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DIAGNOSTIC TOOLS FOR CORONAVIRUS DISEASE 2019 (COVID-19): AN OVERVIEW

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease was first identified in December 2019 in Wuhan, the capital of China's Hubei province, and has since spread globally, resulting in the ongoing 2019–20 coronavirus pandemic. The COVID-19 pandemic has placed incredible tension on healthcare systems around the globe. Common symptoms include fever, cough, and shortness of breath. Other symptoms may include muscle pain, diarrhea, sore throat, loss of smell, and abdominal pain. It is challenging for doctors and physicians to distinguish COVID-19 from other acute respiratory tract infections via clinical symptoms because those who are infected display a wide range of symptoms. An effective, point-of-care (POC) diagnostic tool could ease healthcare system strain, protect healthcare professionals, and support quarantine efforts. With a lack of effective medical treatments or vaccines for COVID-19, early identification and patient isolation has been the most effective means of disease management to slow the spread of the pandemic. COVID-19 testing involves analyzing samples to assess the current or past presence of SARS-CoV-2. The two main branches detect either the presence of the virus or of antibodies produced in response to infection. Test analysis is often performed in automated, high-throughput, medical laboratories by medical laboratory scientists. In this article, we have discussed the currently used diagnostic tests for detection of Coronavirus and COVID-19.

Keywords – COVID-19; Coronavirus; Diagnostic tools; Rapid antigen test; RT-PCR.

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) was discovered in Hubei Province, China in December 2019.[1] A cluster of patients were admitted with fever, cough, shortness of breath, and other symptoms.[2] Patients were scanned using computed tomography (CT), which revealed varied opacities (denser, more profuse, and confluent) in comparison to images of healthy lungs.[3] This finding led to the initial diagnosis of pneumonia. Additional nucleic acid analysis using multiplex real-time polymerase chain reaction (PCR) of known pathogen panels led to negative results, suggesting that the cause of pneumonia was of unknown origin.[1] By January 10, 2020, samples from patients' bronchoalveolar lavage (BAL) fluid were analyzed to reveal a pathogen with a similar genetic sequence to the beta-coronavirus B lineage. It was discovered that this new pathogen had ~80%, ~50%, and ~96% similarity to

the genome of the severe acute respiratory syndrome virus (SARS-CoV), Middle East respiratory syndrome virus (MERS-CoV), and bat coronavirus RaTG13, respectively.[1,4] The novel coronavirus was named SARS-CoV-2, the pathogen causing COVID-19. As of April 2, 2020, the disease has spread to at least 202 countries, infected over one million people, and resulted in at least 45,526 deaths globally. It is suspected that the total number of reported COVID-19 infections is underestimated, as there are many mild or asymptomatic cases that go undetected.[5] From the Diamond Princess cruise ship case study, an estimate of 17.9% of asymptomatic cases were reported. Asymptomatic individuals are as infectious as symptomatic individuals and are therefore capable of further spreading the disease.[6]

SARS-CoV-2 is an RNA virus, and thus all available RNA detection formats can potentially be applied to detect the virus.[7,8] For adaption towards the more frequently used diagnostic DNA detection formats, the viral genome needs to be transcribed into a DNA complement by reverse transcriptase. Currently, the preferred SARS-CoV-2 test is DNA amplification by PCR, and the real-time versions of such tests were among the earliest available. Such tests were previously developed during the emergence of SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), and therefore a PCR-based testing approach for SARS-CoV-2 was an obvious route to take.[9] Moreover, monitoring the host response is important in identifying individuals who have already been infected with SARS-CoV-2 as well as for assessing future vaccine efficacy. For that purpose, again similarly to tests previously developed for SARS-CoV and MERS-CoV, a broad variety of tests detecting specific SARS-CoV-2 antigens and antibodies were developed. Over the past months, all currently available technologies have been exploited to rapidly develop highly sensitive and highly specific detection and characterization assays for SARS-CoV-2.

With a lack of effective medical treatments or vaccines for COVID-19, early identification and patient isolation has been the most effective means of disease management to slow the spread of the pandemic. In point-of-care (POC) diagnosis, a test is performed at the time and place of patient care to inform the treatment plan. An effective POC diagnostic tool for COVID-19 could address current diagnostic limitations and improve healthcare and quarantine efforts.

One of the most significant challenges for clinicians has been distinguishing COVID-19 from other acute respiratory tract infections because of its wide range of symptoms. Currently, the gold-standard approach is real-time reverse transcriptase-polymerase chain reaction (qRT-PCR), which detects the presence of viral ribonucleic acid (RNA).

In this review article, we have discussed various tests and tools which can be used for diagnosis of COVID-19.

