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Sensors International 3 (2022) 100197

Contents lists available at ScienceDirect

Sensors International

journal homepage: www.keaipublishing.com/en/journals/sensors-international

Analytical performances of different diagnostic methods for SARS-CoV-2 virus - A review

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ARTICLE INFO

Keywords:
Covid-19
SARS-CoV-2
RT-PCR
Biosensor
Lateral flow immunoassay

ABSTRACT

Covid-19 is a dreadful pandemic of the 21st century that has created fear among people, affected the whole world, and taken thousands of lives. It infects the respiratory system and causes flu-type symptoms. According to the WHO reports, 2,082,745 deaths and 96,267,473 confirmed cases were perceived all around the globe till January 22, 2021. The significant roots of transmission are inhalation and direct contact with the infected surface. Its incubation period is 2–14 days and remains asymptomatic in most people. However, no treatment and vaccine are available for the people, so preventive measures like social distancing, wearing personal protective equipment (PPE), and frequent hand-washing are the practical and only options for cure. It has affected every sector of the world, whether it is trade or health all around the world. There is high demand for diagnostic tools as high-scale and expeditious testing is crucial for controlling disease spread; thus, detection methods play an essential role. Like flu, Covid-19 is also detected through RT-PCR, as the World Health Organization (WHO) suggested, but it is time taking and expensive method that many countries cannot afford. A vaccine is a crucial aspect of eradicating disease, and for SARS-CoV-2), plasma therapy and antibiotics therapy are used in the early spreading phase. The later stage involves forming a vaccine based on spike protein, N-protein, and whole-viral antigen that effectively immunizes the population worldwide until herd immunity can be achieved.

In this review, we will discuss all possible and developed techniques for identifying SARS-CoV-2 and make a comparison of their specificity, selectivity, and cost; thus, we choose an appropriate method for fast, reliable, and pocket-friendly detection.

1. Introduction

Novel SARS-CoV-2 or COVID 19 is a new problem of the world that emerged in a short period and is named corona virus disease 2019 (COVID-19) by WHO. It affects the respiratory system of patients like swine flu. It started in Wuhan, China, at the end of 2019. Now it has spread all over the world and has taken the lives of thousands of people. On March 11, 2020, WHO declared it a pandemic as a rapid increase in cases of COVID-19 (nearly 13 times) observed outside China and showed its presence in 114 countries with more than 118,000 cases and over 4000 deaths [1].

It's a zoonotic disease, first reported in bats or wild animals in the Wuhan market. Bats are the natural host of this virus, and employing any intermediate host, has reached humans (https://www.theguardian.com/science/2020/jan/22/what-is-coronavirus-wuhan-china). It is an enveloped, single-stranded, positive-sense RNA virus having a spherical or pleomorphic shape with a club-shaped glycoprotein [2]. It is a giant RNA virus of 30 kb in length, with a 5′-cap structure and 3′poly-A tail. It is a member of the subfamily *Orthocoronavirinae* of the family *Coronaviridae.* Corona viruses are four subtypes: alpha, beta, gamma, and delta

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Production and hosting by Elsevier

https://doi.org/10.1016/j.sintl.2022.100197
Received 12 April 2022; Received in revised form 19 July 2022; Accepted 19 July 2022
Available online 30 July 2022

corona virus. Although it arises from the same genus as SARS-CoV and MERS-CoV, there are still many genetic variations between SARS-CoV, MERS-CoV, and SARS-CoV-2 [3]. A study revealed that SARS-CoV-2 and SARS-CoV attach to angiotensin-converting enzyme II (ACE2) as a receptor [4,5]. The basic structural features of the corona virus is depicted in Fig. 1. SARS-CoV-2 binds with ACE2 in animal cells [6], and its spike (S) protein binds ACE2 with high affinity [7,8]. In a study, the gene sequence of SARS-Cov-2 was compared with SARS-CoV genes and observed changes in transmembrane helical segments of the ORF1ab, which encode 2 (nsp2) and nsp3. The alteration occurred at positions 723 and 1010, which initially encodes glycine and isoleucine, but SARS-Cov-2 has a serine and proline at 723 and 1010, respectively [9]. A study confirmed its homology 96% with bat coronavirus and 79.5% with SARS-CoV after the full-length genome sequencing with SARS-CoV-2 taken from earlier patients [6]. This study helps in determining the pathogenesis and clinical treatment of COVID-19.

