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"CURRENT UPDATES ON DIAGNOSTIC METHODS AVAILABLE FOR COVID-19 INFECTION"

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Review Article

ABSTRACT

The abrupt pandemic set off by the novel coronavirus 2019 (COVID-19) has caused severe chaos among people worldwide. ''SARS-CoV2'', a previously unknown strain of coronaviruses caused a severe respiratory disease called Coronavirus disease (COVID-19) which emerged from Wuhan city of China on 30 December 2019 and declared as Universal health problem by the World Health Organisation in a month. COVID-19 has created panic, and scientists are urged to test the efficiency and safety of drugs used to treat this disease. Various diagnostic kits to test for COVID-19 are available, and repurposing therapeutics for COVID-19 has shown to be clinically effective). In the analytic stage, real-time reverse transcription-PCR (RT-PCR) assays remain the molecular test of choice for the etiologic diagnosis of SARS-CoV-2 infection while antibody-based techniques are being introduced as supplemental tools. In the post-analytical stage, testing results should be cautiously interpreted using both molecular and serological findings. This review discusses the updates on specimens/samples and recent efficient diagnostics to control the disease. It covers the latest issues and challenges for the laboratory diagnosis of infections caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Keywords: COVID-19; diagnostics; SARS-CoV-2; RT-PCR; biosensor; Convalescent plasma (CP).

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1. INTRODUCTION

The novel coronavirus disease 2019 (COVID-19), an outbreak caused by the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), continues to spread, and as per the World Health Organization (WHO) data on August 14, 2020, it has reached 213 countries and territories around the world have reported a total of 21,095,532 confirmed cases of the coronavirus COVID-19 that originated from Wuhan, China, and a death toll of 757,779 deaths. At present, the COVID-19 pandemic has entered a dangerous new phase. The spread of COVID-19 is more severe than that of the previous Severe Acute Respiratory Syndrome (SARS) and the Middle East Respiratory Syndrome (MERS), because of the increased industrialization, which led to virus evolution [1]. This is the third coronavirus epidemic after the SARS and MERS coronavirus outbreaks. Structural studies revealed that there is a close relationship between the receptor-binding domains of SARS-CoV-2 and SARS-CoV. SARS-CoV-2, which causes COVID-19, is spherical, and the viral envelope entails a bilipid layer, which has the membrane (M), envelope (E), spike (S) proteins, and positive-sense RNA as a genome, with an RNA-dependent RNA polymerase sequence. On 11 February 2020, International Virus Classification Commission renamed this Novel CoV-19 as ‘‘severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)" based on its genetic composition and similarity with other coronaviruses, and the disease caused by the virus was renamed COVID-19 [2]. Coronaviruses are a group of large-sized (100–160 nm), spherical, positively sense, non-segmented, single-stranded RNA with genome sized 26–32 kb (the largest among known RNA viruses), and known to infect both animals and humans [2,3,4,5]. Coronavirus has been classified into four genera (a-alpha, b-beta, c-gamma, and d-Delta), out of which only two genera–alpha which contains CoVNL63 & CoV-229E, and -beta contains CoV-OC43, CoVHKU1, Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV, found to be infectious for human [3,6]. The genome of the COVID-19 virus constitutes 29,903 nucleotides which upon fresh reannotation and mapping of the RNA-sequences acquired, presented the 123,613 reads assembly and was very similar to SLCoVZC45 an already known bat strain, and SARS-CoV [5,7].

This group of viruses can easily undergo mutation and recombination to adapt to any environment and thus survive by altering wide host range [8] causing constant and long-term health threats, therefore it is necessary to understand its virology to prevent its rapid spreading and safety of mankind. Coronavirus is among the top ten deadliest viruses known for human beings with a high fatality rate of up to 36% by MERS-CoV during 2012 and 10% by SARS-CoV in 2002–2003. Further, the COVID-19 pandemic has forced scientists to rework strategies to combat infectious diseases through drugs, treatment, and control measures. Asymptomatic carriers can uncontrollably increase disease transmission if they will not be identified and quarantine in the early stage. In such a situation, mass screening for the disease becomes necessary and hence the fast testing devices are strongly advised to prevent the spread of the virus. The entire world is taking necessary steps to develop a vaccine for this dreadful virus, and for this kind of research, governments are providing copious funds for scientists and institutions. Recently, President Vladimir Putin claimed and announced Russia had approved a coronavirus vaccine, but World Health Organisation (WHO) says that the vaccine approved by Russia this week is not among the nine that have been considered as the advanced stages of testing. WHO and partners have included nine experimental COVID-19 vaccines in an investment mechanism it is motivating countries to join, known as the Covax facility.

