International Journal of Medical and Health Research
www.medicalsciencejournal.com
ISSN: 2454-9142
Received: 30-07-2021, Accepted: 14-08-2021, Published: 30-08-2021
Volume 7, Issue 8, 2021, Page No. 62-65

Molecular diagnostics and serological methods for detecting COVID-19

Jude N Okoyeh[1*], Teena Thomas[2]
Department of Biology and Clinical Laboratory Science, School of Arts and Sciences, Neumann University, Aston, Pennsylvania, United States

Abstract
Coronavirus (COVID-19) is an ongoing pandemic disease that is caused by acute respiratory syndrome coronavirus2. WHO declared it has a public health emergency in early 2020 and since then many tests and methods have been developed to best prevent and possibly manage the spread of the virus. The disease has now been reported in over 223 countries globally. Detection of the virus in early stages will allow to stop the spread of the virus which would ultimately lower both the incident rates and the mortality rates. WHO have given Emergency Use Authorization (EUA) for various diagnostics methods and this review is mainly focused on the molecular diagnostics and serological methods of COVID-19 and to compare the advantages and disadvantages of these methods in clinical setting.

Keywords: molecular diagnostics, serological methods, Covid-19

Introduction

Coronavirus (CoVs) are a family of viruses that are classified as Coronaviridae and are spherical shaped, enveloped single stranded positive-sense RNA genome ranging from 26 to 32 kb in length. Coronavirus can be divided into 4 subgroups, namely alpha, beta, gamma and delta[1]. Gamma and delta affect mostly birds, while alpha and beta coronavirus infect only mammals and humans. They cause respiratory infections in humans and enteritis in mammals. SARS-CoV and MERS-CoV (beta virus) causes severe acute respiratory syndrome and middle east respiratory syndrome respectively. The subgenus of beta coronavirus in which SARS-CoV and COVID-19 are seen is in one of the genus among the 5 subgenus names sarbecovirus [1, 2]. COVID-19 is considered the 7th member of the coronavirus family that affect humans. Covid-19 was first detected in Wuhan, China, in December 2019 and it has since spread as a pandemic across the world. As of March 13 2021, the confirmed cases and confirmed death cases were more than 119 million and 2.63 million respectively[4]. The symptoms mostly appear 2-14 days after exposure and the incubation period is considered to be approximately 5 days. The most common symptoms seen are cough, fever, shortness of breath, headache and many other acute respiratory disorders [4, 5]. COVID-19 spreads through close contact, mainly from person to person through respiratory droplets from coughing or sneezing from an infected person. It can be spread by airborne transmission also. An increase in the cases is seen to have transmitted from asymptomatic patients as well [4]. The entire genome consists of a 5'-terminal non-coding region with at least 6 open reading frames (16nsps) which occupies approximately two-thirds of the viral RNA and the remaining third contains four structural proteins spike, envelope, membrane and nucleocapsid proteins with a 3' -terminal non-coding region and other accessory proteins are included in it as well [3].

The growing incidence and the rate at which COVID-19 started increasing in the first few months created a surge in demand for diagnosis, treatment and management. This surge led to the evaluation of laboratory tests and approaches to detect SARS-CoV-2(COVID-19). The gold standard method in molecular diagnostics is rRT-PCR. Although serological methods may not detect the virus in the early stages, it is used to monitor and assess the progression of the disease.

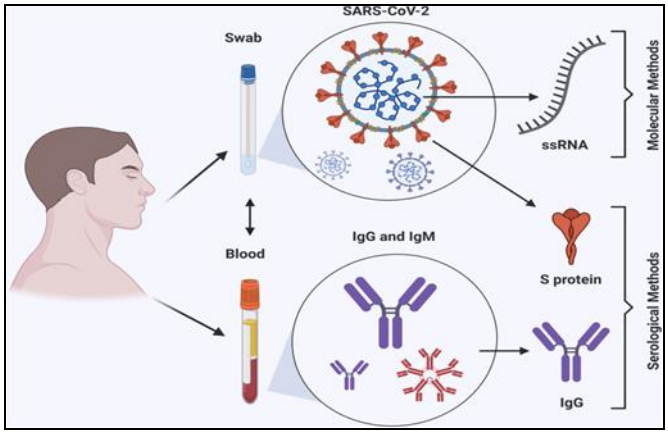

Fig 1: Molecular methods and serological work flow. Ahmad *et al*., 2020.

