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COVID-19 diagnosis—myths and protocols

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1. Introduction

Coronavirus disease 2019 (COVID-19, officially named by the WHO on February 11, 2020) is a highly pathogenic transmittable viral infection, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged as a serious threat to global public health. This newly identified virus has marked the world as the third highly pathogenic coronavirus after SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), capable of causing large-scale epidemic in the 21st century [1]. The pathogen, first identified from Wuhan city, China [2], has now achieved pandemic status infecting a large number of people worldwide due to person-to-person mode of transmission. Clinical manifestations of COVID-19 patients may include dry cough, sore throat, mild fever, headache, nasal congestion, and absence of dyspnea in mild cases. But seriously ill patients can develop severe fatal pneumonia, acute respiratory distress syndrome, or septic shock. Case fatality rate for critical patients remained 49% in China according to the Centers for Disease Control and Prevention (CDC), especially in patients suffering from comorbidities such as diabetes, respiratory disease, cardiovascular disease, and oncological complications as compared to healthy people [3]. Subclinical manifestation of this disease is reported to be 30%, which requires testing and quarantine measures. Furthermore, prediction of spread/end spectrum of any disease is only possible when 1% of the total population is randomly tested. Availability of testing facilities with valid outcome is an unavoidable measure to adopt in order to cope up with this malaise.
It is evident that discrepancies exist in different diagnostic techniques that impart direct influence on the control of infection. In case of COVID-19, mainly two types of tests are being done in laboratories that include serologic tests to check the presence of antibodies against the virus and oronasal swab samples tested for presence of RNA from live virus. Specimens such as nasal secretions, expectorated sputum, endotracheal aspirate, blood, and bronchoalveolar lavage collected from suspected individuals are subjected to the serologic and molecular tests specific for COVID-19 diagnosis. Serologic tests have the ability to detect infections even in recovered patients, but PCR from swab samples of oronasal source is the recommended test for COVID-19. Swab test samples are processed by reverse transcription polymerase chain reaction (RT-PCR) to selectively amplify DNA strands produced by the RNA of SARS-CoV-2. RT-qPCR is preferred to simple PCR because of its additional ability to measure the quantity of the RNA material present in the sample [4]. Noncontrast chest computed tomography (CT) can also be used to diagnose viral diseases at initial stages [5,6]. But chest CT scan can give negative results for viral pneumonia of COVID-19 [7] at initial presentation. Sensitivity of RT-PCR can vary from 42% to 71%, while sensitivity and specificity of CT for COVID-19 ranges from 60% to 98% and 25% to 53%, correspondingly [8,9]. However, it has been found that the sensitivity of RT-PCR may not be able to detect COVID-19 in its initial stage to treat probable patients [10]. But the positive and negative predictive values of chest CT for COVID-19 are reported at 92% and 42%, respectively, in a population with a high probability of disease before testing. The comparatively low negative predictive value does not show efficacy of CT scan to diagnose COVID-19 at early stages of the disease [8]. RT-PCR is a costly technique, requires well-trained staff, and requires more time, leaving many cases untested thus enhancing gap in disease prevention efforts. Moreover, traveling to laboratory for testing increases the risk of spreading the disease and creates problems for resource-limited healthcare systems. For this purpose, a recent novel closed-tube (Penn-RAMP) technique is designed to diagnose COVID-19 following viral nucleic acid amplification and identification. Currently, two primary methods are under development to diagnose COVID-19, including lateral flow immunoassay, which is a common point-of-care (POC) diagnostic method to identify antibodies against SARS-CoV-2 in patient samples, and a molecular based assay [11]. It is thus necessary to describe various possible testing techniques to reach effective, user-friendly, and economical diagnostic techniques. This chapter discusses different tests with their ability of accuracy to detect the virus and concludes with authentic approaches to identify COVID-19 with valid precision.