Symptoms become visible after 2–14 days of entry of the virus into the patient body. These symptoms are cough, difficulty in breathing, fever, sore throat, chills, loss of taste, muscle pain, fatigue, dyspnea, and pneumonia. Some symptoms are less reported, like diarrhea, headache, runny nose, hemoptysis, and phlegm-producing cough [10,11]. Chances of death increase in the case of aged people and having comorbidities. The virus layouts through aerosols/droplets produced by infected persons after sneezing and through direct contact. It indicates early transmission from animal to human [12–15]. Fig. 2 shows the primary transmission way of viruses in humans. A study reported another route of transmission through the digestive system since patients with abdominal discomfort and diarrhea symptoms manifest that ACE2 is highly expressed in the large intestine absorptive cells [16]. In this review article, we will discuss various available detection methods for SARS-CoV-2, including traditional methods, modified traditional methods, recently developed systems, and Biosensors, in detail so that we can compare their efficiency, sensitivity, and cost of SARS-CoV detection. Thus, the best-suited method can be applied according to the situation demand.

2. Diagnostic methods for COVID-19

SARS-CoV-2 detection has become a herculean challenge in the current time because of its newness. Real-time PCR is the WHO recommended test for covid-19. But it takes time and requires expensive instruments and chemicals. Serological methods are based on antibody detection, and antibody production takes approximately 5–10 days. Sensors are also developed, but no specific sensor is used for regular hospital testing. Although, sensors can provide a good detection tool because they are quick, sensitive, and provide specific results. Fig. 3 shows all developed detection methods for the Covid-19 virus.

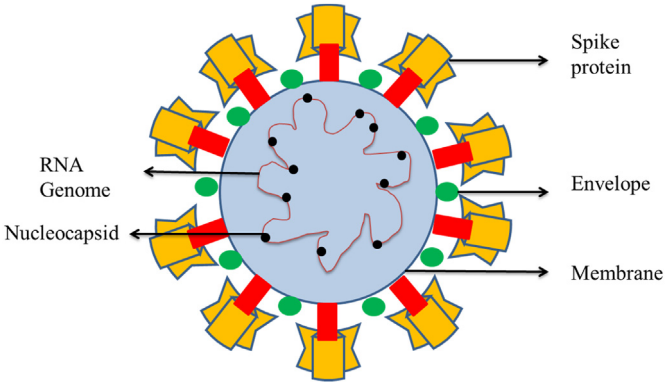

Fig. 1. A basic structure of SARS-CoV-2 virus. Spike protein and envelope protein are present on the external surface of virus and spike protein is used for receptor binding.

Significant features of all the developed techniques for COVID-19 diagnosis have been described in Table 1.

2.1. PCR

Polymerase chain reaction (PCR) is a widely used diagnostic method for rapid, precise, and selective detection of diseases, including influenza, malignant neoplasm, autoimmune disorders, and SARS-CoV-2. This method can be employed even at a paucity concentration of the sample, and reliable results are observed within a short period. However, new approaches like nanoPCR, multiplex PCR, and RT-PCR have been developed by modifying the traditional PCR method. The new system of nanoPCR is dense, fast, and possesses low weight. For intensified signal detection and rapid amplification of nucleic acid, magneto-plasmonic nanoparticles (MPN) are used [17,18].

PCR tests have some shortcomings; they can detect in the acute phase of infection of SARS-CoV-2 during viral shedding, which is not certain [19]. Still, data showed that 53% of cases got virus clearance from nasopharyngeal samples after 21 days of the symptom [20]. Thus, PCR testing alone gives doubtful results in case of convalescent cases.