Molecular Methods

The 3 main types of molecular methods are real-time reverse transcription polymerase chain reaction (rRT-PCR), isothermal amplification and clustered regularly interspaced short palindromic repeats (CRISPR). These nucleic acid-based methods are widely used for virus detection and has played a vital role in early diagnosis of many similar infections. Based on the structure and sequence, RNA is the virus that is detected in the SARS-CoV-2. Collection of specimens from COVID-19 patients recommended by the CDC is same for all these methods. Since pneumonia was one of the main symptoms for COVID-19, nasopharyngeal swab was used as the preferred specimen collection technique for the sample collection however later on, specimens from the upper respiratory tracts such as

oropharyngeal were also used. Lower respiratory tract, serum, urine and stool were also used for testing in later stages [4, 6]. There are over 100 manufactures of diagnostic testing kits that are globally available testing COVID-19 using the nucleic acid-based method [6].

rRT-PCR method

rRT-PCR (RT-qPCR) is the primary reliable screening method for detecting SARS-CoV-2. It is considered the gold standard out of all the methods due to it low LOD, high sensitivity and specificity for the detection and quantification of the virus in the early stages of infection7,13. The sample obtained from the nose or throat is treated with several chemical solutions to remove all the proteins and fats to extract only the RNA present in the sample. The RNA is reversely transcribed to cDNA using a specific enzyme reverse transcriptase. These cDNA is used as a template for the PCR reaction and a fluorescent detection is seen. The fluorescence due to the amplification of cDNA confirms the presence of SARS-CoV8. It has one-step or a two-step procedure. One-step consists of only a single tube that contains the primer to run the rRT-PCR. The Two-step method consists of initial reverse transcription on the RNA template followed by amplification as a separate reaction. Even though this test is more flexible and higher in sensitivity the one-step is faster and easier to set up and involves less handling and decreases contamination error [7, 9]. Some of the manufactures who developed the test for rRT-PCT includes Cepheid (Xpert Xpress SARS-CoV-2 test, ThermoFischer (Taqpath covid-19 combo kit), Roche Diagnostics (cobas SARS-CoV-2), Qiagen GmbH (QiAstat-Dx Respiratory SARC-CoV-2 panel,) Hologic (Panther Fusion SARS-CoV-2 assay and Labcorp (COVID-19RT-PCRtest). All these tests are targeting different genomic regions of COVID-19 including ORF, nucleocapsid gene(N), Spike (S) protein, RdRp or envelope(E)genes [7]. Most of the tests can identify two or more genes and if both the markers are amplified the test is considered to be positive and confirms the presence of RNA. Hence, if only one target is positive the test is considered as an inconclusive result [7]. The disadvantage of rRT-PCR is that it takes 24-48 hours to generate results and it is not cost effective as the reagents and instruments are very expensive, also after 3 weeks of infection the detection rate decreases with the decrease in viral rate resulting in false negative values [13].

Isothermal amplification-based method

Isothermal nucleic acid amplification is done at constant temperature without the need of a thermal cycler thereby simplifying and accelerating the diagnostic process**[10]**. The common methods based on this amplification for detecting COVID-19 are Reverse Transcription Loop-Mediated Isothermal amplification (RT-LAMP) and CRISPR-based method. All of these eliminates the heat denaturation steps in PCR reaction.

RT-LAMP (Reverse Transcription Loop-Mediated Isothermal Amplification)

RT-LAMP requires four primer sets that are specific for the target and combines the LAMP with reverse transcriptase to detect RNA. The general steps include the two inner primers (FIP, BIP) that serves as a base and the outer primers (F3, B3) that anneal the template strand in proceeding the reaction. Strand displacing DNA polymerase initiates the synthesis to form loop structure to form the next cycle of amplification [10]. A positive result on RT-LAMP method can be detected by photometry, calorimetry and turbidimetry. The cloudiness is due to byproduct magnesium pyrophosphate during the reaction [9]. It has higher sensitivity because of its exponential amplification. It also has high specificity because of the 4 primer sets that can identify the 6 different target regions on the template for amplification [10]. They are very cost effective and requires less time to run the test as there are lesser steps and doesn't require thermal cyclers compared to PCR. The sample diagnostics turnaround time is 30 mins with a visible color change [13]. It is also considered as a point-of-care (POC) diagnostic test. ID Now COVID-19(Abbott) is most widely used instrument to detect SARS-CoV-2 in less than 15 mins [9, 11]. The POC instrument is meant for easier accessibility and simpler to operate as the test is conducted with a single cartridge and is also limited to one sample per run. The results can be accessed using smart phones and various other similar technologies, which makes it very attractive by being easily accessible to common users and patients [13]. Limitation of RT-LAMP method is the necessity of sequence-specific primer. This might cause a non-specific amplification which would give inaccurate results or false negative results [10, 11]. The sensitivity of RT-LAMP (at 75%) compared to RT-PCR (at 85%) is low [13].