2. Diagnostic methods

SARS-CoV-2 is a single-stranded, positive-sense RNA genome that is ~30,000 nucleotides in length. A total of 27 proteins are present in the genome of SARS CoV-2, including four structural proteins and an RNA-dependent RNA polymerase (RdRP). Focused molecular targets to diagnose SARS-CoV-2 through RT-PCR include structural proteins, including the spike (S), envelope (E), transmembrane (M), and nucleocapsid (N) glycoproteins.
these, nucleocapsid (N) and spike (S) proteins can act as immunodominant antigens to develop serologic assays for COVID-19 because antibodies (IgM and IgG) are detected in COVID-19 patients. To promptly identify the positive or suspected COVID-19 patients and to prevent further spread, it is crucial to be aware of a patient’s physical status, exposure and travel history to epidemic areas, and direct contact with or eating of wild animals. Fig. 18.1 shows the salient diagnostic techniques used to detect SARS-CoV-2, which are further discussed in detail.

2.1 Nucleic acid-based testing for SARS-CoV-2

2.1.1 Reverse transcription polymerase chain reaction

Nucleic acid-based identification is considered as a golden clinical method to diagnose COVID-19. In this method, RT-PCR kits convert RNA into complementary DNA (cDNA) strands and then specific regions of the cDNA are amplified [12]. There are two ways of performing RT-PCR (Fig. 18.2). In the one-step assay, reverse transcription and PCR amplification both occur together in one reaction. It produces fast and useable results for high-throughput analysis, but it is difficult to optimize the reverse transcription and amplification steps at the same time, which may reduce target amplification product.
While in the two-step assay, the reaction takes place successively in separate tubes. This test pattern has higher sensitivity as compared with the one-step assay because it requires more time and has need to optimize additional parameters [12]. Furthermore, controls should be selected cautiously to confirm the dependability of the assay and to eliminate experimental errors. The detailed mechanism of RT-PCR for COVID-19 has been discussed by focusing on the identification of envelope protein gene (E gene), nucleocapsid protein gene (N gene), and RdRP gene in the open reading frame, ORF1ab region and in SARS-related viral genome sequences.

2.1.1.1 Myths and protocols of RT-PCR for COVID-19
RT-PCR is primary method used to diagnose COVID-19 from respiratory samples (Fig. 18.3) [11]. Upper respiratory tract samples including nasopharyngeal and oropharyngeal swabs are mostly preferred, while lower respiratory tract samples are suggested only in case of productive cough, including expectorated sputum, bronchoalveolar lavage fluid, and tracheal aspirates [13]. The obvious amount of virus is dependent on the days after start of infection. COVID-19 virus can be detected preferably in sputum
and subsequently in nasal swabs in the first 14 days of infection; however, throat swabs do not provide effective results until 8 days after the start of symptoms [14,15]. This variation in the amount of virus or negative test result from respiratory samples does not exclude COVID-19, but this may occur due to inappropriate sampling procedures, lower viral load in the sample, or mutations in the viral genome [8,16].

The collected samples are put to extraction of RNA, which is in turn converted into cDNA synthesis by reverse transcription. For reverse transcription, the time required for thermal cycling is 10 min at 55°C, followed by 3 min at 95°C, then 45 cycles at 95°C for
15 s and 58°C for 30 s. Nucleic acid extraction is performed on 300-μL sample with final elution of 50 μL and pooled in 100-μL extract for concurrent use. The target sequences are then amplified from cDNA template. The conditions for amplification are 50°C for 15 min, 95°C for 3 min, then 45 cycles at 95°C for 15 s, and lastly at 60°C for 30 s [17,18]. The amplification speed depends upon cDNA quality and concentration, thus indicating the viral load in the sample. Amplification failure shows early status of disease or poor quality of cDNA. Thermocyclers (automated or semiautomated) are used to run RT-PCR [19,20].

2.1.1.2 Reverse transcription polymerase chain reaction linkage with COVID-19 diagnosis

COVID-19 can be diagnosed by using the three-step workflow, which includes first-line screening, confirmation, and discriminatory assays [18]. The envelope gene of the virus is the target for probes and primers for first-line screening, while confirmatory test target is RdRP assay [21]. In the latest one-step RT-PCR, N-region and ORF1b region of SARS-CoV RNA act as screening and confirmatory targets, respectively. These regions are present in all coronaviruses. So sequencing is required for differentiation among COVID-19 and closely related viruses [22]. Although RT-PCR technique is used as the gold standard for COVID-19 diagnosis, it has some disadvantages including shortage of PCR reagent kits, lack of PCR setup in all public hospitals, the ability to quantify valuable amount of virus present in the sample, and the lack of ability to detect the virus in recovered patients [18].

