INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

REVIEW ARTICLE

Diagnostic Tools for COVID-19

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OPEN ACCESS

Received: 13-06-2021
Accepted: 25-09-2022
Published: 14-11-2022

Citation: Sehgal S, Aggarwal S, Nagpal N, Sen A, Aafreen , Chakraborty A, Venugopal M, Bhalla R, Rawat Y (2022) Diagnostic Tools for COVID-19. Indian Journal of Science and Technology 15(42): 2240-2251. https://doi.org/10.17485/IJST/v15i42.1075

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Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objective: To review the current diagnostic methodology available for the Coronavirus Disease 2019 (COVID-19) caused by new Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). **Method:** This review critically analyses the merits and limitations of the recent COVID-19 diagnostics being used for disease prophylaxis and its mitigation. Multiple research papers from 2019-2022 were consulted. Many novel diagnostics have been included, such as LAMP, CRISPR, AI and other POC techniques, along with conventional RT-PCR and CT -SCAN. These have been compared based on principle, protocol, sensitivity, specificity, cost-effectiveness and their pros and cons. **Findings:** Mass Spectrometry and Loop mediated isothermal amplification (LAMP) were seen to be the most sensitive and specific. They were also very rapid. However, Mass Spectrometry is expensive, as it requires sophisticated instrumentation. LAMP on the other hand, does not require expensive machinery, and thus is a better choice. **Novelty:** This review has covered most of the techniques, which were not earlier covered in reviews. We have compiled all data in one manuscript for the ease of readers. We have also talked about the diagnostic protocols made in India and compared their sensitivities and specificities.

Keywords: COVID-19; Diagnostics; Coronavirus; Pandemic; Vaccine; Therapeutic

1 Introduction

Several infectious viral disease outbreaks such as SARS, Zika, MERS, Ebola and Influenza A (H1N1) have occurred throughout the world in the past two decades. A 7th coronavirus found to infect human first in China in 2019 was designated as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses. The virus has been proven to cause a severe illness called Coronavirus Disease 2019 (COVID-19)[(1)]. Humans and vertebrates are infected by coronaviruses. Various organs including lungs, intestine, liver, and brain of hosts are adversely affected by coronaviruses[(2)]. The virus is mutating fast and several variants with multiple mutations have been found in different regions around the world[(3)]. These

mutations have been categorized as variant of interest, variant of concern and variant of high consequences by CDC [4].

As the coronavirus cases surge across the globe, speedy and precise diagnosis of COVID-19 is very significant in suppressing the outspread of disease and its better management. The various diagnostic methods that are being used for COVID-19 are rapid antigen tests [5], RT-PCR [6], and CT scans [7].

The positive test result relies heavily on the moment at which the test was administered. Neither serological tests nor molecular tests are useful for virus detection in the first 5-6 days of suspected infection as the virus is said to be in the eclipse phase, hence enough copies of the viral genome are not present that can be detected by molecular assays. Antibodies and viral proteins are also not detectable by serological methods; therefore, prior to occurrence of symptoms, the chance of detecting the presence of virus, by molecular techniques is low. Molecular assays performed two weeks post suspected infection, considering occurrence of symptoms, on nasopharyngeal swabs or bronchoalveolar lavage fluid (BALF) have an increased probability of testing positive because of active multiplication of the pathogen. However, the possibility of positive response declines with time due to curing of the infection. In serological assays, the antibodies, in particular IgM and IgG show positivity at around 30 days after the suspected infection and the abundance of these proteins persists for a large span of time. IgM levels lessen drastically after approximately 1.5 months from occurrence of symptoms. The reports of increase in viral proteins and mucosal IgA during the early phase of the infection suggested their testing for early detection of corona infection [8]. Some ELISA tests have been devised to detect the presence of IgA antibodies.

Since early detection is a more favourable option than mitigation of the disease, there needs to be methods of diagnosis that are rapid, efficient, sensitive, specific, cost effective, and easily available. They should have an ability to detect and distinguish between multiple strains and newly emergent mutants of the virus. In this review, we have compiled all the recent developments in COVID-19 diagnosis, and compared them based on principle, protocol, sensitivity, specificity, cost-effectiveness and their pros and cons.