2. DIAGNOSTIC TOOLS FOR DETECTING SARS-CoV-2

COVID-19 testing involves analyzing samples to assess the current or past presence of SARS-CoV-2. The two main branches detect either the presence of the virus or of antibodies produced in response to infection. Molecular tests for viral presence through its molecular components are used to diagnose individual cases and to allow public health authorities to trace and contain outbreaks. Antibody tests (serology immunoassays) instead show whether someone once had the disease. They are less useful for diagnosing current infections because antibodies may not develop for weeks after infection. It is used to assess disease prevalence, which aids the estimation of the infection fatality rate.[10,11]

Positive viral tests indicate a current infection, while positive antibody tests indicate a prior infection. Other techniques include a CT scan, checking for elevated body temperature, checking for low blood oxygen level, and the deployment of detection dogs at airports. [12,13]

Figure 1 provides a graphical summary of the various approaches used for detection of SARS-CoV-2 and diagnosis of COVID-19.

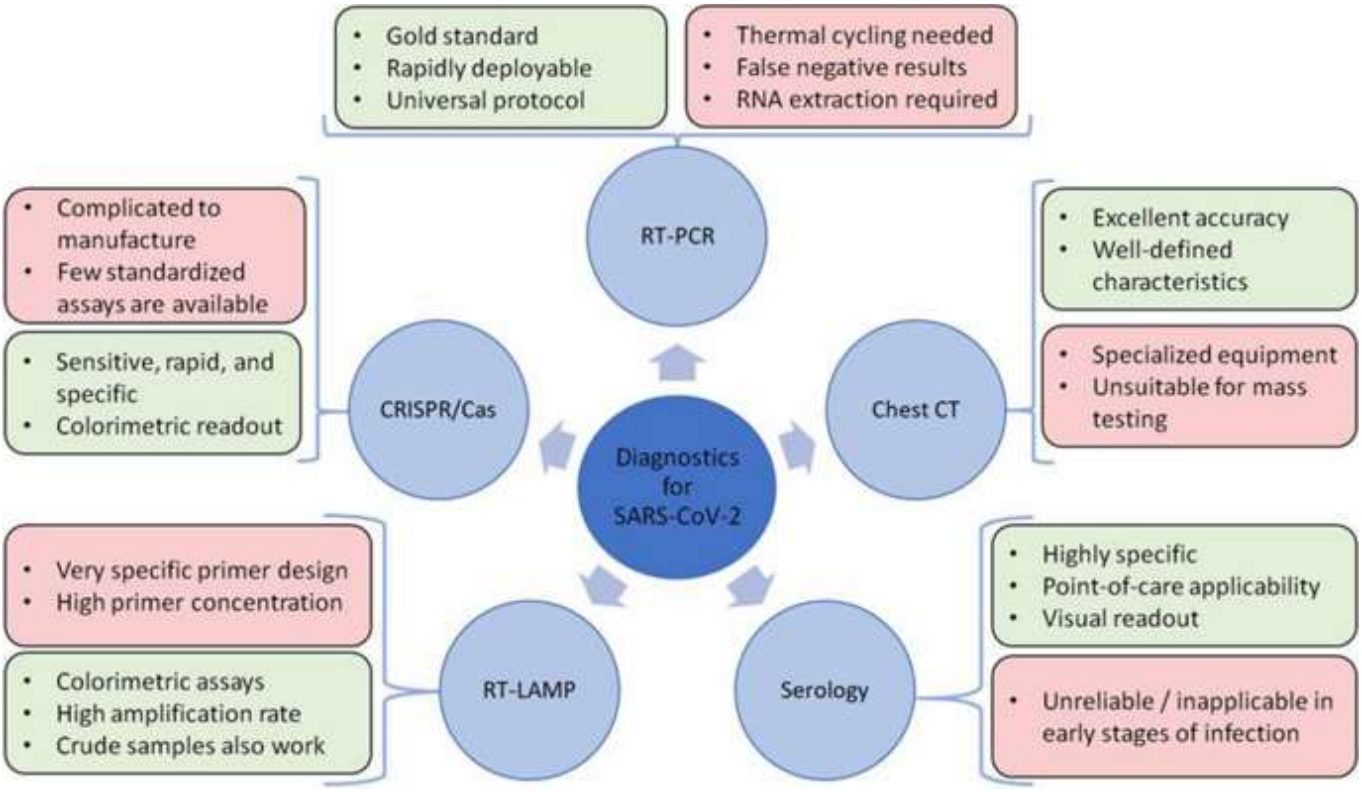

Fig. 1: Summary of the various approaches towards diagnosis of SARS CoV-2 infection.[14]

Figure 2: depicts the adequacy of the principal assay types used or proposed for COVID-19 for 4 key use cases.