2.1.2 High-throughput sequencing technique

RT-PCR and high-throughput sequencing techniques are widely used to diagnose COVID-19. However, the confirmatory method to diagnose COVID-19 is high-throughput sequencing of the whole genome [23]. But it has limited application in clinical diagnosis because of its dependence on equipment and high cost. So RT-PCR is the most effective and preferable method used to identify pathogenic viruses in respiratory secretions and blood [18].

2.1.3 Isothermal nucleic acid amplification technique

Isothermal nucleic acid amplification technique is currently in its developing phase to detect SARS-CoV-2. This technique works at a single temperature and there is no need of particular laboratory instruments to examine diagnostic sensitivity just like PCR [24]. Loop-mediated isothermal amplification (LAMP) technique has been tested clinically to diagnose COVID-19 [25,26]. In this technique, DNA polymerase and 4 to 6 primers are used for binding on 6 discrete regions on a target genome. LAMP is a specific diagnostic technique because of the use of a large number of primers [27]. During this technique, the sample is inserted into the tube to amplify DNA, which is then further identified by examining turbidity, color, or fluorescence of the product [28]. This reaction takes place in <1 h at 60–65°C and can amplify ~75 copies/μL. This method has advantages of easy performance, simple understanding, and mainly it does not require a thermocycler for
working [27]. The major problem of LAMP is to maintain primers and reaction conditions. Briefly, a rapid LAMP assay can process 2 to 2.5 more clinical samples as compared with simple RT-PCR, so it can be used in high-throughput screening applications [28].

2.1.4 Emerging reverse transcription polymerase chain reaction-based diagnostic tests for COVID-19

Some emerging tests are being modified and developed for the detection of SARS-CoV-2 nucleic acid by using the same principle as RT-PCR. These techniques are discussed in the following.

2.1.4.1 Specific high-sensitivity enzymatic reporter unlocking

Specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) is a detection approach that uses Cas13a ribonuclease to sense RNA [29]. In this method, viral RNA is converted into cDNA by reverse transcription, followed by isothermal amplification of cDNA. Amplified cDNA is again converted into RNA by transcription, which is the target site for binding of Cas13a complexes [30]. Cas13a is activated after binding to the target, which cuts adjacent fluorophore-quencher probes to give rise to a fluorescent signal [31]. The SHERLOCK procedure has been modified and clinically tested to diagnose SARS-CoV-2 RNA sequence in a range between 10 and 100 copies per microliter [32,33].

2.1.4.2 Recombinase polymerase amplification

Due to COVID-19 emergency, the demand for instrument-free nucleic acid detection technology is increasing day by day for isothermal amplification. To overcome this issue, another emerging technique, recombinase polymerase amplification (RPA) can be used for COVID-19 detection. But it requires optimizing conditions and is unable to differentiate between single base pair differences in target sequences to identify pathogenicity. Recently, enzymes from CRISPR-Cas (CRISPR-associated proteins) systems have been used for specific, quick, sensitive, and transportable sensing of nucleic acids. These methods depend on Cas13 or Cas12 enzymes that show nonspecific endonuclease activity after binding to a specific target through programmable CRISPR RNAs [34]. When programmable specificity of Cas13 or Cas12 is attached with a reporter molecule that is responsible for the activation of enzymes upon identification of target, it causes specific and sensitive indication of the existence or amount of viral nucleic acid [29]. So it can be used as an emerging diagnostic technique for COVID-19 for rapid and portable sensing of nucleic acids.

2.1.4.3 Real-time nucleic acid sequence-based amplification

A multitarget real-time nucleic acid sequence-based amplification (NASBA) application is being developed to identify SARS-CoV-2 polymerase and nucleocapsid (N) genes. It can perform transcription-based amplification for RNA targets [35]. In case of SARS-CoV, nucleocapsid genes show more sensitivity than the polymerase targets, and the real-time NASBA test exhibits sensitivity equal to real-time RT-PCR. The major benefit of real-time
NASBA assay platform is that the reaction is isothermal, and it has the ability to test many common respiratory viruses instantaneously on the same plate. Incubation time of this test is also almost an hour less than that for RT-PCR tests [36].