2 Conventional Diagnostic Methods of COVID -19

Rapid antigen test, RT-PCR and CT scan are the most commonly used methods for diagnosis of Covid-19

2.1 Rapid Antigen Test

The standard testing procedure for the detection of SARS-CoV-2 has been the RT-PCR test. However due to long processing time and unavailability of enough testing kits, other testing methods have been devised and one such method is rapid antigen testing. Rapid antigen testing is used usually for point-of-care testing (POCT), because of its easy performance and result interpretation without the use of specialized equipment and much training of the personnel. It is also not that expensive and provides results faster [9]. The test detects current infections and the specimen used is nasal/ nasopharyngeal. In case a person has received a positive result for the test within the first 5 days of symptoms, it may be understood as the person has a coronavirus-2 infection and contracted a heavy viral load.

The specificity of the test was reported to be 99.8%, which is high. Sensitivity of the test, which is the ability to detect true positives, was 71.4%. The range depends upon the viral load carried by the patient and was more in symptomatic as compared to asymptomatic patients. The higher the capability of the test to detect true negatives, the more reliable is the positive result [10]. The test is used for the detection of protein fragments that are specific to coronavirus and they can be conducted in doctors' clinics or in a hospital. The turnaround time of the results is generally very fast, and the results may be obtained within 15 minutes.

The test depends on binding of SARS- CoV-2 antigen in the specimen with the antibodies coated on the test strip which becomes visible by a color reaction. The samples are usually collected in the form of nasopharyngeal swabs, but many tests are being developed which can use other samples such as saliva, oral fluids etc. The antigen usually detected is nucleocapsid protein due to its high abundance. Antigens-detecting rapid diagnostic test kits usually consist of strips with sample and buffer wells covered by nitrocellulose membrane and two pre-coated lines are present- control and test lines. Both the lines are visible before applying any specimen. The test line is coated with coronavirus-2 specific monoclonal antibodies coupled with color particles like gold nanoparticles [11].

During the test, the antigen reacts with chromophore-conjugated monoclonal antibodies to form colored antigen-antibody complex. This complex travels by capillary action and is captured by the antibodies on the test line producing blue or red color. A colored test line will appear if antigen is present in the specimen and the control line would appear in case the test has been carried out properly and if the reagents used in the test are working. The intensity of color is directly related to the quantity of SARS Co-V 2 antigen in the sample [11]. People that are tested with rapid antigen testing for coronavirus can be divided into 3 groups:

- People that are symptomatic

Those people that show the presence of antigen have SARS-CoV-2 infection. People showing negative results are further tested using NAAT. Positive NAAT results suggest people are infected with the virus, whereas those showing a negative NAAT are further divided into 2 categories - those having known contact with an infected person and those having no known contact with an uninfected person. Those having known contact are said to have no current evidence of virus, but infection cannot be ruled out and those having no known contact are said not to be infected by the virus.

- People that are asymptomatic and had close contact with an infected person

Those people that show the presence of antigen are further tested using NAAT. Those people that show a positive NAAT result are infected with the virus and those showing a negative NAAT result are said to have no current evidence of virus, but infection cannot be ruled out. Those people that show a negative rapid antigen test are said to have no evidence of the virus, but infection cannot be ruled out.

- People that are asymptomatic and had no known contact with an infected person

Those people that show the presence of antigen should be further confirmed using NAAT[12]. Those people that show a positive NAAT result have the virus infection and those shows a negative NAAT are not infected by the virus. Those people that show a negative rapid antigen test are not infected with the virus.

The technology on which rapid antigen testing is based is called lateral flow immune-chromatography (LFIC). It provides advantages over the other technologies that utilize molecular assays, as they are easy to operate, cost effective and require no instrumentation. An increased number of these assays are being used these days[13].

2.1.1 ***Merits and Limitations***

The test is cost effective, and results are available in short periods of time. No intricate equipment is required. No sensitive and specialized environments are required to conduct the test. The study of the output of the test does not require skilled professionals. These tests are used in economically weak countries where NAAT is unavailable and for large scale testing especially in hotspots[14].

However, there are high chances of false negatives because of low sensitivity. The test result output is stable for only an hour and the interpretation needs to be made within that hour itself[15].

2.2 RT-PCR

RT-PCR or Reverse Transcriptase- Polymerase Chain Reaction- is nucleic acid amplification test (NAAT). It is the widely accepted standard diagnostic test for early virus detection and confirmation of virus infection. Three regions of coronavirus that are commonly amplified during the test include conserved sequences present in the envelope protein (E gene), nucleocapsid protein (N gene) and the RNA dependent RNA polymerase (RdRP gene) on the ORF1ab, which is basically the open reading frame. High analytical sensitivity is reported with RdRP and E gene while the N gene has poor analytical sensitivity[11].