1.1 Indian Scenario

The Indian government is using compulsory measures to ensure that people are well prepared to conquer this deadly infection of COVID-19. The government of India has announced the call for research projects from its various funding sources and has embarked on some major research projects on COVID-19 that are relevant to national needs. Clinical laboratories play a major role in this crisis, contributing to patient screening, diagnosis, monitoring, and treatment. Hence, cooperation with various institutions, academics, governments, and pharmaceutical companies is inevitably necessary to control the virus, combat the situation, and provide solutions for any future pandemic outbreaks. In this review, we aim to discuss the updates of current diagnostics and therapeutic approaches to COVID-19 patients. Depending on the available data, bats may be the initial hosts of COVID-19. It may be transmitted to humans through pangolin [9] or other wild animals confronted at the Huanan seafood market then disseminated through human to human transmission. COVID-19 outbreak brings back memories of the Spanish Flu Pandemic in 1918–1920, which was caused by the H1N1 strain of the influenza virus. As for many respiratory viruses, diagnosis relies on two main compartments: clinical manifestations as fever, fatigue, dry cough, dyspnea, and gastrointestinal symptoms, while the paraclinical diagnostic tools vary between the Polymerase chain reaction (PCR) and computed tomography (CT) [10]. Rapid and accurate

diagnosis is crucial in such a pandemic. From one side, rapidity may enhance management effectiveness and accelerate the application of more suitable isolation measures, leading to less contiguity. As rapid diagnostics and development of vaccines and drugs for this dreadful virus are important interventions in the management of the COVID-19 outbreak, the updates on this topic are essential in the current scenario for the betterment of patients. Besides, we discuss the types of sample/specimens collected and rapid diagnostics methods for accurate results to guide treatment options. Currently, chloroquine and hydroxychloroquine are the most-used drugs. Thus, the present review paper summarized the various detection methods available for the detection of COVID-19, their advantages, disadvantages, and the urgent need for a rapid POC detection method, on the spot biosensor their feasibly and importance in the presence of ever-increasing countdown of COVID- 19 infected patients. Besides, Convalescent plasma therapy, which is promising as a way to improve the clinical outcomes of COVID-19 patients, is also discussed.

2. DIAGNOSTICS FOR COVID-19

Coronavirus detection approaches are generally based on the travel history of the person from the affected areas as well as the analysis of their clinical symptoms along with some auxiliary examinations. Clinical symptoms like pneumonia, due to COVID-19 are highly atypical, and quite similar to diseases due to other respiratory viral pneumonia. Rapid and sensitive diagnosis of COVID-19 is still unavailable, although some diagnosis methods are available presently for virus detection, each having a different degree of specificity and based on single or multiple target molecules from the SARS-CoV2. These methods use the pathological changes in the patient's organ by imaging like CT, or viral nucleic acid-like RT PCR using one or more gene, or Next Generation Sequencing whole genome, immunological molecules produced by the patient or by the virus in the patient's body- Antigen-antibody reaction-based tests like ELISA and utilizing each of these diagnosis approaches has it's their advantages and shortcoming in the present scenario.

Out of these, some methods were already established and considered as gold standard methods which could be replicated for this novel virus also while others are being developed and evaluated for the diagnosis of this virus. On the other hand, there are other methods, technologies also which have been developed for the use in covid-19 methods are described here.

3. NUCLEIC ACID BASED METHODS

Nucleic Acid-based technologies are utilizing genetic material such as DNA/RNA and are based on the principle of their highly specific base pairing with homologous strands. Genetic materials-based detection and diagnostics are comparatively faster than traditional culture-based methods and very useful for high-throughput testing and also provide clinically useful information like drug resistance, virulence factors, or strain sub-types within some hours, but are relatively expensive. These technologies such as polymerase chain reaction (PCR), DNA microarrays, and high throughput automated sequencing methods have a tremendous role in the routine clinical diagnosis and discovering novel strains of bacteria and viruses, and other pathogens. Here, the current state of the art in the nucleic acid-based technologies for diagnostics of COVID-19, and their future advancements along with the pro and cons of using these technologies are being described.