2.1.1. Real-time PCR

It is the gold standard test for SARS-CoV-2 virus detection. Firstly, cDNA is synthesized using the SARS-CoV-2 RNA genome and used to amplify specific gene fragments with the help of specific primers. Fluorescence signals provide us with the copy number of the virus target sequence, which is easy to interpret during the process. The Centers for Disease Control and Prevention (CDC) has recommended primers and probe against nucleocapsid genes to use 2019-nCoV_N1, 2019-nCoV_N2, and 2019-nCoV_N3 (CDC, 2019). Early detection is possible with conventional PCR, but real-time-PCR is a predominant detection method for all kinds of coronaviruses [21,22]. A study used RT-PCR with in vitro-transcribed RNA to match this sequence with 2019-nCoV for quantification. The reported different Limit of detection (L.O.D) for E gene assay and RdRp assay of 3.9 copies per reaction from replicate tests and 3.6 copies per reaction, respectively [23]. But some studies also reported false negative and positive results with this test [24,25]. Another study compared seven commercially available real-time PCR kits against Covid-19. All kits showed promising results with the highest concentration of the sample ($Ct \leq 34.5$)and no cross-reactivity with SARS-CoV-1 E-gene. But, a 6-fold range (3.8–23 copies/mL) variation in their L.O.D was observed [26].

2.1.2. RT-LAMP

Reverse transcription loop-mediated isothermal amplification has emerged as a cost-beneficial and quick alternative test for SARS-CoV-2. This technique merges LAMP with a reverse transcription step and four specific primers for the target region to enhance the technique's sensitivity for RNA detection. Detection of amplification end product involves using photometry and magnesium pyrophosphate solution for turbidity production. Intercalating dyes are used for turbidity measurement and fluorescence, following the process in real-time. Due to the visual inspection and heat requirement, RT-LAMP is a simple and sensitive tool for viral diagnosis [27]. Some presently accessible molecular assays for SARS-CoV-2 detection exploit real-time RT-LAMP technology, for example, the ID NOW COVID-19 test. This method rapidly detects the virus in the upper respiratory tract but is confined to one sample per run. Zhang and coworkers developed RT-LAMP using reverse transcriptase to convert cDNA from viral RNA, amplified by Bst2.0 for fast colorimetric detection with a DNA-binding dye. An RNA-directed DNA polymerase was designed in-silico and coupled with a reversibly bound aptamer which restricts reverse transcriptase activity below 40°Ctemperature and is well suited to employ in LAMP. The calorimetric LAMP technique can detect viral RNA at a low level at approx. 480 RNA copies, without interference, provide a rapid and simple diagnosis of SARS-CoV-2 compared to RT-PCR [28].

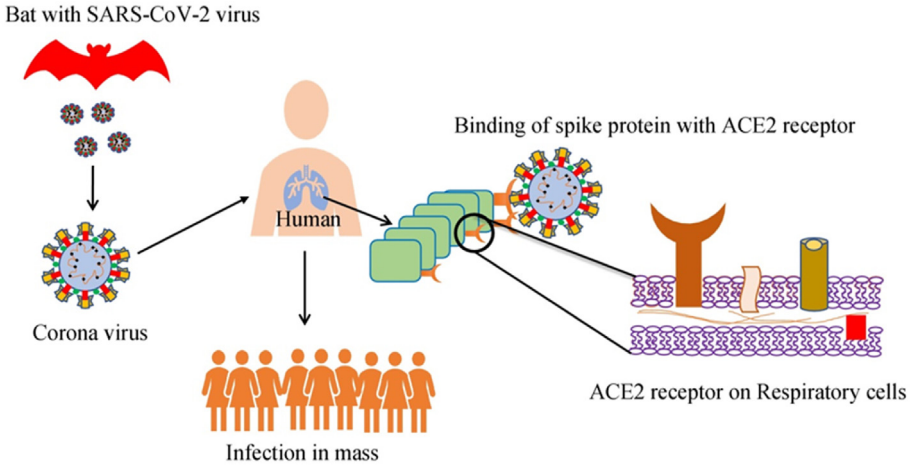

Fig. 2. A basic transmission route of Covid-19 virus in human. Virus binds with ACE-2 receptor on respiratory cells via spike protein which is located the surface of SARS-CoV-2 virus. A partial segment of respiratory cell is showing ACE2 receptor located in plasma membrane.

