International Blood Research & Reviews

12(4): 1-7, 2021; Article no.IBRR.66962
ISSN: 2321–7219

Coronavirus: A Laboratory Perspective

Ransom Baribefii Jacob [1], Chukwu Christopher Ifunaya[1*] and Emeji Roseline[2]

[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.
[2]School of Medical Laboratory Science, Rivers State College of Health Science and Management Technology, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IBRR/2021/v12i430155
Editor(s):
(1) Dr. Dharmesh Chandra Sharma, J. A. Groups of Hospital and G. R. Medical College, India.
(2) Dr. Juan Carlos Troiano, University of Buenos Aires, Argentina.
Reviewers:
(1) Ritu Sharma, Govt Medical College Amritsar, India.
(2) Eman Khalifa Sedeek, Matrouh University, Egypt.
(3) Walid Saad Mousa, University of Sadat City, Egypt.
(4) 4. Nezihe Gökhan, Turkey.
Complete Peer review History: https://www.sdiarticle4.com/review-history/66962

Review Article

Received 12 March 2021
Accepted 20 July 2021
Published 02 August 2021

ABSTRACT

COVID-19 is a virus of the species of the Family coronaviridae known as as SARS-COV-2. This virus is easily contracted and/transmitted from an infected person to another healthy individual and has continued to spread rapidly. The aim of this review is to identify laboratory methods used in the diagnosis of COVID-19 infection. COVID-19 test are aimed at detecting active infection, or past infection, or an immunization so as to treat and curb the further spread of the virus. The initial viral detection is typically carried out with the upper respiratory tract (URT) sample. Repeated testing is particularly helpful and essential if a patient has a clinical appearance of viral pneumonia, radiographic results consistent with pneumonia and/or a history of potential exposure. The Centre of Disease Control and Prevention, CDC recommends the collection of specimens from the lower respiratory tract, upper respiratory tract, and the blood. The lower respiratory tract sample includes; the sputum, broncheoalveolar lavage, bronchial wash, tracheal aspirate, and pleural fluid. The upper respiratory tract specimens include; the nasopharyngeal swab, and oropharyngeal swab (NP/OP swabs). Some laboratory techniques developed and in use for the detection of Covid-19 are; nucleic acid amplification tests (NAATs), antibody detection, and viral antigen detection. The role of the laboratory assay in diagnosis of COVID-19 infection or disease cannot be under-estimated, timing and site of specimen collected must be followed by adequate

**Corresponding author: E-mail: greatchrisng15@gmail.com;*

professional training to ensure result accuracy. This review provides information on available laboratory techniques for the diagnosis of the viral infection and their potential merits and limitations.

Keywords: COVID-19; SARS-COV-2; gene; Antigen; Antibody; Laboratory method.

1. INTRODUCTION

COVID-19 is a contagious disease caused by viral infection with the specie of coronavirus known as SARS-COV-2 (Shrestha and Pokharel, 2020). This virus is easily contracted and/transmitted from an infected person to another healthy individual and has continued to spread rapidly since it was discovered in Hubei, China in late 2019. As a result of this widespread, the World health Organization (WHO) declared it as a global pandemic in March, 2020. COVID-19 infection varies widely from host to host. While some patients may be asymptomatic and may not feel sick, others develop mild symptoms or severe and sometimes potentially life threatening signs. The clinical symptoms of COVID-19 include; fever, dyspnea, fatigue, dry cough, headache, myalgia, and loss of taste or smell. Severe and critical conditions result to acute respiratory distress syndrome, respiratory failure, and other serious health complications. Some acute cases lead to death. Patients with COVID-19 have been shown to manifest high viral load in their upper respiratory tract samples within the first 5-7 days of infection [1].