CRISPR-based method

The clustered regularly interspaced short palindromic repeats belong to the nucleic acid family of prokaryotic organisms such as bacteria, archaea. Bacterial enzymes called CRISPR-associated enzymes (Cas9, Cas12, Cas13) can cut and recognizes the CRIPSR sequences [12]. Cas-12 cleaves ssDNA and Cas-13 cleaves ssRNA. For CRISPR-Cas 12 based method, the extracted RNA virus and the specific regions of the gene are amplified through RT-LAMP method. Cas12 RNA moves the Cas12 protein to the amplified genes where a ssDNA probe reporter molecule is cleaved. For positive results two or more regions need to be amplified. The result is detected by fluorescence or lateral flow strip**[8]**. Two manufactures have come up with CRISPR based method for testing of COVID-19. Sherlock Bioscience uses Cas13 and Cas12 with the Cepheid's GeneXpert test-processing instruments. Mammoth Biosciences (DETECTR) uses the Cas12 based method and a lateral flow assay is seen in 30-40 mins. CRIPSR-COVID requires only 40 minutes to run the diagnostics, doesn't require the thermal cyclers and hence provides an alternative for rRT-PCR [12]. It is also considered as a point of care testing method and also costs much less than RT-LAMP and rRT-PCT tests. Some of the other molecular diagnostics methods that are available include Transcription Mediated Amplification (TMA), which is similar to isothermal amplification and high throughput technologies such as metagenomic base sequencing, next generation sequencing. Compared to the above-mentioned methods, the other methods have limitations and not much work has been done on them to be validated and deployed for wide-spread use in clinical settings [7].

Serological methods

Serological test, otherwise known as antibody tests, is used to detect Immunoglobulin IgM and IgG antibodies in blood,

saliva or sputum and to detect a past or current infection [7]. Antibodies are created in the human body as proteins to fight infections caused by viruses or when vaccinated against an infection. They are formed as a defense mechanism to fight against the virus, in this case SARS-CoV-2. Antibodies can help in identifying the infected individuals from those that are not affected by the virus. It can also help to understand the extent of the virus spread in communities and also aids in vaccine development [8].

The structural protein S and N results in the creation of antibodies IgM and IgG. The presence of these antibodies in the plasma or serum is used to indicate COVID-19 infection. IgM is first produced in the early stages of infection and IgG shows a current or prior infection [8]. One of the advantages of the serology tests is that they can give us an estimate of the rate of occurrence of SARS-CoV-2 in a community. Disadvantage of serology could be the crossover that occurs as a result of other infections and thus a false positive [7]. Since the viral concentration changes over time, another limitation could be the viral load. The other various methods are Enzyme-Linked Immune Absorbent Assay (ELISA), lateral flow assay, etc. There are also rapid antigen tests where antibodies are used to check the presence of viral antigens in the specimen.

ELISA

ELISA or enzyme immunoassay is plate-based microwell assay that has been detecting and quantifying substances such as proteins, antibodies and hormones. It includes direct, indirect, or sandwich formats. Direct and indirect uses both antigen and antibody to detect the virus. In sandwich ELISA, the antigens are used to detect the virus by binding to two different antibodies [13]. The plate well is coated with viral protein and Patients diluted sample is added onto the plate. The sample and the protein have an enzyme substrate reaction. The antibody protein complex can be detected with an additional substate that can detect the color change using an Elisa plate reader [8]. Compared to RT-PCR, ELISA has an average TAT of 2-5 hours (shorter processing time), higher sensitivity and specificity after 2 weeks of infection. An infected patient on day zero shows IgG 50% and IgM 81%. On day five of onset, IgG increases to 81% and IgM to 100% [7]. Some of the disadvantages are that the test kits which are not apt for the non-clinical settings due to different manufacturer criteria that is being used and the antibodies not able to detect the mutated virus in ELISA [13].