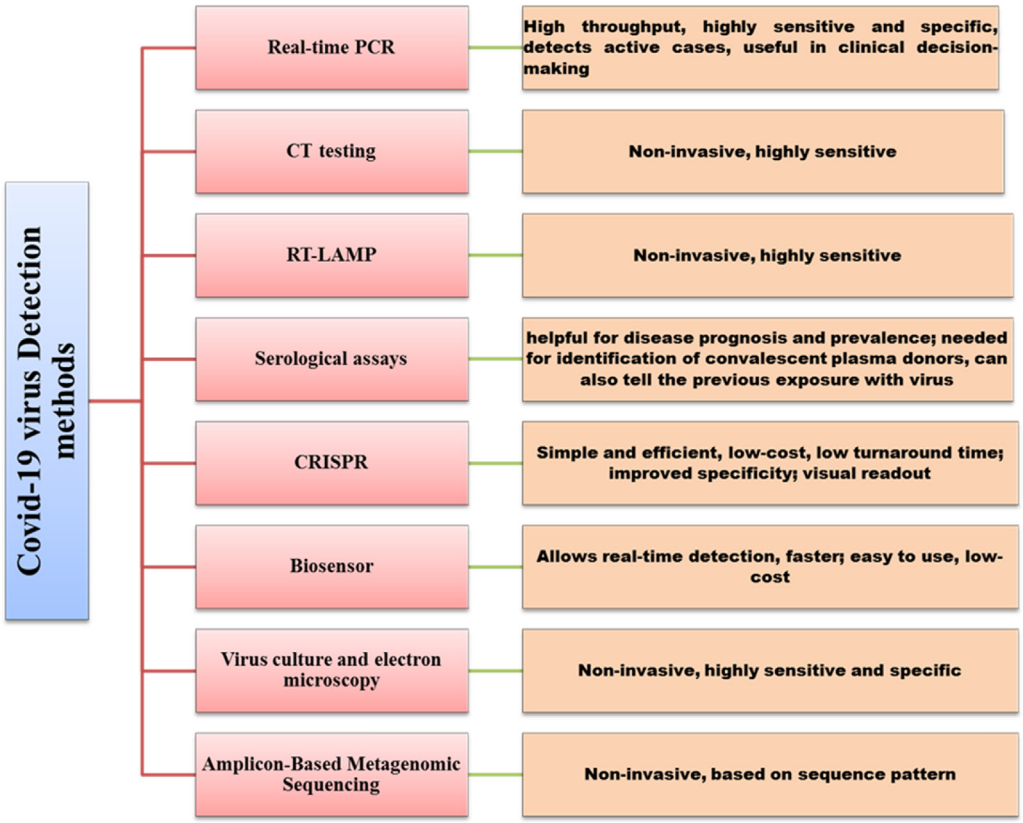

Fig. 3. A flow chart showing different detection methods for COVID-19. Serological assays are subdivided into separate types and explained with their advantage.

2.2. CT testing

Chest Computed Tomography (CT) is viewed as the first essential tool in the diagnosis of COVID-19 and acts as a complement to the RT-PCR test. A study reported that the sensitivity of Chest CT is high compared to an initial RT-PCR for the diagnosis of COVID-19 in swab samples. The positive results rate of RT-PCR assay was compared with chest CT testing and 59% (601/1014) and 88% (888/1014) in suspected patients, respectively. The study found a sensitivity of 97% (580/601) when RT-PCR took as a reference. Many cases show negative results for RT-PCR but give positive chest CT scans in 48% (147/308 patients). In many cases,60%–93% of patients show positive CT scans before the initial positive RT-PCR results. After health improvement, 42% of patients showed refinement in chest CT scans before the RT-PCR showing negative results for the SARS-CoV-2 virus [29]. Another study shows the result of a CT scan of COVID- 19 positive patients' respiratory tracts. Another study used both a CT scan and RT-PCR to check COVID-19. The sensitivity specificity of initial RT-PCR was 79% (27/34) and 100% (48/48) and 77% (26/34), and 96% (46/48) for chest CT testing. They don't observe any statistical difference in the analytical performance of RT-PCR and chest CT testing. This comprehensive testing showed greater sensitivity of 94% [30].