The samples collected for RT-PCR include nasopharyngeal and oropharyngeal swabs and respiratory secretions in which the viral RNA can be detected during acute phases of infections. The lower respiratory samples such as sputum, tracheal aspirates, and bronchial alveolar lavage can also be tested by RT-PCR. Viral RNA can be detected in throat swabs within a week after beginning of symptoms, while in sputum and nasal swabs it remains detectable for the first 2 weeks days after symptoms appearance[16].

The number of cycles required to amplify viral RNA so that it can be detected is known as Cycle Threshold (Ct) and is proportionate to the quantity of viral RNA in the sample. If Ct value is less than 35, then PCR result is considered positive as per ICMR recommendations. In patients with Ct value > 35, possibility of culturing virus declines to 6% after 10 days of onset of symptoms. However, as Ct values are not standardized and cannot be compared among various RT-PCR assays, Ct value is not recommended for use in disease management. The bias may arise because of different sampling times, transportation conditions, test protocol used, and expertise of laboratory workers[17].

A three-step protocol involves screening in which all SARS related viruses are detected by targeting the E gene. Two distinct types of primers and probes are used for detecting the RdRP gene if the result of screening is positive. In case these tests also turn out positive, one probe sequence is used to do the discriminatory tests. Another protocol involves screening by detection of N gene followed by confirmation with detection of ORF1ab gene. If N gene test comes out to be positive and ORF1ab gene

test comes out to be negative, then it can only be confirmed by other diagnostic methods such as antibody tests [11]. Prolonged viral RNA detection after recovery may occur due to disease severity and variable duration of viral RNA shedding.

RT-PCR may be carried in one or multiple steps. In the former one, both the steps i.e., reverse transcription followed by amplification are conducted in a one tube, producing rapid and high throughput outcome. In two- step assay, reverse transcription and amplification are performed in different test tubes and thus giving better flexibility and more sensitivity than the single step assay. We need a lesser quantity of starter culture, but it has been proven to be prone to pipetting errors and cross-contamination [18]. For verification, a repeat RT-PCR test is recommended. Repeat test is also required in clinically suspicious cases if the test result is negative. Being less sensitive, negative antigen tests are required to be reconfirmed with NAAT.

RT - PCR has false negative rates ranging in between 30-40 %. The test has such elevated false negatives due to problems in sample collection, transportation, and errors during processing of sample. A test may also be negative in case the amount of virus is on the lower side, however in subsequent RT - PCR tests it may give a positive outcome. RT - PCR shows maximum efficacy between the 5th and 7th day of the person contracting the infection. In underdeveloped areas, the time between sample collection and report may be as long as 48 hours [19].

Besides RT-PCR, other NAAT tests like CRISPR based assays, next generation sequencing, isothermal amplifications etc. permitted to be used as EUA (emergency use authorization) by FDA [20].

2.2.1 ***Merits and Limitations***

Advantages of the RT-PCR test include high sensitivity and high specificity. RT-PCR are constantly being evolved with more automated 'sample-to-answer' molecular diagnostic platforms which eliminate the need of complex laboratory instruments and has rapid turnaround time. Saliva based tests are being developed as a non-invasive test to prevent shortage of collection swabs and personal protective equipment [9].

However, it is an expensive method that is susceptible to contamination and takes a longer time. False negatives may occur if testing is done too early or too late in the infection cycle. Sensitivity of the test also varies with the sample type [21]. There is a requirement of expensive laboratory instruments and highly skilled laboratory personnel. Moreover, supply of personal protective equipment, collection swabs, and extraction kits may be limited due to increased need of testing.

2.3 CT Scan

CT scans (Computed Tomography) also known as CAT scans (Computerized Axial Tomography) are a type of medical imaging procedure that uses a machine producing X rays to create a cross-sectional view of anybody part (brain, lungs, spine, etc.). This method is highly used as it is pain free, non-invasive and a relatively fast way for examination of organ systems, bones, etc. by medical practitioners [11].