1.1 The Importance of Covid-19 Tests

COVID-19 tests are aimed to detecting active infection, or past infection, or an immunization. Since the recent outbreak, scientists and molecular laboratories all over the world have developed multiple techniques for detecting COVID-19 in infected persons. The need for identifying people with active infection is necessary to curb the spread because people with COVID-19 can transmit the virus to others. Also, it is estimated that about 40% of SAR-COV-2 patients who are asymptomatic or subclinical are potentially capable of transmitting the disease to healthy people [2,3]. According to CDC [4], testing those with COVID-19 symptoms can help to determine whether those symptoms are as a result of COVID-19, a different respiratory virus, or another health condition entirely. Early detection of COVID-19 in people plays a role in the effectiveness of the treatment of administered patients. The existence of asymptomatic SARS-COV-2 patients calls for the necessity for laboratory testing so as to isolate and treat them and to reduce the spread of the infection.

1.2 Specimen Collection Technique

The type of specimen collected when testing for current or past SARS-COV-2 infection depends on the test being performed and the instruction of the manufacturer. James and his team noted that collecting the right sample at the right time and conveying it to the laboratory under the right conditions play a critical role in the pre-analytic components of the specimen examination process [5]. The initial viral detection is typically carried out with the upper respiratory tract (URT) sample. The specimen may cover healthcare personnel (HCP)-collected nasopharyngeal (NP), oropharyngeal (OP), mid-turbinate (MT), or anterior nares swabs, as well as NP or nasal wash/aspirate samples. However, it should be noted that in some scenario, early infections may be missed by saliva or NPs or OPs and in subsequent infection, the main site of replication may have adjusted to the lower respiratory tract [6]. The solution to this will be to carry out repeated testing or getting lower respiratory tract samples. Repeated testing is particularly helpful and essential if a patient has a clinical appearance of viral pneumonia, radiographic results consistent with pneumonia and/or a history of potential exposure [6].

In some cases, patients are permitted to collect their own mid-turbinate or nasal swabs or saliva (1-5ml) under the guidance of healthcare providers, with step-by-step protocol of the process. The swabs should be made up of synthetic fibers and have plastic or wire shafts. The OP and NP, if collected at the same time can be combined in the same tube to maximize test sensitivity and equally conserve transportation devices [5]. The specimen quality and maintenance of cold chain during transportation is vital for obtaining accurate result during testing. There have been cases of false positive results which occurred due to improper timing of specimen collection and shortfalls in

specimen proficiency, especially those of nasopharyngeal swabs [7]. Proper measures must also be taken to protect the healthcare personnel while they work towards obtaining reliable test results. To make the latter possible, the laboratory staffs, clinicians, nurses and paramedics are educated on the standard operating procedure (SOP) for proper method of sample collection, packaging and transportation. The CDC also advised that healthcare providers directly involved in the collection of samples should maintain proper infection control and use recommended personal protective equipment (PPE),which covers an N95 of higher degree of aspirator (or face shelter where an aspirator is unavailable), eye protection, gloves and a gown. For those only handling the specimens being collected or who are not directly involved in collecting the samples, the standard operating procedure should be maintained [8].

1.3 Types of Specimens

The CDC recommends the collection of specimens from the lower respiratory tract, upper respiratory tract, and the blood. The lower respiratory sample includes; the sputum, broncheoalveolar lavage, bronchial wash, tracheal aspirate, and pleural fluid. The upper respiratory specimens include; the nasopharyngeal swab, and oropharyngeal swab (NP/OP swabs). Research suggest that sputum sample or broncheoalveolar lavage used for collecting lower respiratory tract samples, give the highest viral loads for the diagnosis of COVID-19. A recent study showed that broncheoalveolar lavage fluid samples yielded the highest SARS-CoV-2 RNA rate. Though it did not compare results to NP swabs [7]. For serum, 1 tube of (5-10ml) of whole blood are collected for children and adults, while for infants, a minimum of 1ml whole blood is collected. 1ml should be collected in a serum separator tube if possible.

2. LABORATORY METHODS

The CDC and other molecular laboratories all over the world have developed various techniques in an attempt to diagnose SARS-COV-2, the virus responsible for COVID-19. Some of these tests also tests for influenza A and B viruses. These tests are aimed at providing the public health officials with the information needed to help lower the spread of these viruses around communities and to conserve resources that are in limited supply. These tests also gives a chance to those who are infected to live as the infections are quickly identified and treated. Some of those techniques developed are; nucleic acid amplification tests (NAATs), antibody detection, and viral antigen detection.

