Journal of Advances in Medicine and Medical Research

32(14): 10-17, 2020; Article no.JAMMR.60513
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Laboratory Diagnosis of COVID-19 Infection: Current Issues and Challenges: An Indian Perspective

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Authors' contributions

This work was carried out in collaboration among all authors. Author APM designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AM and AK managed the analyses of the study whereas authors NK and SK managed the literature searches. All authors read and approved final manuscript.

Article Information

DOI: 10.9734/JAMMR/2020/v32i1430559
Editor(s):
(1) Chan-Min Liu, Xuzhou Normal University, P. R. China.
(2) Kate S. Collison, King Faisal Specialist Hospital & Research Centre, Saudi Arabia.
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Reviewers:
(1) Ozgur Kasapcopur, Istanbul Univeristy, Turkey.
(2) Raphael Oliveira Ramos Franco Netto, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60513

Review Article

Received 10 August 2020
Accepted 24 August 2020
Published 25 August 2020

ABSTRACT

COVID-19, a disease caused by SARS-CoV-2 has become a public health emergency affecting more than 215 countries worldwide. It originated from Wuhan district of China and in a very short span of time, it has spread rapidly causing millions of deaths worldwide. India reported its first case on 30th January 2020 and since then the numbers have been increasing exponentially every day. As of 9th August 2020, India had recorded 21, 09,631 confirmed cases and 43,379 deaths. Because of the complex dynamics involved in its infection and immunity, proper diagnosis is imperative in order to unravel this ongoing mystery. It has clearly told us the importance of establishing and strengthening a strong network of molecular virology laboratories in the country

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enabling us to strengthen the diagnostic arm which is the first step in disease control. The addition of serological tests has helped in surveillance and also to estimate the overall burden of disease.

Keywords: COVID-19 infection; diagnosis; SARS-CoV-2; disease control.

1. INTRODUCTION

SARS-CoV-2 is a novel coronavirus that was identified in late 2019 as the causative agent of COVID-19. On March 11, 2020, the World Health Organization (WHO) declared the world-wide outbreak of COVID-19 a pandemic [1]. It has presented with pressing diagnostic challenges and emphasized the importance of the laboratory diagnosis to limit the spread as well as to treat those patients who have a serious infection. COVID-19 patients present with a wide range of clinical symptoms (e.g., cough, fever, and dyspnea) that are similar to influenza or other respiratory infections [2,3]. The diagnosis of COVID-19 cannot be definitely made without specific microbiological testing. Several diagnostic strategies are available to identify current infection, rule out other infection, identify people in need of care escalation, and to test for past infection and immune response [4]. The main tests are the Nucleic Acid Amplification Testing (NAAT) and Serological testing. This article will discuss the current issues and challenges about the laboratory diagnosis of COVID-19.

2. MATERIALS AND METHODS

In this review, all research articles published in the three months from April-July 2020 were analyzed and discussed to better understand the laboratory diagnosis of this virus. Three researchers independently searched through the literature and rest two of them collected all the relevant articles as well as reviewed all the selected abstracts. Literature for this review was identified by searching the following online databases: PubMed, Google scholar, Embase as well as CNKI and WangFang data (the two primary databases for research in China). We searched scientific publications from 1 April to 31st July 2020 using the keywords “coronavirus,” “RT-PCR,” “NAAT,” and “COVID-19.” Non-scientific commentaries, reports and news articles were excluded from this analysis.

3. SPECIMEN TYPES AND SAMPLE COLLECTION

The collection of specimens from the surface of the respiratory mucosa is a procedure used for the diagnosis of COVID-19 in both adults and children. A Nasopharyngeal (NP) rather than an Oropharyngeal(OP) swab is recommended for early diagnosis or screening because it provides higher diagnostic yield, is better tolerated by the patient, and is safer for the operator [5]. A NP swab can be combined with OP swab to increase sensitivity but requires twice the number of swabs. Self-collected saliva or nasal washes could be used as an alternative specimen type for epidemiological screening and for the “worried well,” who are asymptomatic persons with no exposure history who wish to be tested just to be sure they are not infected. The other specimens are sputum, saliva, bronchoalveolar lavage (BAL) fluid, fibrobronhoscope brush biopsy, stool, blood, or urine [6]. The role of rectal swabs in testing patients with late infection or as a test of infectivity or cure is currently not well studied. There are no specific contraindications for collecting specimens with nasopharyngeal swabs. However, clinicians should be cautious if the patient has had recent nasal trauma or surgery, has a markedly deviated nasal septum, or has a history of chronically blocked nasal passages or severe coagulopathy [7]. The rate of positivity of different samples is given in Table 1.