4. NEXT GENERATION SEQUENCING (NGS)

Next-generation sequencing (NGS) is also called high-throughput sequencing (HTS). By this method, we can determine the genomic sequence, even more than 1 million base pairs in a single experiment. By this technique, we can diagnose inheritable diseases, cancer, and infectious diseases. Earlier, also the same technology was used in the UK for tracking an outbreak due to the Methicillin-Resistant Staphylococcus aureus (MRSA) [11,12] with high precision and traceability even in a single patient, while other routine surveillance techniques could not do it with that much precision. NGS helps not only in the discovery of novel viral strains on a large scale but also provides very rapid detection of these viruses which link with human diseases.

The NGS technology along with bioinformatics tools has largely influenced the modern viral parthenogenesis studies and viral diagnostics. This technology also played a great application in the present COVID-19 outbreak. At the initiation of the current outbreak of SARS-CoV2, the samples from the patients admitted acute respiratory distress syndrome were negative for the all suspected already known pathogens, the etiological pathogen was identified by only NGS by doing metagenomics, RNA sequencing and the phylogenetic analysis of its complete genome generated could conclude that it is a new strain of an RNA virus which belonged to the *Coronaviridae* family and was designated as SARSCoV2 after nucleotide similarity and genome matching with the existing pathogen’s genome [13].

Therefore, this technology has great importance for identifying unknown pathogens, and mutation or recombination in the genome of the pathogen in a short period, but the huge cost of the equipment an chemicals required in this technique restricts its utilization in routine laboratory diagnosis of the diseases.

5. REVERSE TRANSCRIPTASE-POLYMERASE CHAIN RECEPTOR (RT-PCR)

Presently, quantitative reverse transcription-polymerase chain reaction (rRT-PCR) is being used for diagnosis of COVID-19 and is a gold standard molecular diagnostic technique for many viruses as well. Single-step quantitative RT-PCR with TaqMan chemistry is more sensitive and specific. As this technology is well established, and so can be used easily, only needs specific primer-probe designed and synthesized, remaining components of the reaction remain same as used for other viruses without or with a little change. Once the first sequence results of the SARS-CoV-2 virus from China were out, candidate diagnostic rRT-PCR assays were designed and made available in the public domain for researchers. Various agencies or manufacturers have opted for a different set of genes out of many genes of SARS-CoV2 (ORF-1a gene, ORF-1b gene, RdRp gene, N gene, E gene, etc.), so every essay has a varying degree of sensitivity. As per the standard protocol, one patient is confirmed of infection when both the selected target genes come to be positive [14]. While in some reported studies two individual single-step RT-PCR assays (Based upon TaqManchemistry) were performed for identification, and amplification of two segments of any two genes, mostly N or ORF1b from viral genome separately, others have used multiplex assays using more than one genes amplification in a single reaction. Although, these methods are very sensitive (almost 100%), but take a longer time for confirmation as the test has to be done in a well-sophisticated laboratory. The testing has to be done in two steps; the first step for screening assay, using