Scientists performed CT scans on patients belonging to different incidence rates category, and three groups were included low (less than

Table 1
All developed methods for Covid-19/(SARS-CoV-2) detection mentioned with their different parameters of effectiveness.

S. No.	Detection method	Sample	Target	Binding agent	Sensitivity	Specificity	Time	L.O.D/ sample amount	References
1	RT-PCR	respiratory secretion specimen	RNA	RNA	79%	100%	–	–	[80]
2	Chest CT	non-contrast high resolution CT thorax images	–	–	77%	96%	–	–	[80]
3	IgM-IgG Combined Antibody Test	human blood	–	–	88.66%	90.63%.	15 min	–	[12]
4	colloidal gold nanoparticle-based lateral-flow (AuNP-LF)	serum sample	IgM	–	100	93.3%,	15 min	10–20 μL serum	[39]
5	Rapid Anti IgM and IgG Detecting Tests	IgM and IgG	IgM and IgG	–	33.3%, 35.48%	72.85%, 85.02%	–	–	[37]
6	Lanthanide-Doped Nanoparticles-Based Lateral Flow Immunoassay	human serum	IgG	nucleocapsidphosphoprotein	–	–	10 min	100-μL	[40]
7	ELISA	plasma	IgM and IgA	–	–	–	–	–	[34]
8									
9	FET (field-effect transistor)	cultured virus, antigen protein and nasopharyngeal swab samples	Antibody against spike protein	Spike protein on virus surface	–	–	–	2.42×10^2 copies/mL clinical samples	[49]
10	Dual-Functional Plasmonic Photothermal Biosensor	Respiratory samples	complementary DNA receptors	RdRp and the ORF1ab gene sequence	–	–	–	0.22 pM	[60]
11	Luminescent immunoassay	Serum	IgG and IgM	–	–	–	–	–	[41]
12	Amplicon-Based Metagenomic Sequencing	RNA	Conserved sequence	–	–	–	–	–	[44]
13	CRISPR based assays	RNA	N and E genes	–	–	–	–	–	[43]
14	Colorimetric RT-LAMP	respiratory swabs	purified RNA or cell lysis	–	–	–	–	480 RNA copies	[16]
15	Dual-functional plasmonic photothermal biosensor		RdRp-COVID, ORF1ab-COVID, and E genes from SARS-Cov-2	–	3.2 copies sensitivity	–	–	0.22pM	[60]
16	Colorimetric biosensor	RNA Samples	Nucleoprotein gene (N-gene)	–	–	–	10min	0.18 ng/μL Dynamic range = 0.2–3 ng/μL	[70]
17	FET-based amperometric biosensor	nasopharyngeal swab	Spike protein	–	–	SARS-CoV protein 100 fg/mL	12 min	$2.42*10^2$ copies/mL	[49]
18	Electrochemical immunosensor	Spiked saliva samples	Spike protein	–	–	1 fM to 1 μM.	10–30 sec	90 fM	[65]
19	Cell-based potentiometric biosensor	Nucleocapsid protein	S1 spike protein	–	–	–	3 min	1 fg/mL Semi-linear range = 10 fg and 1 μg/mL	[67]
20	Gr-FET immunosensor	–	S1 spike protein	–	–	2×1011 M^{-1}	–	0.2pM	[64]
21	TMDC-based 2D-FET biosensor	Antigen spike protein	SARS-CoV-2 Spike protein	–	–	–	–	25 fg/μL	[50]
22	Label-free Electrochemical immunosensor	Spiked concentration used	Spike subunit 1 protein	–	–	5.5×10^5 PFU/mL	45 min	20 μg/mL at 260 nm	[66]
23	DT-based electrochemical sensor	Serum	RdRp-gene of SARS-CoV-2	–	–	1 fM to 100 pM	–	2.67 fM	[57]
24	Dual-aptamer based electrochemical biosensor	Serum	Nucleoprotein (n-CoV-NP)	–	–	–	–	8.33 pg/mL	[61]
25		cDNA	RdRp-gene	–	–		–	0.843 nF/nM	[69]

(*continued on next page*)

