ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 3 Issue 7 July 2020

Research Article

Molecular Screening and Diagnosis of SARS-CoV-2: Recent Advances and Future Prospective

Avinash Kumar[1], Abhishek Kumar Verma[2] and Mayadhar Barik[3]*

1Assistant Professor, Department of Paramedical Sciences, Mewar University, Chittorgarh, Rajasthan, India

[2]Assistant Professor, Department of Biochemistry, Mewar University, Chittorgarh, Rajasthan, India

[3]Chief Researcher Basic and Clinical Sciences underScience and Technology Division andAssociate Professor in Department of Biochemistry and Head of the Department (HOD) Paramedical Sciencesat Mewar University, Chittorgarh, Rajasthan, India

***Corresponding Author:** Mayadhar Barik, Chief Researcher Basic and Clinical Sciences underScience and Technology Division andAssociate Professor in Department of Biochemistry and Head of the Department (HOD) Paramedical Sciencesat Mewar University, Chittorgarh, Rajasthan, India.

Received: May 26, 2020

Published: June 18, 2020

Abstract

Background: COVID-19 is a new form of Coronavirus which is resembling the same as SARS-CoV-2. This virus leads to cause severe respiratory diseases among the people. Coronavirus is declared as the pandemic by the WHO on March 11, 2020. Transmission of the disease is mainly due to the person to person contact by any means.

Materials and Methods: We detect the disease primary screening is necessary for a speedy recovery. PCR and other nucleic acid amplification are mainly used to diagnose the Covid-19. For the detection of the SARS, its gene is targeted in the amplification. The genes which are targeted are E, N, S, RdRp and ORf. Mutation in the SARS-CoV-2 genome is also detected by the various nucleic acid detection tests. We improve the detection of this pandemic, the only thing is to increase the involvement of Serological testing and molecular methods.

Results: The majority of 85-95% factor becomes useful to overcome this disease is early detection rate of the disease SARS-CoV-2. Molecular and serological findings should need to work simultaneously for the improvement in the diagnosis and the treatment approximately 85% laboratory.

Conclusion: The only factor which becomes useful to overcome this disease is early detection of the disease.Molecular and serologicalstudyshould need to work simultaneously for the improvement in the diagnosis and the treatment. The need of the hour is to maintain and introduced laboratory networking and its application.

Keywords: COVID-19; SARS-CoV-2; Laboratory Diagnosis; Molecular Method; Serological; Patients Management

Introduction

Coronavirus disease(COVID)is a modified form of severe acute respiratory syndrome and now known as SARS-COV-2, it assumed that this originated from the animal market of the Wuhan city of china and this disease is now spread throughout the World and causing deaths to the millions [11]. This disease is transmitted through the close contact of the two individuals in which one is the carrier and the second one will get affected. Due to the limited study and unavailability of adequate monitoring, it has become a challenge to diagnose and start treatment globally. Every country applying there kind of possible method to tackle the pandemic. The only method to decrease the impact of the pandemic is early diagnosis. The individual who has doubts or suspected to be in touch with the affected person or having symptoms should prescribe the 14-21days quarantine. The person having symptoms should urgently contact the physician. Treatment of the patient should be started with immediate effect with Government health protocols, and they should be closely monitored the pattern of risk associated with pandemic to community.

Based on clinical assessment of the suspected individual it should be decided that they need admission in the hospital or the

home quarantine. Officers of police should ensure all the procedures of home isolation. If the isolated individual show any symptoms during isolation, medical support should be provided. Suggested treatment and signs need to be carefully monitored during home isolation. According to WHO (World Health Organization) COVID-19 cases divided into three groups Probable, Suspected and Confirmed. In Probable group including a suspected case for whom test not done due to some reason and testing for COVID is inconclusive, In suspected group a patient with acute respiratory illness (e.g. Cough, Shortness of breath, and with no other etiology that fully explains the clinical presentation and a history of travel to or residence in a country/area or any territory reportingto local transmission of COVID-19 disease during the 14 days before the onset of symptoms or a patient with severe acute respiratory diseases (SARD)associated with sign of cough, shortness breath, throat infection, and fever and pain requiring hospitalization and with no other etiology that fully explains the clinical presentation. In the confirmed group of personswith a laboratory of COVID-19 infection, irrespective of clinical signs and symptoms.