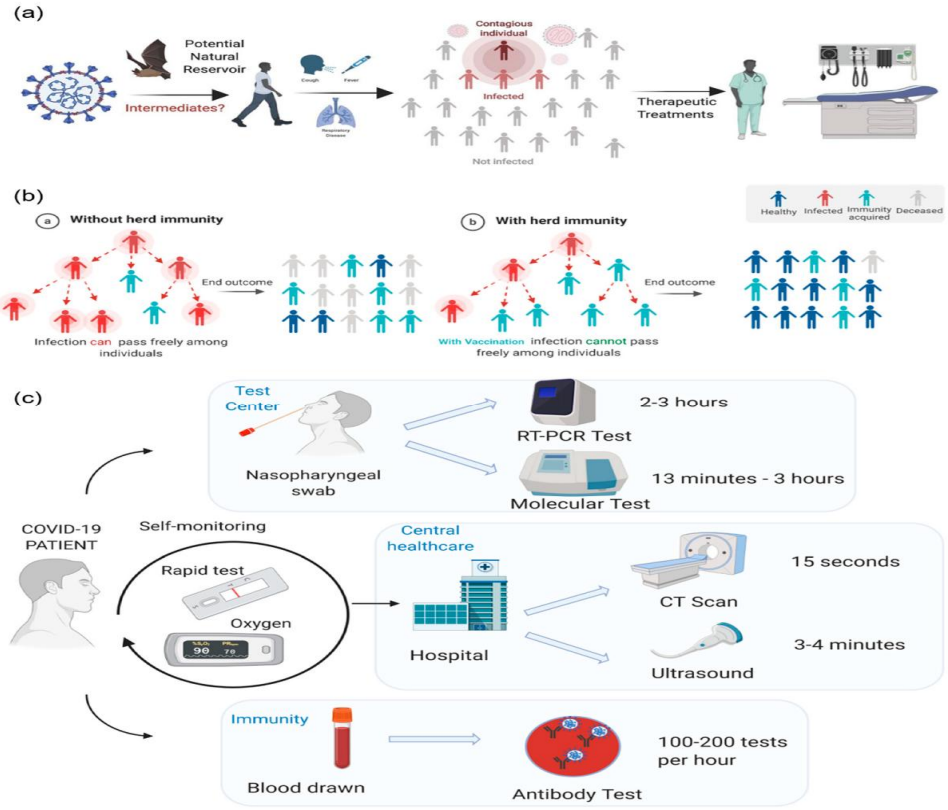

Fig. 1. COVID-19 origins, transmission, immunity, and diagnostics. (a) Animals such as bats are a potential natural reservoir, and other intermediates facilitate the infection of the coronavirus to humans. (b) Herd immunity plays an essential role in controlling the transmission of the disease. (c) The three main categories of COVID-19 diagnostics and the time-frame of each: laboratory test centers for current infection, medical screenings, antibody tests to determine immunity [1, 19]

the SARS-CoV-2-specific E gene, and the second step for confirmatory assays targeted the 'RdRp gene', 'N gene' and 'ORF1b'. The "Positive control" material used for these assays was in vitro transcribed RNA of known copy numbers. RNAse P gene detection as used in other most respiratory viruses is being used as an internal control to give the information of the quality of sample collection, RNA extraction process. The standard testing protocol as per WHO involves 5 steps, (1) sample collection from the patient; (2) Proper transportation of collected samples to the laboratory; (3) Providing demographic and clinical information to the laboratory; (4) Sample testing by the laboratory; (5) preparing and reporting the correct and appropriate test results. Testing is carried out at specific centers which further delays the diagnosis and makes the condition of the patient severe. These PCR assays provide good results but on the other hand, they are laborious and expensive as well. Some studies using RT-PCR SYBER green dye-based assay were found to be less specificity than TaqMan probe-based assays. Similar results were reported recently in China by patients who self-collected saliva and showed 91.7% positive SARS-CoV-2 while diagnosing by SYBR based RT-qPCR. RT-qPCR assays have been reported highly sensitive and specific for SARS-CoV, MERS-CoV detection, and also same for COVID-19, but this technology is prone to its false-negative rates which could result in severe consequences due to a missed diagnosis of COVID-19. The real example is present from the current outbreak of SARS-CoV2 where five patients were reported as negative by RT-qPCR but found positive when CT scan examination of their chest was done and recollected samples repeated RT-qPCR, all patients were confirmed positive for SARS-CoV-2. The sensitivity of RT-qPCR for detection of SARS-CoV was reported between 50% and 79%, that too depends on their adopted protocol, quality of the sample (time of collection, amount, maintenance of

Table 1. Current Diagnosis method available for COVID-19

Method available	Working principle	Advantage	Disadvantage
Next-generation sequencing (NGS)	Whole-genome sequencing	Highly sensitive and specific, Provide all related information; Can identify a novel strain	High expertise Equipment dependency and high cost Highly sophisticated Lab required
RT-PCR[1]	Specific primer-probe based detection	Fast results Higher sensitivity Needs a small amount of DNA Can be performed in a single step Well established methodology in viral diagnostics	Higher costs due to the use of expensive consumables Expensive lab equipment Detection is also complex and time-consuming
LAMP[2]	More than two sets of specific primers pair based detection	Highly repeatable and accurate Single working temperature	Too sensitive, highly prone to false positives due to carry-over or cross-contamination
Serological (traditional)	Antigen/Antibodies IgG/ IgM	Sensitive and specific	Testing comes after 3-4 days of infection False positive
Rapid serological	Antigen/Antibodies IgG/ IgM	POCT	Testing comes after 3-4 days of infection False positive
CT scan[3]	Chest images	Enhance sensitivity of detection if findings combined with RT-PCR results	Indistinguishability from other viral pneumonia and the hysteresis of abnormal CT