2.1 Procedures for Each Methods

2.1.1 Nucleic acid detection technique (NAAT)

This is the one of the tests developed by the CDC to identify SARS-COV-2. It is the recommended technique for the testing of acute or current COVID-19 infection and is designed such that it detects one or more RNA gene targets particular to the virus. This single test that diagnoses current infection with one or more viruses will give room to the public health laboratories to continue influenza surveillance while they continue to test for SARS-COV-2. Those that can be tested using this method includes; patients with obvious COVID-19 symptoms, individuals with known or suspected COVID-19 exposure, and those from areas with high disease prevalence.

At the moment, there are 5 SARS-COV-2 assays that have Emergency Use Authorization approval for testing asymptomatic patients or for use in community surveillance [9].

2.1.2 Multiplex and gene target NAATs

In the detection of SARS-COV-2, some of the most frequently tested gene targets include the envelope (E), spike (S), and nucleocapsid (N) genes and open reading ORF1a/1b [10]. According to Corman and his team, E gene detection seems to have the highest testing sensitivity [11], with detection capability of this gene being far lower than the estimated viral load for SARS-COV-2 positive patients [12]. Chu and his team in their research found that this assays can detect as few as 10 copies per reaction [12]. Different NAAT assays however, vary in terms of clinical sensitivity, independent of the gene detected [10]. Apart from detecting SARS-COV-2, many NAATs have received approval as multiplex assays, which can detect other respiratory viruses such as influenza A and B, as well as bacteria that cause atypical pneumonia [9].

2.1.3 Sensitivity and viral load NAAT

The duration between the infection of a patient and the testing for the disease can impact the

sensitivity of nucleic acid analysis. Viral RNA present in the upper respiratory tract samples of patients infected with SARS-COV-2 is at highest during the early infection [13]. The viral load of patients who are asymptomatic at the time of infection but manifest symptoms subsequently do not appear differently from that of persons with symptomatic infections [14,11,15]. This implies that the sensitivity of nucleic acid would be highest in the early course of the infection and should also be effective at the onset of the symptoms, although how soon after the exposure to the virus that this assay can readily sense viral RNA of SARS-Cov-2 remains a question. NAATs generally can detect SARS-Cov-2 samples obtained weeks to month after the onset of COVID-19 symptoms [16]. However, the probability of recovering replication-competent virus greater than ten days from the onset of the symptoms in those with mild disease and greater than twenty days in those with severe disease is very low [14]. In some patients, saliva has been shown to have higher viral loads and RNA that persists longer than do paired nasopharyngeal samples [17].

2.1.4 Isothermal amplification NAATs

The isothermal amplification differs from the polymerase chain reaction (PCR) in that it is achieved using a constant temperature, while PCR requires cycling of temperature [18]. Although there are several isothermal amplifications, the two most important ones for the diagnosis of SARS-COV-2 are the reverse transcription loop-mediated isothermal amplification also called the RT-Lamp, and transcript-mediated amplification or TMA.

Isothermal amplification methods provide detection of nucleic acid sequence in a streamlined, exponential manner, and are not limited by the constraint of thermal cycling. The COVID-19 pandemic provided the opportunity for the adoption of isothermal methods as the foundational technology for the detection of SARS-COV-2 RNA in the clinical and home testing market. One application of colorimetric LAMP can be seen in the Color Genomic SARS-COV-2 Diagnostics Assay, which has received FDA Emergency Use Authorization.

2.1.5 RT-PCR NAATs

There are over 200 NAATs with EUA approval from the FDA which has been classified into two broad groups according to the FDA [9]: nucleic acid amplification based reverse transcription polymerase chain reaction (RT PCR) assays, and SARS-COV-2 RNA detection via isothermal amplification. Considering that all coronaviruses have an RNA genome, it is necessary to synthesize complementary DNA or cDNA, from the RNA genome via the reverse transcription of the complementary DNA with specific primers for the SARS-COV-2 genes of interest. The real-time RT-PCR is the most common, which employs fluorescence to detect the amount of amplified DNA in real time. In real-time RT-PCR assay, the number of PCR cycles is determined by the amount of gene target present in the sample needed before SARS-COV-2 is detected [5].