Material and Methods

Types of specimen and its collection

The sample collection of any microbiological specimen or sample, it requires the proper knowledge and the standard protocol known as SOPs. SOP stands for standard operating procedures. The staff of the lab should be well trained and know the primary guidelines given by the WHO for the COVID samples. The sample is highly contagious that's why it requires proper handling of the collection container and its proper protection and transport [14]. We assuredthat these protocols are made for suspected clinical sample should be only processed by the designated medical professional. The minimize risk detection ratein the laboratory and the officials should follow the described standard procedures by the National Laboratories. Biosafety cabinets of stages 2 and 3 are required for the molecular and viral diagnosis. Specimens from the upper respiratory organs are required for the detection of the virus. There is various type of specimen which we collect for the diagnosis such as nasopharyngeal, oro-pharyngeal and Broncho alveolar lavage, urine sputum whole blood and convalescent plasma [17]. The pathophysiology of the disease, atthe lungs are extracted from the post-mortem of the dead patients [13].

Results

Biosafety actions

Samples should be processed by the well-trained staff to minimize the risk of contamination through the specimen. The person should wear proper PPE which include mask, gloves, apron, etc. Proper sanitation should be done in the lab and at the workplace [3].

The correct steps to put out the PPE

Carefully change your clothes and wear a hospital suite. With the help of sanitizer sanitize your hand and the exposing body for preventing any contamination; a person can also apply alcohol-based hand wash. Then PPE should be applied, the order of the putting PPE is shoe cover, apron, N95 Mask, goggles or shield, Headcover, and gloves [4].

The correct steps of PPE doffing

After your working hours, put off the PPE in designated areas only to prevent any type of contamination. After this workplace is sanitized and hand hygiene of the person should be maintained. The order of doffing is started with the shoe cover, gloves, glasses or shield, head cover, apron, and mask [4].

Specimen processing

- The final processing of the specimen is done in the biosafety cabinet of type 3 or type 4 [4].
- Type 2 or Biosafety cabinet level 2 is used in the working of the molecular method like PCR sample preparation or nucleic acid amplification [4].
- For the disinfection of the workplace, the disinfectant which users are (e.g. hypochlorite [bleach], ethanol, hydrogen peroxide, quaternary ammonium compounds, and phenolic compounds) because it works on the enveloped viruses also [4].
- "Infectious agents impacting humans" tag should be placed on the viral samples before transporting to the labs [4].

Specimen packaging and transport

The specimen should be transported to the labs immediately after the collection of the samples. The specimen should be carried carefully without any chance of contamination to the lab. The temperature of 2-8°C would be required for the transport of the specimen. If there is any chance of delay in the processing of the specimen it should be maintained on the -20 degree Celsius to -70 degree Celsius. Transport within national boundaries must conform to applicable laws and regulations (Table 1). The standard protocol for the selection, packaging, and transport of specimens were provided by the designated authorities [5]. The proper-packaged sample delivered at the laboratory by ensuring all the transport protocol(Li X.,*et al*. 2020).

Sr. No.	Specimen Type	Collection Materials	Transport to Laboratory	Storage Temperature till Testing	Comments	References
	(NOS)	Dacron or Polyester flocked swabs	4°C	≤ 5 days : 4°C >5 days: -70°C	To increase the viral load both nasopharyngeal and oropharyngeal swabs should be placed in the same tube.	(Padni., *et al.* 2019)
	(BL)	Sterile container	4°C	≤ 48 hours : 4°C > 48 hours : -70°C	Some dilution of pathogen may be there but a important specimen in patients with serious infection.	(WHO, 2020)
	TS, NPS or nasal wash	Sterile container	4°C	≤ 48 hours : 4°C > 48 hours : -70°C	-	(Padni., *et al.* 2019)
	Sputum	Sterile container	4°C	≤ 48 hours : 4°C > 48 hours : -70°C	To ensure if the material is from lower respiratory tract.	(WHO, 2020)
	Tissue from biopsy Or Autopsy including from lung	Sterile container with saline or VTM	4°C	≤ 24 hours : 4°C > 24 hours : -70°C	Important for post mortem diagnosis.	(Padni., *et al.* 2019)
	Serum ((ACSs)	SST (Adults: collect 3-5 ml whole blood)	4°C	≤ 5 days : 4°C > 5 days : -70°C	Paired samples to be collected: Acute- First week of illness Chronic- 2 to 3 weeks later	(Padni., *et al.* 2019)
	WB (5 ml)	Blood in EDTA vial	4°C	≤ 5 days : 4°C > 5 days : -70°C	-	(Padni., *et al.* 2019)
	Stool	Stool container	4°C	≤ 5 days : 4°C > 5 days : -70°C	Important sample to rule out gastrointestinal infection.	(Padni., *et al.* 2019)
	Urine	UCC	4°C	≤ 5 days : 4°C > 5 days : -70°C	-	(WHO, 2020)

Table 1: In this table include Specimen type, Collection materials, storage temperature and comments.