4. BIOSAFETY MEASURES

The samples should be collected by well-trained healthcare personnel after putting on Personal Protective Equipment (PPE), following adequate infection control measures, and adequate biosafety precautions to protect self and the environment. Initial processing of all specimens should take place in a biological safety cabinet (BSC) or primary containment device. Laboratory work involving non-propagative procedures like sequencing, nucleic acid amplification test (NAAT) should be conducted in a Biosafety Level 2 (BSL-2) facility. In contrast, propagative methods like virus culture, isolation or neutralization assays should be performed at a containment laboratory with inward flow in a Biosafety Level-3 (BSL-3) facility. Disinfectants having action against enveloped viruses should be used, and patient specimens from suspected or confirmed cases should be transported as per guidelines [10].

5. DIAGNOSTIC METHODS OF COVID-19

The different diagnostic methods of COVID-19 are

1. Nucleic Acid Amplification Testing (NAAT)
a. Real-time RT-PCR Test
b. TrueNAT and CBNAAT
2. Viral Sequencing
3. Viral Cultures
4. Serology of COVID-19
a. Antigen detection test
b. COVID-19 Antibody test
5. CRISPR based assay

1. Nucleic Acid Amplification Testing (NAAT)

Early diagnosis is the key for prompt management and control of the spread of the COVID-19 infection. Currently, the laboratory diagnosis of SARS-CoV-2 is based on the detection of viral RNA by nucleic acid amplification tests (NAAT) like Real-time reverse transcriptase-polymerase chain reactions (RT-PCR) and cartridge-based nucleic acid amplification test (CBNAAT). Various genes targeted so far include E, N, S, ORF and RdRp as a part of screening and confirmation of cases [11].

One of the following conditions should be met to consider a case as a laboratory confirmed by NAAT.

- A positive NAAT result for at least two different targets on the SARS-Cov-2 virus genome, of which at least one target is preferably specific for SARS-CoV-2 virus using a validated assay; or
- One positive NAAT result for the presence of betacoronavirus, and SARS-CoV-2 virus further identified by sequencing partial or whole genome of the virus as long as the sequence target is larger or different from the amplicon probed in the NAAT assay used.

When results are ambiguous, the sample should once again be collected from the patient and, if appropriate, sequencing of the virus from the original specimen should be done. Several factors could lead to a negative result in an infected individual, including;

- Poor quality of the specimen, containing little representative material.
- The specimen was collected late or very early in the course of infection.
- The specimen was not handled and transported appropriately.
- Technical reasons inherent in the test, e.g., virus mutation or PCR inhibition.

If a negative result is obtained from a patient with a high index of suspicion for SARS-Cov-2 virus infection, particularly when only upper respiratory tract specimens were collected, additional specimens, including from lower respiratory tract if possible, should be collected and tested.

a. Real-Time RT-PCR

Reverse transcription PCR (RT-PCR) is the most common and straightforward method for the detection of SARS-CoV-2 owing to its advantages as a specific, sensitive and simple quantitative assay, which significantly helps in the diagnosis of early infection [12]. It is the gold standard test for detecting cases of COVID-19 and typically targets the viral RNA-dependent RNA polymerase (RdRp) or Nucleocapsid (N) genes [13]. Viral load in upper respiratory tract secretions peak in the first week of symptoms but may decline below the limit of detection in those presenting later. In individuals who have recovered, RT-PCR provides no information about prior exposure or immunity. The test requires a specialized laboratory with molecular virology facilities with specific bio-safety and bio-security precautions, skilled laboratory staff, specialist equipment, and PCR reagents. The average time taken is around 4-6 hours from receipt of the sample to get the result. The sensitivity of this test is 68-80%, and specificity is 90-95% [14]. The advantage of this test lies in the accuracy of detection as well as its ability to run up to 100 samples in a single run.

b. TrueNAT and CBNAAT

These systems are widely available as these are already being used for the diagnosis of tuberculosis and other infectious diseases. These tests use customized cartridges and have quick turnaround time (30-60 minutes), but only 1-4 samples can be tested in one run, limiting the maximum numbers that can be tested to 24-48 samples per day only. The sensitivity of this test is 50-80%, and specificity is 90-95%. Because of closed nature platforms and minimum sample handling, these tests pose a minimum bio-safety hazard and have increased access to testing [15].