		Selected Use Case			
		Screening during incubation/ asymptomatic phase	Diagnosis of symptomatic disease	Screening for viral shedding in convalescence phase for de-isolation decisions	Epidemiologic surveillance
Assay Type	Laboratory-based RT-PCR or NAAT assay	Unknown/insufficient negative predictive value	Current reference standard	Unknown/insufficient negative predictive value	Passive surveillance Unknown/insufficient negative predictive value for case finding
	POC sample-to-answer NAAT assay	Unknown/insufficient negative predictive value	Likely comparable to reference standard	Unknown/insufficient negative predictive value	Passive surveillance Unknown/insufficient negative predictive value for case finding
	Antigen detection POC*	Unknown/insufficient negative predictive value	Yet to be developed	Likely insufficient negative predictive value	Likely lower sensitivity than NAAT will hamper predictive value with low prevalence
	Serology IgM/IgG detection (POC or laboratory based)*	Likely false-negative in early disease	Likely false-negative in early disease†	Typically do not mirror disease activity	Serosurveys could assess individual and population immunity*

Figure 2: Heat map showing the adequacy of principal assay types (rows) for 4 key use cases.[15]

2.1 Detection of Virus

Detection of the virus is usually done either by looking for the virus' inner DNA, or pieces of protein on the outside of the virus. Tests that look for the viral antigens (parts of the virus) are called antigen tests. There are multiple types of tests that look for the virus by detecting the presence of the virus's DNA. These are called molecular tests, after molecular biology. As of 2021, the most common form of molecular test is the reverse transcription polymerase chain reaction (RT-PCR) test. PCR-based methods are considered the gold standard for viral detection. SARS-CoV-2 requires RT-PCR-based approaches, by virtue of being an RNA virus. Polymerase chain reaction (PCR) is a process that amplifies (replicates) a small, well-defined segment of DNA many hundreds of thousands of times, creating enough of it for analysis. Test samples are treated with certain chemicals that allow DNA to be extracted. Reverse transcription converts RNA into DNA. Reverse transcription polymerase chain reaction (RT-PCR) first uses reverse transcription to obtain DNA, followed by PCR to amplify that DNA, creating enough to be analyzed. RT-PCR can thereby detect SARS-CoV-2, which contains only RNA. The RT-PCR process generally requires a few hours. These tests are also referred to as molecular or genetic assays. Real-time PCR (qPCR) provides advantages including automation, higher-throughput and more reliable instrumentation. It has become the preferred method. The combined technique has been described as real-time RT-PCR or quantitative RT-PCR. Samples can be obtained by various methods, including a nasopharyngeal swab, sputum (coughed up material), throat swabs, deep airway material collected via suction catheter or saliva. [16,17]

There are three issues that have arisen with RT-PCR. First, the availability of PCR reagent kits has not kept up with demand. Second, community hospitals outside of urban cities lack the PCR infrastructure to accommodate high sample throughput. Lastly, RT-PCR relies on the presence of detectable SARS-CoV-2 in the sample collected. If an asymptomatic patient was infected with SARS-CoV-2 but has since recovered, PCR would not identify this prior infection, and control measures would not be enforced.

2.2 Antigen tests

Mucus from nose or throat in a test liquid is placed onto a COVID-19 rapid antigen diagnostic test device (COVID-19 Antigen Rapid Test Kit). An antigen is the part of a pathogen that elicits an immune response. Antigen tests look for antigen proteins from the viral surface. In the case of a coronavirus, these are usually proteins from the surface spikes. SARS-CoV-2 antigens can be detected before onset of COVID-19 symptoms (as soon as SARS-CoV-2 virus particles) with more rapid test results, but with less sensitivity than PCR tests for the virus.

Antigen tests may be one way to scale up testing to much greater levels. Isothermal nucleic acid amplification tests can process only one sample at a time per machine. RT-PCR tests are accurate but require too much time, energy and trained personnel to run the tests. Samples may be collected via nasopharyngeal swab, a swab of the anterior nares, or from saliva. The sample is then exposed to paper strips containing artificial antibodies designed to bind to coronavirus antigens. Antigens bind to the strips and give a visual readout. The process takes less than 30 minutes, can deliver results at point of care, and does not require expensive equipment or extensive training.[18]

2.3 Chest CT scans

Chest CT (Computerized Tomography) scans can occasionally aid to depict the lung pathology. An organized review of chest CT scan findings in 919 patients revealed the distinctive primary manifestation of COVID-19 as "bilateral multilobar ground-glass opacification (GGO) with a peripheral or posterior distribution". In one of the investigations, it was found that the sensitivity of CT for COVID-19 infection was 98% compared to RT-PCR sensitivity of 71%; however, this was carried out in Wuhan province of China and is not generalizable.