2.1.4.4 Magnetic beads and quantum dot barcode
Multiplexing of isothermal amplification techniques can be done by using polymeric beads encrypted with distinctive visual signs like organic fluorescent molecules for barcoding. Various biomarkers can be developed from these barcodes to examine numerous analytes from a single patient sample in one reaction tube by improving its clinical sensitivity and specificity [37]. Barcoded-bead multiplex panels can be used to diagnose cystic fibrosis and respiratory diseases [38,39]. These tests are designed for use in the laboratory, but they can be used for POC testing of COVID-19 in the future. But the major problem is to design a specific display device operated by a distinct instrument to detect the codes. Scientists are doing research to overcome this drawback through the use of inorganic quantum dots for barcoding because it is excited by battery and the emission signal is controlled by a smartphone camera. Clinical specificity and sensitivity of 91% and 95%, respectively, has been discovered to diagnose hepatitis B in patients by using quantum dot barcodes after isothermal amplification by reverse polymerase [37]. Pieces of barcodes can be expressed into tablets to distribute reagents easily to diagnose coronaviruses, especially COVID-19, and sexually transmitted diseases [35].

2.2 X-ray-based testing for SARS-CoV-2

2.2.1 Computed tomographic scan
CT scan is radiographic technique used to build cross-sectional images of the tissue by passing an X-ray beam. These cross sections are reconstructed by measuring the attenuation coefficient of the X-ray beam passing through the scanned area of the object. Attenuation coefficient is the penetrating ability of a material by passing X-ray beam. In this scanning procedure, the emitter of X-rays rotates around the patient and the detector that is placed in completely reverse side measures the transmission of X-ray beam throughout the scanning to take images from different angles. Tomographic images of patients can be taken from computer using complex mathematical algorithms for image reconstruction [40]. Most of the COVID-19-infected patients have common epidemiologic background of lung-related issues, hence high-resolution chest CT scan has been performed for the assessment of pathologic changes happening in the area. It provides a clinical picture as well as some characteristic signs of COVID-19 pneumonia. During this method, the inspiratory phase of chest CT scan is performed at full inspiration breath hold. The technique uses different scanning parameters according to the detection of SARS-CoV-2, which are mentioned in Table 18.1 [41].

Abnormal attenuation of lung lobes with lower and peripheral distribution, ground-glass opacity, and enrichment of vasculature in the area are prominent [42]. Moreover, signs of lung consolidation, air trapping, and interlobular septal thickening can also be seen in the patients with COVID-19.
2.2.1.1 Comparative efficacy analysis of computed tomographic scan

CT is the most common technique used to diagnose and monitor patients suffering from COVID-19 pneumonia. It can be used as the first screening test for quick diagnosis of lung deformities in highly suspected persons whose early RT-PCR test result is negative. Radiographic imaging results can differ in patients according to their age, immunity level, stage of disease at the time of scanning, underlying diseases, and drug interferences. Chest X-ray images show manifold small patchy shadows and interstitial changes notable in the lung periphery during early stage of pneumonia [43]. However, bilateral multiple ground-glass opacity, infiltrating shadows, and pulmonary consolidation, with infrequent pleural effusion, can be present in X-ray images of severe cases [22]. But chest CT scan shows more clear pulmonary lesions as compared with X-ray examination, which include ground-glass opacity and segmental consolidation in both lungs, specifically in the lung periphery. Besides, multiple lobar lesions can also be present in both lungs of severely infected children. CT imaging analysis of 21 patients with COVID-19 showed 21% patients with normal CT scans, 57% with ground-glass opacity only, and 29% with ground-glass opacity and consolidation in both lungs [44]. In another study of confirmed COVID-19-infected persons, bilateral lung involvement has been reported on chest radiographs. Briefly, imaging analysis for COVID-19 shows results similar to SARS [45] and MERS, and this is not unexpected because SARS-CoV-2 is also a coronavirus [46].

2.2.2 Automatic detection of COVID-19 using X-ray images and deep convolutional neural networks (ResNet50, InceptionV3, and Inception-ResNetV2)

CT scan of the chest is an important method used to diagnose COVID-19 pneumonia. Artificial intelligence-based automated CT image analysis techniques have been developed to detect and quantify COVID-19 [47]. Timely forecast of COVID-19 patients is important to adopt safety measures for preventing disease spread to other people. For this purpose, a deep-learning-based system has been developed for automatic segmentation of all lung and infection sites using chest CT and to extract the graphical features of COVID-19 for early clinical diagnosis before pathogenic testing to save time required to diagnose the disease [32].