Table 1. Corona testing positivity rates [8,9]

Sl. no	Types of Specimen	Positive % (Wenling et al.)	Positive% (WHO)
1	Bronchoalveolar lavage fluid	93%	>90%
2	Fibrobronhoscope brush biopsy	46%	-
3	Saliva	-	90%
4	Sputum	72%	70%
5	NP and OP Swabs	-	70%
6	Nasal swabs	63%	60%
7	Pharyngeal swabs	32%	30%
8	Throat washing	-	30%
9	Stool	29%	30%
10	Blood	1%	15-30%
11	Urine	0%	-

2. Viral Sequencing

Sequencing does not have a role in the initial laboratory diagnosis of SARS-CoV-2but can be helpful in the following circumstances:

- Confirming the presence of the virus.
- Monitor for viral genome mutations.
- Countermeasures, including diagnostic tests.
- Virus whole-genome sequencing can also inform molecular epidemiology studies.

3. Viral Cultures

Viral culture is not recommended for the laboratory diagnosis of SARS-CoV-2 but can be used for research purposes like isolation of the virus, studying the properties of the virus and development of vaccine. Human airway epithelial cell lines were used for the initial isolation of the virus [16].

4. Serology of COVID-19

Members of the coronavirus family have four structural proteins: The spike(S), membrane(M), envelope(E) and nucleocapsid (N) proteins. Serological methods have focused on detecting serum antibodies against S proteins from the coronavirus spike. The other protein that appears to be an essential antigenic site for the development of serological assays to detect COVID-19 is the N protein which is a structural component of the helical nucleocapsid. Antibodies to the N protein are frequently detected in COVID patients suggesting that the N protein may be one of the immunodominant antigens in the early diagnosis of COVID-19 [17].

a. Antigen Detection Test

The Antigen detection test detects the presence of infection by targeting specific viral proteins present in the patient's sample. It takes about 15-30 minutes for the result and hence it is a rapid point of care (PoC) test. In medical diagnosis, the sensitivity of a test is the ability of the test to correctly identify those with the disease (True positive rate), whereas the specificity is its ability to correctly identify those without the disease (True negative rate). Most of the COVID-19 Antigen detection tests have been found to have a very high specificity (99.3-100%) with moderate sensitivity (30.2-84%) [18]. In India, the Standard Q COVID-19 antigen detection kit, has been validated by ICMR and is being used widely as a point of care diagnostic assay in several government and private institutions with excellent results [19]. It has been recommended to be used in the containment zones or hot spots as well as in healthcare settings. A positive test should be considered as a true positive, whereas all symptomatic individuals testing negative through the rapid antigen test should be confirmed with a real-time PCR test. The advantages and disadvantages of the antigen test are shown in Table 2.

Recommendations of Rapid Antigen PoC test:

A. All containment zones or hotspots

a. All symptomatic Influenza-Like Illness (ILI)
b. Asymptomatic direct and high-risk contacts with comorbidities of a confirmed case to be tested once between day 5 and day 10 of coming into contact.

B. Healthcare setting

a. All symptomatic ILI presenting to a healthcare setting and suspected of having COVID-19 infection.

b. Asymptomatic patients who are hospitalized or seeking hospitalization in the following high-risk group:

i. Patients undergoing chemotherapy
ii. Immunosuppressed patients including those who are HIV+
iii. Patients diagnosed with malignant disease
iv. Transplant patients
v. Elderly patients (>65 yrs of age) with comorbidities.

b. COVID-19 Antibody Test

Seroconversion occurs after 7 days of symptomatic infection in 50% of patients (14 days in all) but is not followed by a rapid decline inviral load. IgG antibodies generally start appearing after two weeks of the onset of infection, once the individual has recovered after infection and last for several months. Therefore, the IgG test is not useful for detecting acute infection. However, the detection of IgG antibodies for SARS-CoV-2 may be helpful to in the following situations

i. To understand the proportion of the population exposed to infection with SARS-CoV-2 including asymptomatic individuals.
ii. Survey in high risk or vulnerable populations (Health care workers, frontline workers, immunocompromised individuals, individuals in containment zones) to know who has been infected in the past and has now recovered.

It is strictly advised to use IgG based ELISA and CLIA assays for conduct of serosurveys. Since test, track and treat is the only way to prevent the spread of infection and save lives, it is imperative that testing should be made widely available to all symptomatic individuals in every part of the country and contact tracing mechanisms for containment of infection are further strengthened. Unlike RT-PCR tests, antibody tests are not intended to identify active SARS-COV-2 infections. Instead of detecting viral genetic material in throat or nasal swabs, antibody tests reveal markers of immune response- the IgM and IgG antibodies that for most people show up in blood more than a week after they start to feel sick, when symptoms may already be waning. Up to a quarter of people with SARS-CoV-2 infection may unwittingly spread the virus because they have mild or no symptoms. The antibody tests are being used to screen donor blood of recovered patients for antibodies to SARS-CoV-2. The plasma containing the antibodies is then transferred to gravely ill patients in an experimental treatment known as Convalescent Plasma Therapy (CPT). Serologic testing could be used to check their antibody status after they have recovered; those with no or low immunity would be prime candidates for a vaccine when one becomes available. Resorting to antibody testing to diagnose active infections is a 'complete misuse.' Not only are antibody tests likely to report false-negative early on, they will also miss infections among people who are immunocompromised and don't produce antibodies. Many believe that antibody testing can also be used to return people with immunity to the workforce or keep them there. Ultimately, a positive antibody test could be a sort of get-out-of-isolation card. In the long run this test can be used for the whole population because everybody who is immune could basically go back to a normal and healthy life because they can't infect anybody else [20].