When a CT scan is performed, the patient lies on a bed that moves through a gantry - a doughnut like ring - which consists of an X ray tube that shoots beams of radiation while rotating around the person under examination. Detectors are placed opposite to the source of radiation and detect the X rays. Multiple 2-dimensional slices gather to create a well-rounded, complete, and sophisticated 3-dimensional image of the part of the body to be examined to narrow down to the existing problem. For increased efficiency and to find out the problem, the person under observation may be administered a contrasting compound- solutions composed of opposing compounds like barium / iodine- either directly (orally or rectally) or injected into the bloodstream.

CT scans performed on multiple people affected by the virus have shown white coloured patches in the right lower lobe of the lungs, also called a nodular glass lesion. The scans are used to see if hazy, ground glass like patches is present in the lungs as these spots are said to be associated with coronavirus infection [22,23]. According to researchers, for efficient treatment and isolation of patients for safety of public health, early diagnosis is imperative. CT scans are preferred over other forms of testing due to the alarming rate of false negatives and day-long wait for results associated with other testing procedures [24].

Different countries have differing views on utilizing CT scan for diagnosing COVID -19. While China used CT scans increasingly for the detection of the infection, the US on the other hand has used the diagnostic tool in a sparing manner due to concerns over contamination of the machine. China did not face this problem as the machines were cleaned thoroughly before each patient was tested, leading to avoidance of infecting the medical practitioners, health care workers or other patients being tested. According to researchers, CT scan is said to be much safer than the swabs used as these swabs often cause extreme coughing, which can lead to dispersion of viral particles into the air, posing a threat to the health care workers. Many viral infections such Influenza, CMV and other coronaviruses, can resemble the CT scan results from COVID-19 infected patients.

Research has shown that the primary diagnostic test - RT-PCR has a lower sensitivity than the sensitivity of CT scan in detection of the virus [25]. The sensitivity was reported to be 98% in CT scan versus 71% in RT-PCR [26]. Mehrabi et al. (2020) suggested the use of CT scan results together with clinical data for effective disease management [27]. For final diagnosis and management of the disease, it is advisable to conduct both RT-PCR test and CT scan [28].

A CT scan cannot be used alone for confirmation of COVID-19[24]. It can only show the signs of an infection, which may not necessarily be COVID-19. Many people infected with the virus show clear CT scans. Thus, CT scans can be misleading and make infected people believe they are healthy, even though they have the potential to spread the virus. Cleaning of machines is imperative, and the machine surface requires disinfection with dilute hypochlorite solution every single time the machine has been used to test a possible infected person. However, the chances of obtaining maximum sterilization are feeble. Patients with the potential viral infection increase the risk of infecting others while going to and fro from the room having the scanning machine. It is not economical to solely keep aside CT scan machines for COVID-19 diagnosis. Thus, facilities with fewer CT scanners might find it difficult to manage the use of machines for COVID-19 detection and for other diseases. Meta-analysis done by Garg et al (2021) suggested that primary diagnosis should not be based on CT scan[29]. The low dose CT scan is recommended for reliable diagnosis of COVID-19 associated pneumonia[30]. More investigations are required to specify the role of CT scan in children[31].

2.3.1 ***Merits and Limitations***

CT scan is a non-invasive, less time-consuming test with high sensitivity. Apart from being used as a diagnostic tool, it can also be used to study progression of the disease[32,33]. However, use is limited by being an expensive procedure, requiring technical expertise, not specific to COVID-19 and exposure to radiation can result in adverse outcomes in the future[34].

2.4 Serological Tests

Serological tests can be performed to find the specific antibodies against coronavirus. The test is less useful for acute patients as a sufficient level of antibodies for detection develops after several days (usually 10-14 days). It will help in identifying the individuals who had earlier infection or current patients having symptoms for 3-4 weeks. As recommended by IDSA (Infectious Disease Society of America), tests based on IgG or total antibody detection are more accurate[35]. EAU status has been granted to several serological tests by FDA[36].

Mostly indirect ELISA based colorimetric tests are used to identify and quantify various SARS - CoV 2 specific antibodies like IgG, IgA, or IgM in the blood sample. This helps in obtaining increasingly specific and sensitive outputs within 1 - 5 hours. Intensity of colorimetric reaction is indicative of the quantity of antibodies. The tests can be used on patients tested negative for infection in a molecular assay to make sure that the patient has acquired immunocompetence against SARS - CoV. The sandwich-based ELISA is used for the identification of viral spike proteins[8].