Currently, all SARS-COV-2 RT-PCR assays with EUA approval are for quantitative measurement of the amount of virus present in the sample and not for qualitative detection of gene targets specific to the virus.

2.2 Antigen Detection Test

A rapid antigen detection test (RADT), also known as rapid test, is a rapid diagnostic test suitable for point of care testing that is used directly for to detect the presence or absence of an antigen. It is commonly employed in SARS-COV-2 detection. They are lateral-flow immunoassays designed for qualitative sensing of nucleocapsid protein antigen directly from nasopharyngeal and/or nasal swabs. The presence of that antigen confirms SARS-COV-2 infection, while its absence implies no infection. While other antigen test systems can be read virtually by the operator, most of them need an instrument reader to determine the results [5].

Generally, the workflow of antigen detection test needs the placement of the swab into a reagent solution that is mixed and then applied to a test cartridge. The sample is then allowed to migrate along the stripe. Specific antibodies to SARS-COV-2 antigen will react with the sample if the antigen is present, and will produce a colorimetric or fluorescent signal within 10-15 minutes. The tests includes built in internal controls that can be seen by the operator or read by the instrument to ensure the validity of the results. To obtain accurate result, the instruction of the manufacturers must be followed. While RADTs may provide the state of health of an infected individual, CDC recommends that it must not be used to make decisions about discontinuing isolation.

2.3 Antibody Detection Test

Antibody assays or tests are usually employed for the identification of patients with current or previous SARS-COV-2 infection. The test detects antibodies in blood and saliva samples. Generally, the detectable levels of antibodies can take many days or weeks to come up. As a result, antibody detection tests have limited utility in acute infection diagnosis, when compared to testing by NAATs. According to the study conducted by Cheng and his team and that carried out by CDC, the use of antibody tests is viable as an aid to COVID-19 diagnosis, though the test should not represent the only basis for the determination of acute SARS-COV-2 infection [19].

The knowledge of the nature, dynamics and timing of antibody response to SARS-COV-2 infection is very crucial for the interpretation of an antibody detection tests. Studies have revealed that seroconversion takes place by two weeks in most patients after the symptoms onset, even though almost all patients have detectable levels of anti-SARS-COV-2 antibodies by the 28th post symptoms onset.

2.3.1 Types of antibody detection assays

SARS-COV-2 commercial serological assays includes the following: automated direct chemiluminescence immunoassay or CIA, enzyme-linked immunosorbent assay or ELISA, rapid lateral-flow assay or LFA, and bioassays such as those requiring plaque reduction and microneutralization [5]. CIA functions by using a combination of recombination of recombinant antigens coated onto magnetic bead with luminescent detection to provide quantification. Data from studies show that this technique offers excellent specificity and sensitivity for the determination of the total antibodies present or selected isotypes. It also provides an option for automation, allowing high-throughput specimen examination [20]. ELISA functions by detecting the total antibody content or immunoglobulin IgA, IgM, or IgG. It however has the disadvantage of limited reproducibility as a result of lack of standardization, use of variable antigens, and variable limits of detection. This creates wide gap in sensitivity and specificity across assays, even within assays validated by different users [20]. Bioassays like those requiring plaque reduction and microneutralization give essential information for candidate validation diagnostic tests, though they need trained and specialized users and are available to limited number of highly specialized laboratories. Similar to ELISA, LFA uses SARS-COV-2 antigen as a capture agent but in the form of lateral flow [21]. It has the advantage of fast turnaround time of between 10 – 30 minutes when compared with ELISA which takes several hours with limited sample processing. On the other hand, it is expensive than the throughput of ELISA. It also exhibits large inconsistencies according to a research by Whitman *et al.* [22] and as such, its result should be interpreted with caution. A follow up testing is also recommended [22].