The advantages and disadvantages of Rapid COVID-19 Antibody Test is shown in Table 3.

Population-based testing by measuring SARS-CoV-2-specific IgG antibody titers can be using four ways [21].

i. To estimate epidemiological variables, such as attack rate or case fatality rate.
ii. To deploy immune HCWs to reduce exposure of the virus to susceptible individuals.
iii. To assess the effects of non-pharmacological interventions at the population level and inform policy changes.
iv. To identify individuals who mounted a strong immunological response to be considered as a plasma donor.

Table 2. Advantages and disadvantages of rapid antigen test

	Advantages	Disadvantages
Time	Rapid (30 min)	-
Sensitivity	-	Low (50%)
Specificity	High (100%)	-
	No need to confirm if positive. Useful in asymptomatic cases.	In symptomatic if negative RT-PCR test to be done.

Table 3. Advantages and disadvantages of rapid antibody test

	Advantages	Disadvantages
Time	Rapid (30 min)	
Safety	Safe sample procedure	
	Detects recovery phase of illness	Not for diagnosis of current infection of COVID-19
IgM	Appears between 7-14 days	-
IgG	Appears from 14 days	

The RDT kit for SARS-CoV-2 antibodies, with 95.3% detection rate after the second week of illness could be used as POCT, a semi-quantitative method, and for seroprevalence studies.

At this point in the pandemic, there is not enough evidence about the effectiveness of antibody-mediated immunity to guarantee the accuracy of an "immunity passport or risk-free certificate," but at some point in the near future antibody testing will become a viable option [22].

To summarize the immunological testing and molecular biology helps in etiological diagnosis whereas the antibody testing helps in epidemiological surveillance.

5. CRISPR based assay:

Another powerful and promising tool coming up is the CRISPR assay which involves clustered regularly interspaced short palindromic repeats (CRISPR) technology. It is being quickly deployed in the molecular diagnostics landscape. It works by programming a CRISPR molecule to detect the presence of a specific genetic signature for SARS-CoV-2. If the signature is found, the CRISPR enzyme generates a fluorescent glow. It is a rapid (<40 min), easy-to-implement and accurate assay for the detection of SARS-CoV-2 from respiratory swab RNA extracts. CRISPR-based diagnostic method shave high sensitivity and specificity with efficiency and no requirement for elaborate instrumentation. However, it is subjected to the same potential limitations with regard to the availability of personal protective equipment, extraction kits and reagents.

6. CHALLENGES FOR DIAGNOSIS AND FUTURE

Though early diagnosis of COVID-19 is essential for the timely management, prompt isolation of confirmed cases to prevent further transmission, sample collection, transport and kit validation are major bottlenecks. A very low positivity of 30–60% has been reported in some cases by initial RT-PCR due to wrong timing of collection. Furthermore, the sensitivity of the testing kits is a matter of debate and thereby a sizeable number of patients may not be identified. In some counties, the healthcare system is not robust enough as a result of which the testing laboratories often face difficulties in the performance of molecular testing. Robust networking of laboratories is required for prompt sample collection testing and reporting. Furthermore, as the pandemic widens its arm a point of care molecular test has proved to be like a holy grail in the rapid diagnosis of cases, there by initiating the treatment at the earliest.

7. CONCLUSION

It seems now clear that we all will be living together with COVID-19 virus for quite a long time. Though the lack of availability of diagnostic tests hampered testing initially, the testing capacity is increasing quickly. Rapid and accurate detection of COVID-19 is essential to initiate the appropriate treatment rapidly, to limit further spread of the virus and to ultimately eliminate the virus from circulation. Molecular-based approaches are the first-line methods to confirm suspected cases. Nucleic acid testing is the primary technique for laboratory diagnosis. As with other emerging viruses, the development of methods to detect antibodies and viral antigens began after the identification of the viral genome. Early diagnosis is the key for prompt management of COVID-19 infection. Molecular and serological assays together will strengthen the diagnosis and in turn, facilitate timely and effective management of COVID-19.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://doi.org/10.1016/j.jmii.2020.07.003

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/60513