2.4.1 ***Merits and Limitations***

The tests are rapid and sensitive. However, these tests may not confirm the infectious state and need to perform molecular tests to confirm the infections.

Various diagnostic tests that can be used for COVID-19 diagnosis are listed in Table 1.

Table 1. Diagnostic Tests for Covid-19

Test	Principle	Methodology	Advantages	Disadvantages
Rapid Antigen testing	Immunoassay directly detect SARS-CoV-2 spike proteins or glycans[37]	Nasopharyngeal or nasal specimens, directly placed into assay's reagent	Low cost Good method for testing in community and remote areas Results obtained in 15 minutes	Sensitivity is low, so results confirmatory tests usually (RT- PCR) need to be used Works best at early phases of infection when viral load is high

Continued on next page

Table 1 continued

Antibody Testing	Antibody detection indicates exposure to SARS-CoV-2 [18,38]. Detection of IgM antibodies implies recent exposure to virus whereas detection of IgG antibodies in the absence of IgM antibodies shows prior exposure to the virus.	Blood sample is added to virus antigen coated microtiter plate. Antibodies in blood bind to antigen and detected by color production on addition of a substrate solution. Detection methods are several viz. Lab based (ELISA) & CLIA (Chemiluminescence immunoassay) or Point-of-care based (Lateral Flow Assay (LFA) [11] Complement Fixation Test- Antigen-antibody complex formed on Antibody reaction with antigen binds with complement. Then lysis or non-lysis of antibody sensitized cells by complement haemolytic reaction, is measured.	Detect antigen. Very specific as can detect even picogram of antigens 96-well plate assay can carried out in 384- well format Easy to perform Help in identifying plasma donors for therapy Used in confirming body response to vaccine Identify individuals previously infected by SAR-CoV	Results must be read readily Give information regarding absence/ presence or amount of antibody in the sample Results obtained are not always correct CFA has low sensitivity and can carry a risk of bias due to selection of patients
RT-PCR	NAAT, involving 2 steps- reverse transcription and amplification [11]	RNA extracted from the sample is reverse transcribed to DNA followed by repeated RT-PCR cycles for amplification to detect viral nucleic acid	Very sensitive, reliable Early virus detection Most accurate method Lower potential for contamination	Does not detect past infections Time-taking Costly test
Mass Spectroscopy (MS)	Recognizes nucleoprotein peptides of SARS-CoV 2 [39]	Precipitation and digestion of proteins present in the highly diluted gargle sample by acetone and trypsin succeeded by targeted MS analysis	High sensitivity Extremely quick method (15 minutes)	Alternatives like triple-quadrupole instruments required for larger quantities of viral protein
LAMP	Based on isothermal nucleic acid amplification [18]	Require up to six different DNA primers, reverse transcriptase & DNA polymerase. It utilizes 6 distinct target sequences for simultaneously amplification in a single reaction.	Highly sensitive and specific Cost effective, Rapid Expensive equipment not required	Susceptible to false positive reactions Less versatile increased primer - primer interactions.
CT scan	Creates a 3D image of the lungs [24,32,33]	Multiple X rays are used to form a cross sectional view of the lungs slice by slice	More accurate Relatively faster	False positives

3 Novel methods used in the diagnosis of COVID -19

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

LAMP technique amplifies target DNA within 30 minutes at a temperature of about 65°C. The technique shows high specificity and is being used for the detection of various pathogens. The time required for amplification has been shortened to half by using two loop primers instead of 4 or 6 primers as required initially in the LAMP. RT-LAMP is now employed for SARS-CoV-2 diagnosis by combining LAMP with reverse transcription at 65°C. The result is obtained in 20 minutes simply as a colour change that is visible to the naked eye [37].

It is a cost-effective alternative to the standard polymerase chain reaction, as it does not need costly thermocycler. This method can synthesize up to 10^9 copies of sample gene in less than 60 minutes. The amplification reaction takes place in a single tube at around 65^O by using 2-3 differing primers that are gene specific along with strand displacement polymerase enzymes [38].

3.2 CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)

CRISPR technology is an effective tool for editing the genomes to change the DNA sequences and alter the gene characteristics. Besides rectifying genetic defects, CRISPR and Cas proteins have found their applications in analyzing and treating infections by RNA viruses [39]. The possible role of the technique is suggested to be in diagnosis and treatment of COVID -19 infections. Diagnostic methods utilizing CRISPR-Cas technology are reported to be sensitive, rapid, and accurate [40].