Kumar, R., Nagpal, S., Kaushik, S., & Mendiratta, S. (2020). COVID-19 diagnostic approaches: different roads to the same destination. Virus Disease, 1-9

[1]*Reverse Transcriptase-Polymerase Chain Receptor*
[2]*Loop-Mediated Isothermal Amplification*
[3]*Computed Tomography (Ct) Scan*

cold chain), and a total number of samples [15], and needs further improvement using synergistic approaches. Besides the sensitivity problem, RT-qPCR has some other drawbacks such as possible biological safety hazards that occurred during transport and sample processing, nucleic acid extraction, and the requirement of sophisticated laboratory equipment like biosafety cabinets that are often available only in few main central laboratories. Technical expertise along with inevitable sample transportation makes the overall process time-consuming. All these drawbacks could make the process less useful in case of a health emergency or present global outbreak situation. Moreover, in PCR we can detect not only the target virus, but it can also perform co-detection of several other respiratory viruses which leads increase in false positive or negative results [16].

6. LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

LAMP is a comparatively novel technique which in process of approval for COVID-19 diagnosis is a molecular amplification technique that can amplify any genomic material with high efficiency and in a shorter time. The technique is based on a synthesis of target DNA at a constant temperature of 60–65 C using specially designed primer sand enzyme (DNA polymerase) having strand displacement activity instead of heat denaturation as in other PCR techniques and in an hour or lesser time can amplify the target sequence up to more than 109 copies forming a cauliflower-shaped structure as a final product consisting a stem and a loop form of DNA with many inverted repeats. LAMP is a user-friendly technology that can provide reliable, sensitive, and specific results in lesser time as compared to other conventional techniques, and therefore become quite popular just after its development focusing its applications in microbial detection [13]. This technique has the advantage of requiring only a single constant temperature and thus eliminating the need for thermocycler and power consumption.

7. COMPUTED TOMOGRAPHY (CT) SCAN

CT Scan is also one of the diagnosis techniques having high sensitivity due to which many researchers recommend its use as one of the necessary auxiliary diagnostic methods for COVID-19, moreover its results come even before clinical symptoms appear. Typical features by CT of COVID-19 patient include bilateral multi-lobar ground-glass opacification with different distributions in posteriors and also in peripheral [17], along with sub-pleura ascendance, thickened lobular septa with variable alveolar filling, and amalgation [18]. According to a recent report from Wuhan, the CT is significantly more sensitive than PCR for SARS-CoV-2 suspected persons. The results concluded that in patients having negative RT-qPCR reports, a more sensitive and accurate conclusion can be achieved using a combination of CT-Scan and other standard techniques like RT-qPCR or other sensitive diagnostic tests. Moreover, the high-resolution CT of the chest is also proved as an essential tool for the detection of SARS-CoV-2, at an early stage and to take a rapid and necessary intervention. Therefore, various studies recently utilizing chest CT images to diagnose the COVID-19 [10]. Earlier also the typical CT images in patients infected with SARS-CoV and MERS-CoV showed similar symptoms as in COVID19 [17]. As per these findings, CT scans found to be a great diagnostic tool for screening of COVID-19 patients, especially in the high prevalence or pandemic areas. The CT scans are indicative and not confirmatory tools for pathogen detection in the COVID-19 diagnosis and are associated with few shortcomings also such as the inability to separate the cases of other pneumonia (viral or non-viral) and the hysteresis of the abnormal CT imaging.

8. ANTIGEN–ANTIBODY BASED METHODS

Serological-based testing methods normally use blood samples for the detection of the virus instead of nasopharyngeal swab samples used in the PCR test. The blood samples contain either a significant and measurable concentration of antibodies or virus-specific antigens. The two main types of antibodies in the blood which the test looks for are the immunoglobin G (IgG) and immunoglobulin M (IgM). The body's way of remembering the prior infection, and how it responded to infection in the previous encounter is very crucial so that the body can attack the same pathogen again is through antibodies. IgM appears within a few days and acts as the first line of active defense, followed by the production of IgG to start clearing the infection. All kinds of infections are fought through IgM and IgG. The body's immune response mechanism can be utilized to detect the particular pathogen. The blood test for COVID-19 detect the protein (signature antigen/biomarkers) or antibodies particular to the virus so as for SARS-CoV2 with the confirmed SARS-CoV2 specific antibodies in case of antigen detection or confirmed SARS-CoV2 antigen in case of antibodies detection, and not produced for the seasonal flu or other viruses. Currently, two types of COVID-19 tests have been reported one direct utilizing antigen based on detection of viral component present during the time of infection and the second indirect using antibodies that appear in patient's serum later due to development of immune