3. CONCLUSION

The role of the laboratory assay in diagnosis of COVID-19 infection or disease cannot be under-estimated. Timing and site of specimen collected must be followed by adequate profession training to ensure result accuracy. This review has provided information on the various available laboratory techniques for the diagnosis of the viral infection and their potential merits and limitations. Further studies are needed with the view of improving on the limitations of the methods so as to enhance result validity.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis. 2020;24:30113–30114.
2. Al-Sadeq DW, Nasrallah GK. The incidence of the novel coronavirus SARS-CoV-2 among asymptomatic patients: A systematic review. Int J Infect Dis. 2020;98:372-80.

3. Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection: A narrative review. Ann Intern Med. 2020;173(5):362
4. Centers for Disease Control and Prevention. Available:https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html Labs COVID-19 resources and guidelines for labs and laboratory workers;2021.
5. James JD, Joanna J, Cameron AB. Laboratory' Diagnostics and Testing Guidance for COVID-19: Laboratory studies, Specimen Selection, Collection, and Transportation, Nucleic Acid detection;2021.
   Available:https://emedicine.medscape.com/article/2500138-questions-and-answers Retrieved June, 7, 2020.
6. Yi-Wei T, Jonathan E, Schmitz DHP, Charles WS. Laboratory Diagnosis of COVID-19: Current Issues and Challenges. JCM. 2020;1128:00512-20
7. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA. 2020;323(18):1843-1844.
8. Centers for Disease Control and Prevention. CDC's influence SARS-CoV-2 multiplex assay and required supplies. Updated;2021.
   Accessed June 9, 2021 Available:https://www.cdc.gov/coronavirus/2019-ncov/lab/multiplex.html.
9. US Food and Drug Administration. Coronavirus Disease 2019 (COVID-19) Emergency Use Authorizations for Medical Devices. FDA; 2020.
   Available:https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices Accessed: June 8, 2021
10. Van Kasteren PB, Van Der Veer B, Van Den Brink S, Wijsman L, de Jonge J, Van Den Brandt A, et al. SARS-CoV-2 asymptomatic and symptomatic patients and risk for transfusion transmission. Transfusion. 2020;60 (6):1119-1122
11. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Peiris M, Poon L. Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia. Clin Chem. 2020;66 (4):549-55.
12. Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, et al Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. JAMA. 2020;323(15):1488-94
13. Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, Spicer K, Bardossy AC, et al. Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility. N Engl J Med. 2020;382(22):2081-90
14. Walsh KA, Jordan K, Clyne B, Rohde D, Drummond L, Byrne P, Ahern S, Carty PG, O'Brien KK, O'Neill M, Smith SM, Ryan M, Harrington P. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J Infect. 2020;81(3):357-71.
15. Xiao AT, Tong YX, Zhang S. Profile of RT-PCR for SARS-CoV-2: a preliminary study from 56 COVID-19 patients. Clin Infect Dis. 2020;71(16):2249-2251
16. Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, Warren JL, et al. Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2. N Engl J Med. 2020;383(13):1283-6.
17. Zhao Y, Chen F, Li Q, Wang L, Fan C. Isothermal Amplification of Nucleic Acids. Chem Rev. 2015;115 (22):12491-545.
18. Cheng MP, Papenburg J, Desjardins M, Kanjilal S, Quach C, Libman M, Dittrich S, Yansouni, CP. Diagnostic Testing for Severe Acute Respiratory Syndrome-Related Coronavirus 2: A Narrative Review. Ann Intern Med. 2020;172 (11):726 – 734.
19. Espejo AP, Akgun Y, Al Mana AF, Tjendra Y, Millan NC, Gomez-Fernandez C, Cray C. Review of Current Advances in Serologic Testing for COVID-19. Am J Clin Pathol. 2020;154 (3):293-304
20. Sheridan C. Fast, portable tests come online to curb coronavirus pandemic. Nat Biotechnol. 2020;38(5):515-8.
21. Whitman JD, Hiatt J, Mowery CT, Shy BR, Yu R, Yamamoto TN, et al. Test performance evaluation of SARS-CoV-2 serological assays. Med Rxiv. 2020;32511497

Available:https://www.cdc.gov/coronavirus /2019-ncov/lab/guidelines-clinical- specimens.html Retrieved June 14, 2021

© 2021 Jacob et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/66962