and the findings revealed a 100% accordance between the two methods. The study also demonstrated that positive patients could be identified as early as after 3 days of fever, whereas 73.6% of patients who tested positive for COVID-19 had detectable nucleocapsid in their urine samples.[17]

2.3 Antibody detection

Tests that detect antibodies play an important role in COVID-19 testing, especially in patients who present late with mild or moderate disease. Research has shown that the positivity of PCR declines with time especially in mild cases. In one study that investigated the viral load dynamics of COVID-19, it was reported that in respiratory samples, a significantly longer median duration of the virus was observed in patients with severe disease (21 days, 14-30 days) compared to that of patients with mild disease (14 days, 10-21 days; P=0.04).[18] This means that RT-PCR may yield false-negative results for the latter and serological testing for antibodies may be useful in this group of patients.

Although some studies have demonstrated the

TABLE 1: Comparing RT-PCR and RT-LAMP in COVID-19 testing

RT-PCR	LAMP
• Requires the use of a thermal cycler and expensive reagents	• Thermal cycler and expensive reagents not required
• Samples often need to be transported to a central laboratory	• Test can be done within a hospital laboratory
• Test is slower and takes hours	• Test is faster and takes as short as 30 minutes
• Gold standard in COVID-19 testing due to its sensitivity and specificity	• Studies have shown that it can be more sensitive than RT-PCR
• Test is more established and has been used on many patients	• Test is less established and less tested on patient samples

observation of antibodies within the first week of symptom onset[19,20], using serological testing for antibodies may yield false-negative results in patients who have a mild form of the disease if the test is done within the first two weeks of the onset of clinical illness. Typically, the levels of IgM and IgG begin to increase in the 3rd and 4th week of symptom onset. However, by week 5, IgM levels begin to decline and nearly disappear by week 7, while IgG levels may persist beyond week 7 from the onset of symptoms.[21] Other than its role in detecting disease in the later stage of COVID-19 in milder cases, antibody testing helps in understanding the disease's extent in the community and identify individuals who have developed immunity to COVID-19.

3. Role of imaging in the diagnosis of COVID-19

To date, RT-PCR remains the gold standard for the diagnosis of COVID-19. Due to its lack of specificity, imaging has not been used as a first-line tool for diagnosis, even though chest X-ray or CT findings are common in patients with COVID-19. Nevertheless, chest CT still plays a role in the early detection and management of COVID-19 as demonstrated in some studies. Some common imaging findings include ground-glass opacities (GGO) and peripheral consolidation with some patients showing the evolution of GGO into consolidation, followed by resolution of lung changes subsequently. However, some of these changes that are visible on chest CT are not visible on chest X-ray, suggesting that chest CT is a more sensitive imaging modality compared to chest X-ray.[22]

In one study, Xie *et al*. reported the usefulness of chest CT in the diagnosis of COVID-19 in 5 patients with high clinical suspicion of infection but showed negative RT-PCR results initially. The patients were all isolated for presumed COVID-19. A repeated swab testing was carried out later and SARS-CoV-2 infection was confirmed subsequently. Hence, a combination of RT-PCR and chest CT may be helpful in patients with obvious clinical features but negative RT-PCR results at the initial stage.[23] Patients in their early phase (≤ 7 days after symptom onset, n=40) of the disease and advanced phase (8-14 days after symptom onset, n=22) demonstrated different chest CT findings in another study. For the former, GGO, consolidation, pleural thickening and pleural retraction sign were more common, while GGO and reticular pattern, fibrotic streaks and pleural effusions were more common in the latter.[24]

It is important to note that a dissociation between clinical, laboratory and imaging findings is not uncommon. Patients who are tested COVID-19 positive with RT-PCR may not show any chest CT changes whereas those with positive chest CT findings may not have a positive RT-PCR test. In addition, imaging findings of COVID-19 are not specific and resemble those of other pulmonary infections.[25] Therefore, imaging findings should be correlated with clinical features and laboratory findings in the diagnosis of COVID-19. A comparison between viral RNA detection by RT-PCR and various approaches in COVID-19 testing and diagnosis is summarised in Table 2.

CONCLUSIONS

From this review, it can be concluded that various methods have been applied in COVID-19 testing, which utilises different technologies. However, to date, the detection of the presence of SARS-CoV-2 via RT-PCR remains the gold standard in the diagnosis of COVID-19. Depending on the approach used, some tests are better suited for diagnosis while others are more useful for screening, monitoring or other purposes. As in many other diseases, no one test is perfect, and each method described has its unique advantages and disadvantages. The choice of the method used often depends on the individual case presentation as there is no such thing as a one-size-fits-all approach. Sometimes, a combination of two or more approaches is used in cases where there are uncertainties. Where there is a late presentation with mild symptoms or a strong clinical suspicion but a negative RT-PCR, the use of antibody testing and imaging may help to diagnose COVID-19. Keeping in mind the advantages and disadvantages of the currently available diagnostic methods, future research should aim at the development of diagnostic kits that are specific, sensitive but at the same time, rapid and cost-effective that can be used in mass quantities.

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TABLE 2: Comparing various approaches in COVID-19 testing and diagnosis

	RT-PCR	Antigen detection	Antibody detection	Imaging
What the test detects	• Detects viral RNA	• Detects viral antigen e.g. proteins from surface spikes	• Detects antibodies produced by the patient in response to infection	• Visualizes lung changes typical of pneumonia
	• Gold standard in diagnosis • Mainly used to detect the presence of infection during the acute phase • Not used in gauging the disease extent in the community	• Mainly used to detect the presence of infection during the acute phase	• May identify mild cases that present weeks after symptom onset where RT-PCR is negative • Can be used to gauge the disease extent in the community and identify individuals who have recovered from the disease and developed immunity	• Not used as a first-line diagnostic tool • May be helpful in patients with obvious clinical features but negative RT-PCR results • Imaging findings need to be correlated with clinical and laboratory findings
Specificity	• High specificity • A positive test indicates the presence of acute SARS-CoV-2 infection	• Specific • Positive test indicates active infection • However, in cases with negative results, RT-PCR is necessary	• Specificity varies with the kits used for detection • A positive test indicates either current or past infection	• Not specific • CT findings are common to many pulmonary infections • Presence of findings does not necessary indicate SARS-CoV-2 infection
Technical requirements	• Requires the use of a thermal cycler and expensive reagents	• Thermal cycler and expensive reagents not required	• Thermal cycler and expensive reagents not required	• Requires imaging machines such as a CT scanner
	• Samples often need to be transported to a central laboratory	• Test can be done within a hospital laboratory	• Test can be done within a hospital laboratory	• Test can be done in the imaging department of a hospital
	• Skilled technician required to perform test	• Skilled technician not required	• Skilled technician not required	• Radiologist required
Duration of test	• Test is slower and takes hours	• Test is faster than RT-PCR	• Test is faster than RT-PCR	• Test requires interpretation by a radiologist

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