response against the virus lists ten rapid antigen detection tests for COVID-19 with EU approval under IVDs directives, but yet to come in the market due to non-availability of distributors for these devices. However, reports from competent authorities indicate the availability of three such CEmarked devices very soon. Antibody Detection tests: There are nearly 60 antibody tests marked rapid SARS-CoV-2 that are expected to come soon in the market along with various other in-house validated tests for SARS-CoV-2 by many researchers, which can help in early diagnostic at a commercial scale. Also, several points of care (POCT) kits based on IgM or IgG, and ELISA for COVID-19 showing higher detection rates compared to nucleic acid-based detection methods, have been developed and pre-tested by many companies but still not in the commercial stage. ELISA based detection kits developed or being developed using antibodies against spike, nucleocapsid, or membrane and envelope proteins are considered as one of the most sensitive methods for COVID-19 diagnosis. Earlier also this method using N-based IgG ELISA and S-based IgG ELISA showed good sensitivity for SARS-CoV i.e. 94.7% and 58.9% respectively. The sensitivity of the ELISA kit for SARS-CoV-2 is still under study. Also, antibodies-based diagnostic assays are not useful for early or active diagnosis of COVID-19, due to their longer time requirement (7 days or more) to be developed by the host to provides positive results, and so, the detectable antibodies are produced late after the appearance of symptoms and once developed can persist long after the infection has been cleared [20]. There is great urgency to develop an auxiliary method for accurate diagnosis of COVID-19 which should be enough sensitive, specific, and cost-effective.

9. RAPID TEST METHOD

Rapid tests are the one which involves non-automated, mostly qualitative but in some cases quantitative also, are used for in vitro diagnostics (IVDs) of many diseases already, and now also being tried for COVID-19 diagnosis. These tests can provide results within 10–30 min, so their results are considered as instant as compared to the molecular tests which generally takes 4–6 h. Moreover, these tests are user-friendly, thus won't require any extensive training or expertise to operate and can be used either in the hospital environment, in the laboratories, or at the patient bedside without any difficulty.

10. POINT OF CARE TESTING (POCT) APPROACHES

Point of care testing (POCT), as the name indicates can be used at the patient's bedside with ease without any experts or trained person to operate. These devices are useful for detecting various diseases including infectious viral-like HIV, influenza, Hepatitis, etc. and bacterial disease in a cost-effective and user-friendly way, and help in finding the source of any health outbreaks quickly and providing enough time to the authorities for taking necessary preventive or therapeutic measures. Out of many types of POC devices, the handheld POCTs are of great importance in medical diagnostics which includes various types of biosensors.

11. BIOSENSOR

A biosensor is a self-contained integrated analytical device consisting of the bioreceptor, transducer, and a signal detector. The interaction of bioreceptor with the target analyte produces an electronic signal and through transduces which can then be further amplified by a detector circuit, processed, and displayed. Biosensors help in the development of point of care, portable devices for sensitive, specific, and rapid diagnosis of disease in a cost-effective way. They use various diagnostics principles, such as PCR involving RNA or DNA sequences, gel electrophoresis, enzyme-linked immunosorbent assay (ELISA) also called sandwich assay involving the interaction of antigen antibodies, and other detection procedures coupled with fluorescent and or radioactive labeling. Nowadays several advanced biosensors-based diagnosis approaches have been utilized for the fabrication of innovative and novel handheld devices that can overcome the drawbacks of a lengthy gold standard detection protocol. These biosensors use the nanomaterials with tunneling and quantum properties leading to enhancement in signal amplification. Further, the nanomaterials are having a high surface-to-volume ratio which enhanced their high sensitivity many fold [21], moreover, the viruses (target analytes) are also in nanoscale, these all features make the nano-sensors a potential diagnostics tool. Nano-biosensors using aptamers are one of such potent analytical tools for rapid diagnosis of diseases with high sensitivity and specificity in a cost-effective and user-friendly manner compared to conventional methods [22]. Such a nano-sensor will have great potential for the detection of SARS-CoV-2 even in person without any symptoms with high sensitivity, specificity, and selectivity only for COVID-19.