Many of these tests including the AIOD-CRISPR system are based on Cas 12 enzymes, which recognizes viral specific sequences and produces fluorescence on cleavage. The test can be performed in a single tube [41].

Another diagnostic test CONAN (Cas3-Operated nucleic acid detectioN) utilized the Cas-3 based detection in combination with RT-LAMP. After performance of RT-LAMP on the isolated RNA from the samples, amplicons cleaved by Cas-3 releases fluorescence signals. The sensitivity and specificity of 90% and 95% respectively was comparable with RT-PCR. It is for the first time Cas- 3 was used in detection of pathogens [41,42].

CRISPER/Cas 13 based technique SHERLOCK (Specific high-sensitivity enzymatic reporter unlocking) is another CRISPER based diagnostic tool for COVID- 19 detection devised in 2017 [43]. CRISPR/Cas-13 system seems to have a potential in therapy of COVID-19 as Cas-13 can cleave the genome of virus and block the protein expression within the cell. [44]

3.2.1 ***Merits and Limitations***

The technique is efficient, easy to use, and needs minimal expense on infrastructure so it is easy to use even in asset restricted regions. The effectiveness of the technique in diagnosis is, however, influenced by the presence of RNAase [45]. The use of CRISPR-Cas in therapeutics is restricted by the availability of suitable delivery systems [44].

3.3 AI (Artificial Intelligence)

AI is also coming up as a worthy detection option for COVID-19 diagnosis and has been taken up by radiologists for recognizing the disease. It detects infection through pattern recognition algorithms, however information obtained solely from radiological data might not completely rule out infection with the virus.

AI is applied for identification of infectious microorganisms through species-specific volatile organic compounds by breath biochemistry. The same test used for detection of COVID-19 gave sensitivity range of 82.4 - 100 % and specificity from 54 - 90 %. Humidity, food habits and background contamination might influence and result in false positives. Even though these AI based breath tests have a lower specificity compared to other tests and are yet to be validated, these can turn out to be cost effective, quick, and non-invasive tools for ruling out COVID -19 in the coming future [46].

Integration of machine learning with applications available on the smartphone has been suggested for self-testing of the COVID-19 infection by utilizing sounds of breathing or coughs. These sounds that appear as acoustic patterns are recognized by the integrated system for early detection of the virus. This system, however, has to be tested further and credible data needs to be collected to validate the same.

3.4 Point of Care Testing

Several assays are available for POC testing that produced the results in a short time and may be performed for mass screening [47].

Xpert Xpress SARS-CoV-2 is the popular point of care test to detect the pathogen, using N2 and E genes as a target in about 45 minutes through the GenXpert bench top system. The specimens present in the upper respiratory systems require less than one minute for preparation. The test may be modified to identify variants that we may be encounter in near future [48].

An Isothermal Nucleic Acid Amplification Test (INAAT) - ID NOW COVID-19 test detects RNA of the virus from samples collected from the upper respiratory system by amplifying the RdRp gene in few minutes to a detectable level (125 genome equivalents/ml). The test has the overall sensitivity and specificity of 80.4 % and 95.9 % respectively [48].

CovidNudge is another fully automated cartridge based multiplex RT-PCR test having a running time of less than 90 minutes from start to finish. This test utilizes dry NP swabs and targets 7 genes of the virus. False negatives decreased by including the host gene as a control. The test has 94% sensitivity and 100% specificity. CovidNudge and ID NOW COVID-19 analyze one sample per run.

TrueNat is a newly devised test that adapts the technology of a test used for detection of pulmonary tuberculosis. It is a portable PCR that is chip based. It is developed to be a quick and cost-effective tool to detect the infection in undeveloped nations. This test has shown to have 100% specificity as well as sensitivity. Its cost effectiveness, small size, easy to use and interpretation make it a favorable option for testing of the pathogen in underdeveloped countries.

Non-material-based biosensors have also been devised to find nucleic acid sequences of SARS - CoV - 2 [46,49].