A deep convolutional neural network (CNN)-based pretrained transfer models (ResNet50, InceptionV3, and Inception-ResNetV2) and chest X-ray images are used for

| Scanning parameter | Specification |
|--------------------|---------------|
| Tube voltage       | 120 kV        |
| Tube current       | 110 mA        |
| Pitch              | 1.0           |
| Rotation time range| 0.5–0.75 s    |
| Slice thickness    | 5 mm (for axial and sagittal reconstruction, and for coronal reconstruction, it is 1.0–1.5 mm) |
automatic detection of COVID-19. Residual neural network (ResNet) model is a better quality version of CNN. It can solve problems by adding shortcuts between layers and it avoids the misrepresentation that normally occurs in case of deeper and complex network. Additionally, this model can become fast by using bottleneck blocks [33]. ResNet50 is a 50-layer network trained on the ImageNet dataset. ImageNet is an image database having greater than 14 million images related to higher than 20,000 categories made for image identification competitions [48]. However, InceptionV3 is a type of CNN model, which is made up of various convolution and maximum pooling steps, comprising a fully connected neural network at the last stage [49]. It is also trained with ImageNet dataset just like the ResNet50 model. The third model Inception ResNetV2 design trained with the ImageNet-2012 dataset requires input as $299 \times 299$ image and gives output by making a list of predictable class possibilities [50]. A deep transfer learning-based method is helpful to predict COVID-19 in patients automatically by using chest X-ray images obtained from both COVID-19 patients and normal individuals. It is proposed that results of the ResNet50 pretrained model are more accurate (98%) as compared with the other two models InceptionV3 (97%) and Inception-ResNetV2 (87%). So it will help doctors for the early diagnosis of COVID-19 and to make decisions regarding its treatment and prevention [32].

2.2.3 Sensitivity of chest computed tomography for COVID-19: comparison to RT-PCR

Early detection of COVID-19 is critical for control and treatment of the disease. Because CT scan and RT-PCR are being most commonly used to diagnose COVID-19 throughout the world, there is need to compare the sensitivity of these diagnostic techniques. While comparing with RT-PCR, CT imaging of chest can be considered as a more practical, reliable, and rapid method of diagnosis and for the assessment of COVID-19 particularly in an area of epidemic. Considering the results of RT-PCR as a reference in COVID-19 patients, the sensitivity, specificity, and accuracy of chest CT scan in determining COVID-19 has been reported to be up to 97%, 25%, and 68%, respectively [10].

According to the currently available diagnostic criteria for COVID-19, detection of the virus by RT-PCR technique is playing a proactive role in determining the isolation and hospitalization of individual patients. However, RT-PCR has insufficient stability and sensitivity and it takes a long time in processing, which is not beneficial to control the epidemic. In a study, 97% of patients who were diagnosed and confirmed by RT-PCR assay indicated positive results of CT imaging of chest [10,51]. Moreover, chest CT is a noninvasive and conventional imaging modality, having high speed and accuracy. It has been demonstrated that several re-examination of CT radiographs can accurately show the evolution of disease and monitor the effect of treatment [52].

2.3 Protein-detection-based testing for SARS-CoV-2

The protein detection approach for COVID-19 diagnosis assists to combat the pandemic as well as rapidly rolling out the heavily infected part of community [53]. Serologic tests
are diagnostic methods that are used to identify whether individuals have been exposed to a particular pathogen by identifying antigens and antibodies in patient’s sample. This testing for SARS-CoV-2 is showing high demand in order to better quantify the COVID-19-affected patients, including those who may be asymptomatic or have recovered. Keeping in view this approach for the diagnosis of SARS-CoV-1 and SARS-CoV-2 with sensitivities and specificities up to 100%, the serostatus approach for protein detection is appealing and reliable [54].