12. SPECIMENS COLLECTION FOR COVID-19

The dramatic COVID-19 pandemic has surged at an increasing rate, where the characteristic transmissibility can only be defined at commencement because confirmed cases of COVID-19 may be

presented with either asymptomatic or symptomatic infection, which could range from mild to severe or life-threatening pneumonia. Particularly, COVID-19 seems to be transmitted mostly during the incubation period, when most of the patients either lack the symptoms or have very mild non-specific symptoms [23]. The common symptoms associated with this disease are high fever, cough, difficulty in breathing, and lesions in the lungs; in the worst cases, it can cause severe pneumonia, acute respiratory distress syndrome (ARDS), and risk for life [24]. Individuals affected with such symptoms or who have had any International travel history or contact with a person who is ill or been quarantine should approach the government and health-care officials to monitor and check their health status for the safety of their family as well as society. The Centre for Disease Control and Prevention (CDC) [25], the World Health Organization (WHO) [23], and the Indian Council of Medical Research (ICMR) [26] has recommended a few guidelines for the collection of the specimens from the affected or suspected COVID-19 patients. The affected or suspected individuals must co-operate with the state or local health-care departments for the collection, the further process like storage, and shipment of the specimens appropriately. It is highly mandatory and recommended that the specimens should be collected only in a BSL-3 laboratory for the safety of the clinicians and researchers. Based on the recommended guidelines of WHO, the sample will be isolated from two major sources, which are the lower respiratory tract and the upper respiratory tract. The specimens, such as a nasopharyngeal swab (NP) or the oropharyngeal swab (OP), will be collected from the upper respiratory tract, whereas the bronchoalveolar lavage, tracheal aspirate, or sputum will be collected from the lower respiratory tract. Nasopharyngeal swabs: The NP specimen is a vital and sensitive sample to test the SARS-CoV-2 virus, as suggested by the CDC. This sample could be used to analyze an asymptomatic patient with the disease. For this type of sample collection, only synthetic fiber swabs with plastic shafts are recommended, because calcium alginate or wooden-shaft swabs might inactivate the virus and could provide a negative result for the nucleic-acid assay. After the collection of the swab, it should be placed immediately into a 2-3 mL of sterile medium or saline for proper viral transport. If the NP swabs are not available, then the anterior nares and mid-turbinate specimens can also be collected from the symptomatic patients for the detection of the viral infection. Oropharyngeal swabs: Another important specimen recommended by the WHO and CDC to detect SARS-CoV-2 infection is the OP. This swab is collected from the posterior pharynx region, avoiding contact with the tongue. According to the CDC, if the NP and OP specimens are collected together, then both specimens should be placed in a single vial for more sensitive and appropriate results of the SARS-CoV-2 infection. Bronchoalveolar lavage and tracheal aspirate: For patients who are severely ill or having severe symptoms for the COVID-19 disease, the bronchoalveolar lavage (BAL) and tracheal aspirate could be used from the lower respiratory tract. The BAL sample is acquired using a bronchoscope or catheter into the bronchus, where the aspirated fluid with the virus will be collected for the testing of the COVID-19 infection. Sputum: The patients who have severe coughing symptoms will be asked to collect their sputum as the sample to detect the viral infection. The patients will be asked to rinse the mouth with sterile water and further expel the deep cough sputum straight away into a sterile, leak-proof, screw-cap tightened sputum collection cup or sterile dry container. Other samples: Since SARS-CoV-2 is present in blood and stool, these specimens can be collected in addition to the respiratory specimens. However, the efficacy of these tests remains unclear, because the data on viral shedding is still preliminary. Moreover, for patients who have died, autopsy material and lung tissue may be used to test for the presence of the virus. Based on the samples collected, the diagnostic techniques will be carried out to verify the presence of the viral infection.