Abbreviations: NOS: Nasopharyngeal and Oropharyngeal Swab; BL: Bronchoalveolar Lavage; TS: Trachial Aspirate; NPS: Nasopharyngeal aspirate; WB: Whole Blood; UCC: Urine Collection Container; ACSs: Acute and Convalescent samples; SSTs: Serum Separator Tubes; EDTA: Ethylene Diamine Tetraacetic Acid.

Laboratory diagnosis

Nucleic acid amplification testing (NAAT):

Today, the only method to diagnose the coronavirus is a molecular method that includes many nucleic acid amplification tests. For the detection of the SARS, its gene is targeted in the amplification. The genes which are targeted are E, N, S, RdRp and ORf(Li X.,*et al*. 2020).

Identification of the sequence:

Identification of the sequence of the virus genome the particular steps are required which are as follows:

- The SARS-CoV-2 viral genome sequence has two distinct matches with it, of which at least one target is ideally SARS-CoV-2 virus-specific using an amplification assay Or
- An amplification test should be said positive for the presence of beta-coronavirus and by sequencing the viral genome partial or full as long.

The tests which are not clear as they are positive or negative the sample is again collected from the patient. If possible, sequencing and amplification of the viral genome are also done [13].

Places where the coronavirus commonly carries a simplified logarithm, there would be adequate to screen one single differ-

ential target (Lim J.,*et al.*2020). There are many factors by which positive samples can give negative results, including:

- Specimen for the diagnosis is not of good quality, it means it doesn't have the deciding factors for the diagnosis. For example for PCR, DNA is required and the sample does not yield enough constituent.
- Maybe the collection of the samples done late after an outbreak.
- Transport of the samples should not be according to the standard protocol.
- Viral genome mutation occurs after the collection and it inhibits the NAAT tests.

If the patient of a high suspect of disease is observed a negative result, then samples from the lower respiratory system are collected for the sequencing of the genome (Lippi G.,*et al.*2020).

Viral sequencing

Although there is no significant part of the sequencing of the targets of the viral genome it is done in the cases of (Zing.,*et al.*2020):

- There is a piece of evidence that a virus is present.
- Genome mutation is diagnosed in the amplification test earlier.

Serology

- Help to resolve an ongoing outbreak and retrospectively assess the attack rate or magnitude of the epidemic.
- Cases where sequencing reports are negative and could be there any link to infection, Availability of the serological test, and convalescent plasma samples are used to help the diagnosis.

Viral culture

Viral culture is not done for the diagnosis of the SARS-COV-2 because the viral culture is done only when there is a need for viral isolation and identification and to find out their properties. The human airways epithelial cell lines were used to narrowly isolate the virus [14].

Future Perspective

By recognizing the above scenario there is a requirement of the laboratory network to overcome this challenge. Our country has a large network of laboratories for virus detection and their studies. During these research phases, these laboratories take the lead in India's COVID-19 testing. This laboratory connectivity could be appropriate for sample collection in low - income countries before the final diagnostic of SARS-CoV-2 and some other diseases. The prior diagnosis of the disease is beneficial for the countries whose per capita income is low or has low resources. Thus, even as pandemic stretches the impact, a molecular biology checkpoint in the rapid detection of cases will be like an ultimate goal, thereby initiating therapy as early as possible [13].

Diagnostic Challenges

Prior detection of the corona cases is necessary and the isolation of the positive case individual is necessary to prevent further transmission to the healthy person. In this procedure, collection and transport of the contagions samples should be done carefully because it plays an important role. The research found that the overall positivity of immediate RT-PCR cases was about 30 - 60 percent [1]. This primarily depends on time specimens were taken, even though the PCR positive result is observed during the early stages of symptoms [24]. The sensitivity of the test kits is a subject of speculation and thus it is not easy to classify a large number of cases, which may potentially be adverse to the early detection and care of coronavirus cases. As a result, laboratories still face challenges in performing molecular synthesis; the healthcare system is not appropriately stable.

Discussion

The effective test and done proper collection of a specimen is to be done. There is various type of specimen which we collect for the diagnosis such as nasopharyngeal, oro-pharyngeal and Broncho alveolarlavage, urine sputum whole blood and convalescent plasma. These are collected in sterile containers such as serum separator tube, EDTA tube, viral transport medium, etc. When these samples are transported and stored it required the optimum temperature according to their function as well. Apart from this, collection to the transport of the samples required proper protocol followed. PPE should be properly applied before collecting any type of sample. After collecting the samples, seal the neck of the collecting container with the para-film. Now primarily vial is covered with the absorbent to resist any kind of leakage and then placed inside the secondary container carefully. Centrifuge tube is placed or packed in the zip-lock pouch and then this pouch is also placed in the second container of plastic and seals it. The secondary package is covered with the frozen gel packed and transported to the designated labs.