2.3.1 Enzyme-linked immunosorbent assay for IgG and IgM
Enzyme-linked immunosorbent assay (ELISA) is generally a laboratory-based test that can be both qualitative and quantitative. This is designed to detect the antibodies produced after initiation of active infection (IgG and IgM). After onset of symptoms, patients are reported to have neutralizing antibodies at day 14 [55]. IgM is produced by the body after initial exposure to the virus, while IgG appears later on [56]. Currently, serologic testing is not routinely offered as part of the screening or diagnosis of COVID-19, as no validated assays are available and these tests cannot detect the early status of the disease because antibody production requires 5–10 days after exposure to the virus [57,58].

In this technique, the sample used for antibody detection is whole blood, serum, or plasma. IgG and IgM antibodies in COVID-19 patient’s serum are detected by using SARS-CoV-2 Rp3 nucleocapsid protein. The surface of a 96-well plate is coated with recombinant proteins, and samples obtained from patients are then incubated with this coated protein for a specific time with subsequent washing. After this, antihuman IgG functionalized with horseradish peroxidase is added and permitted to bind to the target. The plate is again washed, and the substrate 3,3′,5,5′-tetramethylbenzidine is added. Product develops due to the reaction of peroxidase enzyme with the substrate and this produces a change in color (Fig. 18.4), which can be identified by a plate reader. As a result, positive signal develops due to packing in between the adsorbed nucleoprotein and antihuman IgG probe, which indicates the presence of anti-SARS-CoV-2 IgG in the sample. But in case of IgM test, antihuman IgM is adsorbed to the plate and an anti-Rp3 nucleocapsid probe is used. On testing SARS-CoV-2-positive patient samples with RT-PCR, higher antibody levels can be detected by using this technique [59].

2.3.2 Newly developed antibody-based rapid test kits
COVID-19 rapid test kit is a qualitative lateral flow assay used to simultaneously identify IgM and IgG antibodies of SARS-CoV-2 in patient’s sample. Recombinant SARS-CoV-2 antigen conjugated to colored particles is present in the test cassette. When adding the sample in the sample well of the cassette, antibodies present in the sample bind to the conjugated antigen and make colored antigen-antibody complexes that flow along the membrane to the test region where antihuman IgM and antihuman IgG have been immobilized onto the membrane. As a result, a colored line will appear in the respective antibody region. Till now, five antibody-based COVID-19 detection tests are available. Of these, BioMedomics and Surescreen rapid test kits are lateral flow immunoassays,
while Assay Genie rapid POC kit and VivaDiag COVID-19 IgG-IgM test kit are colloidal gold assays. Goldsite diagnostic kit is a time-resolved fluorescence immunoassay. The test procedure of all assays is same (Fig. 18.5). The sample used for antibody detection is whole blood, serum, or plasma. A few drops of the sample are pipetted onto the immunoassay, then two to three drops of buffer solution are added. After 10–15 min, results can be visualized as lines [19,55]. Only clinical data-based results of BioMedomics IgM-IgG rapid test showed 89% sensitivity and 91% specificity but no laboratory validation is available for this [60]. Antigen testing could be a good option, but no antigen test is available till now in the market [40].

2.3.3 Western blot
Western blot is used to identify the presence of a protein of interest by emitting a colored or fluorescent product due to the interaction of an active antibody with the viral protein. In the context of COVID-19, the spike (S) protein and nucleocapsid (N) protein act as primary proteins to cause infection in host cells. Hence, antibodies can be produced against these proteins in Western blot to diagnose COVID-19 [61].

2.3.4 DNA-assisted immunoassay
DNA-assisted immunoassay technique is now modified for COVID-19 diagnosis, which works on the principle of indirect detection of protein signal by amplifying DNA bound to gold nanoparticles. This assay can exhibit one to six times
more sensitivity as compared with conventional ELISA on the basis of the complexity of target and sample [62]. Hence, it is helpful to identify biomarkers at higher level in comparison to ELISA and it can be used to diagnose COVID-19 antibodies [59].

2.3.5 Immunofluorescence assay
Immunofluorescence assay has been used to detect SARS CoV-2 antigens present in the upper respiratory tract by using antiviral antibodies. If fluorescent label is conjugated to the antiviral antibody, it is called direct immunofluorescence, but if it conjugates to an antiantibody then it is called indirect immunofluorescence. The amount of fluorescence can be observed with ultraviolet light in this test. The amount of antibody bound to the antigen is directly associated with the amount of fluorescence produced [63].