3.5 Next Generation Sequencing (NGS)

NGS is generally used for finding novel variants & investigating their epidemiology and not as a diagnostic test. Few organizations, however, have developed commercial kits based on next generation sequencing of SARS - CoV 2 genome. The method involves fragmentation of the sample followed by amplification and sequencing of each fragment. The innumerable small fragments then joined together to generate readout of the genome. Even the full length of unknown or poorly characterized

viral genome can be reconstructed. The technique provides an insight about mutations occurring in virus and plays an imperative role in timely detection of the pathogen[50]. The major obstacle of using this approach is that it is expensive and requires highly specialized technology & extensively trained professionals[8]. Also, this technique is not very rapid. NGS is based on the sample to be tested to be fragmented into fragment libraries. Each of these fragments are independently amplified and sequenced. This leads to the accumulation of innumerable small pieces or fragments that can be joined together that leads to generation of a readout of the genome.

The technique can be used to reconstruct full length viral genomes that are not known or not characterized properly. Hence plays an imperative role in timely detection of the pathogen and provides unique insights such as if the genome of the pathogen has undergone mutations or not.

3.6 Fecal Tests

Increased number of cases has been reported where there is increased persistence of the virus in the fecal sample even though the samples collected from nasopharyngeal swabs came out to be negative. The virus can be detected in fecal samples up to 4 weeks post infection. Healthcare workers have a higher risk of infection because of exposure to fecal matter, especially in procedures that generate aerosols[48].

3.7 Digital PCR [dPCR]

Asymptomatic patients usually have a low viral load, and as a result the RT-qPCR tests are not sensitive enough to detect virus in their samples. Hence, newer approaches such as digital PCR can be used to detect such low loads of virus. The dPCR is more sensitive as it separates the sample into many sub-reactions before the process of amplification. Standard curves or reference genes are not a prerequisite for this technique and this technique is resistant to factors like specific template amplification inhibitors. The digital polymerase chain reaction can be divided into 3 further subcategories - droplet based, chip based and microfluidic digital PCR. dPCR is considered a point of care test as it is convenient and gives quicker results. The disadvantage of this technique is that it requires high end equipment[50].

3.8 Nanomaterial-Based Techniques

Rapid and precise detection of viruses can be achieved by use of different nanomaterial-based procedures, wherein the viral RNA is extracted by coprecipitation via magnetic nanoparticles followed by subsequent polyamine ester functionalization. This preparation can then be used for up to 50,000 diagnostic tests. The binding dynamics of the viral S protein to the human ACE2 and the subsequent viral internalization can be detected using Quantum dots (QDs) owing to their photostability, relatively small size, and ability of surface functionalization with biomolecules for FRET biosensors. For example, AuNPs (gold nanoparticles) absorb electromagnetic radiation in the visible range of the spectrum. When conjugated with thiol-modified antisense oligonucleotides, a visible colorimetric change occurs, that can be used to detect the N-gene RNA of SARS-CoV-2. This method of diagnosis only takes 10 minutes to be performed. The method is also very sensitive, with the ability to detect as low as 0.18 ng μl^{-1} of RNA[51].

Another sensor combines the photothermal effect of gold and silver nanoparticles with localized surface plasmon resonance sensing transduction. Diagnosis is performed on two-dimensional sheets called gold nano islands which contain the complementary DNA receptors that can hybridize to nucleic acids of SARS-CoV-2. It can detect envelope (E), RdRp-COVID and F1ab-COVID genes from SARS-CoV-2. This type of biosensor can detect as low as 0.22 pM of viral protein, and thus has significantly lower false-positive results. These techniques allow portable and rapid diagnosis of SARS-CoV-2.

3.9 Other Tests

Colorimetric analysis - In recent times, various tests used for diagnosis of COVID 19 such as RT - PCR, CRISPR/Cas9, etc. are combined with colorimetric probing systems leading to development of point of care tests that decrease detection times and do not require high end equipment[52].

Nicking and extension chain reaction system-based amplification (NESBA)- It is a unique isothermal nucleic acid amplification method where a nicking recognition sequence is added to the primer enabling amplification aided by the nicking enzyme[53].

Mass Spectrometry of nasopharyngeal and salivary samples is being developed as a diagnostic tool for detecting viral peptides or proteins of SARS-CoV2, based on mass and charge. The novel host breath test measures patterns of volatile organic compounds that are released upon infection. Infection can also be measured in sebum samples via the host skin test, wherein

SARS-CoV2 infection associated dyslipidemia is measured [54].