None of the study has yet validated the accuracy and discriminatory value of CT scans to differentiate COVID from other viral pneumonia. Consequently, the CDC does not endorse CT for initial screening. CT systems are expensive, require technical expertise, and cannot specifically diagnose COVID-19.[19]

2.4 Antibody-based techniques

The body responds to a viral infection by producing antibodies that help neutralize the virus. Blood tests (also called serology tests or serology immunoassays) can detect the presence of such antibodies. Antibody tests can be used to assess what fraction of a population has once been infected, which can then be used to calculate the disease's mortality rate. They can also be used to determine how much antibody is contained in a unit of convalescent plasma, for COVID-19 treatment, or to verify if a given vaccine generates an adequate immune response.[20]

Serologic tests that identify antibodies (such as IgA, IgM, and IgG) to SARS-CoV-2 from clinical specimens (such as blood or saliva), such as enzyme-linked immunosorbent assays, may be less complex than molecular tests and have the potential to be used for diagnosis in certain situations. However, their utility for diagnosing acute infections is probably limited around the time of symptom onset, when viral shedding and transmission risk seem to be highest. Antibody responses to infection take days to weeks to be reliably detectable. Negative results would not exclude SARS-CoV-2 infection, particularly among those with recent exposure to the virus. Cross-reactivity of antibody to non–SARS-CoV-2 coronavirus proteins is also a potential problem; whereby positive results may be the result of past or present infection with other human coronaviruses. Serologic assays might be more relevant in scenarios in which patients present to medical care with late complications of disease, when RT-PCR may be falsely negative, because viral shedding drops over time.[21-23]

2.5 Sniff tests

Sudden loss of smell can be used to screen people on a daily basis for COVID-19. A study by the National Institutes of Health showed that those infected with SARS-CoV-2 could not smell a 25% mixture of ethanol and water. Because various conditions can lead to the loss of the sense of smell, a sniff test would not be definitive but indicate the need for a PCR test. Because the loss of the sense of smell shows up before other symptoms, there has been a call for widespread sniff testing. Health care bureaucracies have generally ignored sniff tests even though they are quick, easy and capable of being self-administered daily. This has led some medical journals to write editorials supporting the adoption of sniff testing.[24,25]

On 15 December 2020, the U.S. Food and Drug Administration (FDA) issued an emergency use authorization (EUA) for the first over-the-counter (OTC) fully at-home diagnostic test for COVID-19. The 'Ellume COVID-19 Home Test' is a rapid, lateral flow antigen test, a type of test that runs a liquid sample along a surface with reactive molecules. The test detects fragments of proteins of the SARS-CoV-2 virus from a nasal swab sample from any individual two years of age or older. The Ellume test uses a mid-turbinate nasal swab (sample is collected further back than the usual nasal swab, but not as far back as nasopharyngeal swabs, which are only appropriate for use by a trained health care provider) to detect certain proteins of the virus known as antigens. The Ellume test uses an analyzer that connects via Bluetooth with a software application on a smartphone to help users perform the test and interpret results. Results are delivered in as little as 20 minutes to individuals via their smartphone. [25-27]

The comparison of various diagnostic tests used for COVID-19 is given below:

Table 1 : Comparison of various diagnostic tests used for COVID-19

Parameters	Rapid Antibody Test	ECLIA Serology Test	Rapid Antigen / Rapid Swab Test	RT-PCR / Swab Test
Test duration	10-15 min	10-15 min	15-30 min	15-30 min
Result out in	30 min to 1 day	1-2 day	1 hour – 1 day	1-7 days
Sample collected	Blood from fingertip / vein	Blood from vein	Mucus from nasal tract / throat	Mucus from nasal tract / throat
What the test detects	Antibodies (IgG and IgM)	Antibodies (IgG and IgM)	Viral antigen	Virus Genetic Material
Best time to test	Minimum seven days after exposure to virus	Minimum seven days after exposure to virus	1-5 days from onset of symptoms	5-7 days after exposure to virus
Advantages	Affordable, widely available	More accurate than rapid antibody test	Positive results usually accurate	Results are highly accurate
Disadvantages	Accuracy rate varies depending on the test kit and timing	Not yet an official travel prerequisite	Negative results should be confirmed with an RT-PCR test	Expensive, facilities not widely available

3. CONCLUSION

The COVID-19 pandemic demonstrates the essential role of diagnostics in the control of communicable diseases. Laboratory-based molecular assays for detecting SARS-CoV-2 in respiratory specimens are the current reference standard for COVID-19 diagnosis, but point-of-care technologies and serologic immunoassays are rapidly emerging. The utility of COVID-19 testing has been diminished by limited resources, reducing the number of tests that could be offered in the face of a rapidly increasing epidemic. There has been difficulty in extending the tests to the populations most in need and possibly a lack of stringent application of the testing guidelines. Urgent clinical and public health needs now drive an unprecedented global effort to increase testing capacity.

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