Conclusion

Early detection of the disease only factor which becomes useful to overcome the SARS-CoV-2. Molecular and serological test, Immunological, and Biomarkers study should need to work simultaneously for the improvement in the diagnosis and the treatment. The need of the hour is to maintain the laboratory networking system in the World.

Bibliography

1. Ai T., *et al.* "Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases". *Radiology* (2020): 200642.

2. Center for Disease Control and Prevention. "Coronavirus disease 2019 (COVID-19) – transmission". In: Center for Disease Control and Prevention (2020).

3. Center for Disease Control and Prevention. "Interim infection prevention and control recommendations for patients with suspected or confirmed coronavirus disease 2019 (COVID-19) in healthcare settings". Centre for Disease Control and Prevention (2020).

4. Center for Disease Control and Prevention. "Interim Laboratory Biosafety Guidelines for handling and processing specimens associated with coronavirus disease 2019 (COVID-19)" (2020).

5. Center for Disease Control and Prevention. "Interim Guidelines for collecting, handling, and testing clinical specimens from persons for coronavirus disease 2019 (COVID-19)". Centre for Disease Control and Prevention (2020).

6. Hopman J., *et al.* "Managing COVID-19 in low- and middle-income countries". *The Journal of the American Medical Association* (2020).

7. WHO. "Naming the coronavirus disease (COVID-19) and the virus that causes it" (2020).

8. WHO. "Novel coronavirus (2019-nCoV) situation reports" (2020).

9. WHO. "WHO Director-General's opening remarks at the media briefing on COVID-19" (2020).

10. WHO. "WHO Director-General's opening remarks at the media briefing on COVID-19 (2020).

11. WHO. "Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases" (2020).

12. WHO. "Guidelines for the safe transport of infectious substances and diagnostic specimens". In: WHO (2020).

13. Wu Y., *et al.* "Prolonged presence of SARS-CoV-2 viral RNA in fecal samples". *Lancet Gastroenterology and Hepatology* (2020).

14. Zhu N., *et al.* "A novel coronavirus from patients with pneumonia in China, 2019". *The New England Journal of Medicine* 382 (2020): 727-733.

15. Padhi A., *et al.* "Laboratory Diagnosis of Novel Coronavirus Disease 2019 (COVID-19) Infection. In: Saxena S. (eds) Coronavirus Disease 2019 (COVID-19)". Medical Virology: From Pathogenesis to Disease Control. Springer, Singapore (2020).

16. Guidelines for the safe transport of infectious substances and diagnostic specimens.

17. Arundhati Sharma., *et al.* "An Overview of Global Pandemic Covid-19 (Corona Virus)". *International Journal of Research and Analytical Reviews (IJRAR)* (2020).

18. Turner LH., *et al.* "Pre conceptual Zika virus asymptomatic infection protects against secondary prenatal infection". *PLos Pathogens* 13.11 (2017): e1006684.

19. Zou L., *et al.* "SARS-CoV-2 viral load in upper respiratory specimens of infected patients". *The New England Journal of Medicine* (2020).

20. Rothe C., *et al.* "Transmission of COVID-19 Infection from an asymptomatic contact in Germany". *The New England Journal of Medicine* 382.10 (2020): 970-971.

21. Singer AJ., *et al.* "Comprehensive bedside point of care testing in critical ED patients: a before and after study". *American Journal of Emergency Medicine* 33.6 (2015): 776-780.

22. Prevention of CCfDCa. The guideline of diagnosis and treatment of COVID-19.

23. Wan ZY., *et al.* "IFA in testing specific antibody of SARS coronavirus". *South China Journal of Preventive Medicine* 29.3 (2003): 36-37.

24. Lee HK., *et al.* "Production of specific antibodies against SARS-coronavirus nucleocapsid protein without cross-reactivity with human coronaviruses 229E and OC43". *Journal of Veterinary Science* 11.2 (2010): 165-167.

25. Racine R and Winslow GM. "IgM in microbial infections: taken for granted?" *Immunology Letters* 125.2 (2009): 79-85.

26. Guan WJ., *et al.* "Clinical characteristics of 2019 novel coronavirus infection in China". *Med Rxiv* (2020).

27. National Health Commission of the People's Republic of China. Novel coronavirus pneumonia diagnosis and treatment protocol (5th edition, trial).

28. Ho PL., *et al.* "A prediction rule for clinical diagnosis of the severe acute respiratory syndrome". *European Respiratory Journal* 26 (2005): 474-479.

29. Rainer TH., *et al.* "The spectrum of severe acute respiratory syndrome-associated coronavirus infection". *Annals of Internal Medicine* 140.8 (2004): 614- 619.

30. Xi M., *et al.* "Understanding the influence factors in viral nucleic acid test of 2019 novel coronavirus (2019-nCoV)". *Chinese Journal of Laboratory Medicine* 43 (2020): E002.