4 The Indian Scenario

In India, suspected patients are selected based on WHO or national guidelines. The required nasal, nasopharyngeal, blood or sera samples have been collected from the patients during the early phase of infection by skilled personnel wearing personal protective equipment (PPE). The transportation of these collected samples to virology laboratories should be at lower temperatures. The samples are processed, and a diagnostic assay is performed as soon as the sample is in the laboratory. In India, COVID-19 diagnosis takes place in more than hundred virology labs that have been approved by NABL (National Accreditation Board for Testing and Calibration Laboratories). COVID-19 is diagnosed by RT-PCR, following the protocols given by WHO and ICMR. Table 2 summarizes the diagnostic tests developed in India.

Table 2. DiagnosticTests Developed in India

Tests	Principle	Methodology	Advantages
Feluda test Developed by (CSIR-IGIB), New Delhi, India	A paper strip test using CRISPR/Cas9 technology	RNA from the sample is reverse transcribed, amplified, and biotinylated. This is incubated with FnCas9 gRNA (labelled with FAM). RNP complex bound to the labelled substrate. The reaction is detected as a positive test line on adding anti-Fam antibodies coupled to gold nanoparticles [55]	Cheap Easy to interpret -No trained personnel required. Quick (45 minutes) Sensitivity is 96% Specificity is 98%
Covid Kavach Merilisa-Developed by National Institute of Virology, Pune, India	Indirect ELISA for detecting SARS-Cov specific antibody [56]	Sample added to virus coated wells followed by addition of anti IgG HRP. Colour develops occur on adding chromogenic substrate (TMB/H2O2)	90 samples can be tested together Assay time- 130 minutes Specificity is 100% Sensitivity is 93.3% Help in identification of patients with adaptive IR
Chitra Gene LAMP-N Developed by Sree Chitra Tirunal Institute for Medical Sciences and Technology, Kerala, India	Based on RT-LAMP	It identifies two regions of the N-gene and the result observed by change in fluorescence [56,57]	It is a confirmatory test No need for a screening test Total time taken is 2 hours In a single run, 30 samples can be tested.
CoviSelf Mylab Discovery Solutions, Pune, India	First self-use rapid antigen test (RAT) kit for testing at home. It does not have the option of amplifying any genetic material in the swab sample [58]	Test strip is coated with specific antibodies to the CoV-2 antigen. Nasal swab is mixed in a pre-filled extraction tube before pouring on the test strip. Two lines will appear on the test strip indicating if antigen is present.	Results are out fast in 15 minutes Low cost (Rs. 250/- per kit). High specificity (a positive test does not need to be re-confirmed through other tests), but a slightly lower sensitivity (about 20-30 % per cent tests false negatives)

5 Future Prospects

The countries are experiencing different waves of the disease outbreak. Rapid mutations in the SARS-CoV impact the effectiveness of the tests [59]. Many NAAT are unable to detect the mutated S gene. Devising newer earlier detecting diagnostic methods that are economical, more sensitive, and specific are required. A group of researchers created an AI (Artificial Intelligence) pre-screening test, in which COVID-19 patients can be diagnosed based on a forced-cough recording. It was seen to discriminate 98.5% of COVID-19 positive patients and 100% of asymptomatic ones as well, at basically zero cost [60]. Even research is going on tests based on cell-mediated immunity like interferon gamma release assays [55]. Cadegiani et al (2020) reported Andro CoV clinical scoring as a sensitive simple test for diagnosing Covid-19 [56]. Devising novel more specific rapid methods and optimization of the existing tests is required for increasing the sensitivity and detection of a range of variants emerging of SARS-CoV-2.

6 Conclusion

Accurate SARS-CoV-2 diagnosis is possible only by laboratory-based assays. In the initial phase, coronavirus diagnosis can be by isolation from virus, serological methods, and electron microscopy. After genome sequencing, molecular assays are done. Isolation of viruses is time-consuming and requires high levels of containment. Electron microscopy is expensive and needs expertise. In today's era, molecular diagnostic assays, such as CT-Scan, RT-PCR, and Rapid Antigen Testing are used, which have high specificity and sensitivity and are rapid and cheap. COVID-19 is detected mostly by performing Real-time PCR. Also, numbers of alternative methods were devised with varied sensitivity and specificity. It is needed to continuously improve the existing diagnostic methods and develop novel ones with high sensitivities and specificities to deal effectively with emergence of new variants of corona virus and with such pandemics in future.

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