2.3.6 Neutralization assay
Neutralization assay is used to detect active antibodies present in the patient serum to prevent viral growth in cell culture system, which is a laboratory-based method to culture SARS-CoV-2 growth like VeroE6 cells. Antibodies can block growth of virus by attaching to important entry protein of cell. This test can detect antibodies present even after recovery of COVID-19 [63].
2.4 Point-of-care-based testing for SARS-CoV-2

These techniques allow clinicians to detect infected patients in home-based settings, and there is no need to send samples to centralized facilities or the use of laboratory setup. POC testing techniques that diagnose COVID-19 are discussed in the following sections.

2.4.1 Loop-mediated isothermal amplification-based COVID-19 near-patient assay (Penn-RAMP)

A new closed-tube Penn-RAMP method has been developed for better sensitivity of COVID-19 diagnostic test. It is a two-stage isothermal double-stranded DNA amplification method that uses both RPA and LAMP techniques in a single tube [64]. To perform the COVID-19-specific LAMP test, first conserved COVID-19 sequences are selected by using Clustal X and according to this, LAMP primers are designed. This identification method can be made simpler by using leucocrystal violet dye as a chromogenic reagent, which can produce a clear, deep violet color change for easy observation with the naked eye. The whole diagnostic procedure is very simple because of the use of single tube for reaction. In this process, the RPA mixture is placed into the inside of the tube cover and the LAMP mixture (ratio of 1:9) is added within the tube itself. The tube is sealed and incubated at 38°C for 15–20 min in a thermal cycler to assist the RPA reaction. The tube is then inverted many times and incubated at 63°C for 40 min. The Penn-RAMP process gives better sensitivity than RT-PCR or LAMP alone. Even Penn-RAMP provides 100 times better sensitivity than a single LAMP test in case of less amount of virus. Additionally, Penn-RAMP assay can be performed in clinical or home setting, as compared to LAMP assay, because it requires less energy and is easy to perform due to no usage of sophisticated equipment [64].

2.4.2 Lateral flow assay

Lateral flow assay is one POC method that is under development for antigen detection of SARS-CoV-2 [65]. In this method, a membrane strip is covered with two lines: one line contains gold nanoparticle-antibody conjugates and the other contains capture antibodies. Blood sample of the patient is dropped on the membrane while proteins are drained by capillary action through the strip. On passing through the first line, the antigens make a complex by binding to the gold nanoparticle-antibody conjugates that further flow through the membrane to reach the second line for attachment with the capture antibodies and as a result produce a visible red or blue line. Red is shown by individual gold nanoparticles, while blue is an indicator of linkage of plasmon band in a solution containing clustered gold nanoparticles. By using this assay, clinical sensitivity of 82% for both IgM and IgG and clinical sensitivity, specificity, and accuracy of 57%, 100%, and 69%, respectively, for IgM and 81%, 100%, and 86%, respectively, for IgG have been reported [65].
2.4.3 Microfluidic devices

Microfluidic devices contain a palm-sized chip imprinted with very small channels and reaction chambers used to detect antibodies against viruses. The chip uses electrokinetic, capillary, vacuum, and other forces to mix and separate liquid samples. These chips are made up of polydimethyl sulfoxide, glass, or paper. Microfluidics are beneficial due to use of minute volume of sample, fast detection ability, and transportability [66]. These devices have been used to test 96 patients in Rwanda for the identification of antibodies against HIV and have shown 100% sensitivity and 87% specificity [67]. Hence, these microfluidic devices can be used to identify SARS-CoV-2 RNA or proteins in the future.

3. Conclusion

The ongoing COVID-19 pandemic has highlighted the significance of laboratory diagnostic techniques to identify coronavirus infections to control the spread, along with proper treatment of seriously infected patients, and to decrease its global economic impact. Clinical diagnosis is done on the basis of symptoms, exposure history, and characteristic manifestations of COVID-19 on chest CT imaging as well as positive results of RT-PCR. Besides CT scan and RT-PCR, serologic tests, rapid detection kits, and POC molecular techniques are also under development for the accurate diagnosis of SARS-CoV-2 infection. These tests will play a significant role in developing immediate diagnosis, patient management, and plans to control infection. Additionally, these tests will be simple, fast, and precise and can be used in local clinics and hospitals that lack sophisticated laboratory instrument.

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