13. CONVALESCENT PLASMA (CP) THERAPY

Although a definitive treatment or specific vaccine for this deadly viral infection is still a question to be answered, an experimental study related to convalescent plasma (CP) therapy appears as a shaft of light to combat the SARS-CoV-2 infection. Doctors and scientists are claiming that CP therapy was effective during the past epidemics; it could be a potential treatment option in this current scenario as well. Even the ICMR has approved for the state hospitals to register for a CP trail protocol, to initiate the clinical trials of CP therapy for the COVID-19 patients. The CP therapy is common adoptive immunotherapy, where the patients recovered from a viral disease with high neutralizing antibody titer against the virus and would be used as plasma donors to treat the affected individuals. In this therapy, when our body gets infected with the SARS-CoV-2, our immune system produces antibodies against it. These antibodies reach out to identify and mark the invading virus as the intruding foreign body inside the human system. This, in the future, triggers the white blood cells to attack the identified intruders (SARS-CoV-2), which leads to the deactivation of the viral infection. The CP therapy has been used to treat several infectious diseases, including Spanish flu (1917-

1918), the Ebola epidemic in West Africa, human coronaviruses, influenza A (H1N1) and A(H5N1) [27]. During the H1N1 viral infection, the CP therapy gave positive results, where the respiratory tract viral load, serum cytokine levels, and mortality levels were significantly reduced in the infected groups compared to their control group [28]. During the SARS and MERS pandemics, CP had provided several satisfactory and successful therapeutic outcomes [29]. In a meta-analysis from 32 studies conducted on SARS-CoV infection, the mortality rate was significantly decreased when compared to their placebo group. Since there is a high sequence as well as virological homology between the SARS-CoV and SARS-CoV-2, the CP therapy could be a promising therapy to treat the severely affected COVID-19 patients [30]. In CP therapy the antibody-rich plasma will be isolated from the COVID-19 recovered patients using an apheresis machine; the plasma-rich antibody against the SARS-CoV-2 would be injected, which could neutralize the viral load in the severely affected COVID-19 patients. For this procedure, around one liter of plasma will be collected, where approximately only 250 mL of plasma is required for one patient. According to a recent study in China, the researchers found that CP therapy could rescue COVID-19 patients from disease severity. During this experimental assay, one dose of 200 ml CP transfusion was readily accepted, which also reduced several symptomatic conditions of the COVID-19 patients. The researchers found that after the introduction of CP therapy, the patients showed negative for the SARS-CoV-2 nucleic-acid test, increased the oxygen saturation levels and lymphocyte counts, and also improved the functions of organs [31,32]. These studies demonstrate that CP therapy could be a promising therapy to improve the clinical outcomes of COVID-19 patients. Since this therapy is in its pipeline stage, more investigations are required in larger cohorts to make it a global and standardized therapy to treat COVID-19 infection.

14. CONCLUSION

COVID-19 is a global pandemic, and currently, it has emerged as the most intense and petrifying viral infection to be handled by the human race. Many countries have taken precautionary measures against the virus, and government officials in all countries continue to make efforts to minimize human contact by facilitating countrywide shutdowns of public places. Several steps have been initiated to ensure the safety of the people, like social distancing and self-quarantine, which limits our social interactions. Since the long-term effects of COVID-19 remain unknown, we still need the exact mechanism of the virus for the preventive, diagnostic, and therapeutic approaches to battle the situation. This will help to diagnose diseases at earlier stages, allowing medical intervention at this time. Early medical intervention will help to prevent the conditions altogether or at least ameliorate its prognosis. As the number of individuals infected with SARS-CoV-2 continues to rise globally, health-care systems become increasingly stressed. The clinical laboratory plays a major role in this crisis, contributing to patient screening. Other than RT-PCR, SARS-CoV2 specific other diagnostic tests like rapid antibodies-based kits being used or under development seems not worthy for mass screening. It is essential to diagnose suspected cases at the clinic or hospital, but results take time anywhere from a few hours to some days which is too slow for front-line screening. As COVID-19 has triggered human casualties and a serious economic crisis posing a global threat, an understanding of the current scenario and developing a plan of action to contain the spread of the virus are urgently needed. Rapid diagnostics, vaccines, and therapeutics are important interventions for the management of the COVID-19 outbreak. Many methodology and devices are aiming for rapid diagnosis of COVID19, are in pipeline for development, and are at different stages. These POCT, Biosensors and other alternative devices have the potential to become the technology of the future with high sensitivity, specificity, and reproducibility. It is essential to move forward with all the information necessary to be more prepared for a pandemic of its kind in the future. At present, scientists are working rigorously to find the solution to treat the SARS-CoV-2 infection at a rapid pace. Hence, more research is needed to adequately care for patients post recovery and to provide a framework of possible physical manifestations of the disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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