Lateral Flow Immunoassay

This test is used for qualitative purpose to detect antigens, antibodies and amplified nuclide acids with different kind of samples like blood, saliva and urine. It generally gives a positive or negative chromatographic assay. It is a rapid diagnostic test, and the results can be obtained in 10-30mins [7]. Once the patient sample is added onto the test plate, the IgG and IgM are moved by the capillary action. Antibody antigen recognition happens as IgG/IgM interacts with the chemicals on the test pad. The two red lines will indicate that the test is positive due to the accumulation of the gold [7, 8]. It can be done with 1-2 drops of blood and is cheap and is used for point of care testing. It is not enough as the only initial diagnostic testing method for COVID-19 as the sensitivity and specificity are not that accurate to be used exclusively for clinical use. The sensitivity compared to other serological methods are low after some weeks following infection. The reagents and the capillary flow and the velocity all are a bit challenging to control during the process of antigen capture [13]. Other tests that are being used include antigenic tests as screening tool to find out the spread of the COVID-19 in the communities. They are capable of detecting a specific viral antigen which would mean that there is a current infection. It is not used as much as the antibody testing but the method is used same as that of LFIA. As their specificity is lower than that of rRT-PCR, the results have to be taken into consideration more carefully consequently, more test kits may need to be validated for it to be used in the clinical setting [9].

Table 1: Comparison between molecular and serological methods for detecting COVID-19 virus. Ahmad *et al*., 2020.

		Molecular methods		**Serological methods**	
Technique based	rRT-PCR	Isothermal amplification	CRISPR-Cas12	LFA	ELISA
Sample	RNA	RNA	RNA	Ag or Ab	Ag or Ab
Accuracy	High	High/Moderate	High	Low	Moderate
Time	Hours	Minutes	Minutes	Minutes	Hours
Professional skills need	Yes	Yes	YES/No	No	Yes
POCT	No	Yes/No	Yes/No	Yes	No
Availability	Limited	Limited	Limited	Available	Available
Cost	Very high	High	Average	Low	Average
High throughput	Yes	No	No	No	Yes

Conclusion

Both Molecular and serological testing are useful in diagnostics purpose for COVID-19. Even though the molecular methods are slower, and not very cost effective, they work very well in clinical settings. The serological tests are simple and fast and they can be used for clinical references to monitor the various disease stages. But in order to confirm the COVID-19 cases, molecular methods are still preferred and considered as the standards. The latest research is looking at producing more efficient test in terms of its cost, reliability sensitivity and specificity and accuracy. With the current known facts from symptomatic and asymptomatic patients and from the previous gained knowledge, further tests with more accurate and established techniques can be developed and a universal test can be finally used for the diagnosis of COVID-19 and the emerging variants of this pandemic virus.

References

1. Malik YS, Sircar S, Bhat S, Sharun K, Dhama K, Dadar M *et al*. Emerging novel coronavirus (2019-nCoV)-current scenario, evolutionary perspective based on genome analysis and recent developments. The veterinary quarterly,2020:40(1):68-76.
2. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nature reviews. Microbiology,2019:17(3):181-192.
3. Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. Journal of medical virology,2020:92(4):418-423.
4. Centers of Disease Control and Prevention (CDC) (2020) Centers of Disease Control and Prevention

(CDC) Resources for laboratories working on coronavirus (COVID-19), 2020.
5. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. Journal of autoimmunity,2020:109:102433.
6. Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B *et al*. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerging microbes & infections,2020:9(1):386-389.
7. Linda J Carter, Linda V Garner, Jeffrey W Smoot, Yingzhu Li, Qiongqiong Zhou, Catherine J Saveson, *et al*. Assay Techniques and Test Development for COVID-19 Diagnosis. ACS Cent Sci,2020:6(5):591-605.
8. Dhamad AE, Abdal Rhida MA. COVID-19: molecular and serological detection methods. PeerJ, 8, e10180.
9. Machado B, Hodel K, Barbosa-Júnior VG, Soares M, Badaró R. The Main Molecular and Serological Methods for Diagnosing COVID-19: An Overview Based on the Literature. Viruses,2020:13(1):40.
10. Ganguli A, Mostafa A, Berger J, Aydin MY, Sun F, Ramirez S *et al*. Rapid isothermal amplification and portable detection system for SARS-CoV-2. Proceedings of the National Academy of Sciences of the United States of America,2020:117(37):22727-22735.
11. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N *et al*. Loop-mediated isothermal amplification of DNA. Nucleic acids research,2000:28(12):E63.
12. Hou T, Zeng W, Yang M, Chen W, Ren L, Ai J *et al*. Development and evaluation of a rapid CRISPR-based diagnostic for COVID-19. PLoS Pathogens,2020:16(8):1-12
13. Nguyen Ngan NT. McCarthy, Colleen; Lantigua, Darlin; Camci-Unal, Gulden. Development of Diagnostic Tests for Detection of SARS-CoV-2. Diagnostics,2020:10(11):905.