Table 1 (*continued*)

S. No.	Detection method	Sample	Target	Binding agent	Sensitivity	Specificity	Time	L.O.D/ sample amount	References
	Label-free DNA biosensor					0.843 nF/ nM			
26	Aptamer-based ECL biosensor	spike-deficient NL4-3 plasmid	Spike protein	–	–	–	40 min	66 pg/mL	[62]
27	Label-free ECL immunosensor	Artificial nasal secretion samples	Spiked RBD antigen protein	–	0.238 kohmpgmL^{-1} cm^{-2}	94.27%–104.95% good recovery rate	-	0.577 fg/mL Detection Range = 0.002–100 pg/mL	[72]
28	PANI-nanowire based genosensor	Serum	N-gene	–	–	–	–	3.5 fM	[71]
29	CRISPR/Cas12a-derived electro-chemistry assay	Human serum	N-protein	–	–	–	30 min	16.5 pg/mL	[63]
30	ECL-based DNA biosensor	Human Serum sample	ORF1ab sequences of SARS-CoV-2	–	–	–	–	514 aM	[74]
31	CRISPR-Cas13a based electrochemical biosensor	Artificial saliva	ORF fragments and S-gene	–	–	–	–	4.4×10^{-2} fg/mL and 8.1×10^{-2} fg/mL respectively	[74]
32	FET-based biosensor	Throat swab	N-Protein S-Protein	–	–	–	–	8 fg/mL	[58]

CRISPR-Clustered Regularly Interspaced Short Palindromic Repeats, RT-LAMP-Reverse Transcription Loop-mediated Isothermal Amplification.

10%), medium (10–70%), and high incidence rate (above 70%). Nearly 70% of individuals were found to be positive in the high incidence rate category, confirmed through RT-PCR. CT scan findings show bilateral abnormalities and vascular enlargement. While in the low incidence group, central lesion distribution, lymphadenopathy, and pleural effusion are 3.6%, 5.1%, and 5.2%, respectively. However, CT scan reports of the high incidence group (10–70%) show halo and reversed halo signs, 34.5 and 11.1%, respectively. The pleural thickening percentage increased to 347%, and various lesions were also observed, including focal, multifocal, and unilateral, with 10.5%, 63.2%, and 15%, respectively [31].

2.3. Serological assays

There are many serological tests, but all are not routinely used for the screening or diagnosis of COVID-19. Now some are being used for screening of population and surveillance. The only reason is that these results will show positive results only after 5–10 days of post-infection when the body has started to make enough antibodies against COVID 19 virus. These test helps in other ways, like to detect how many people have encountered the virus, but these test gave sensitive and specific results if the body has made antibodies or have enough time for the virus in the body. These tests cannot detect the virus in the early stages of the disease. When molecular test gives negative results, these serological tests are beneficial if further validation is required [32]. It helps in detecting mild and asymptomatic cases and also helps in the detection of a suspected person who has already recovered. Here we mentioned such validated serological assay for detection of covid-19. It can be difficult for some people to diagnose those who have mild respiratory and have recovered without treatment [33].

2.3.1. Enzyme-Linked Immunosorbent Assay

Enzyme-Linked Immunosorbent Assay (ELISA) is based on the analysis of specific binding between antigen and antibody and is often used for many disease diagnoses, including COVID-19 detection. Although the qPCR-based technique is in use currently, it can give false results too when the viral nucleic acid is not present in sufficient quantity. A study explained time kinetics for different antibody production against novel coronavirus (SARS-CoV-2). It can help in diagnosis using antibodies. IgM and IgA detection require five days (IQR 3–6), and IgG has 14 days median duration for detection in the body. Confirmed cases show positivity of 75.6% for IgM, providing a 98.6% increased detection rate when IgM ELISA detection is done with PCR compared to a single qPCR test (51.9%). Thus humoral immunity can help in detection very specifically [34].

In a study, recombinant nucleocapsid protein expressed in pcDNA3.1 vector of SARS-CoV and SARS-CoV-2 was used for ELISA assays. Wells were coated with 100 ng of the recombinant receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. Horseradish peroxidase-conjugated goat anti-human IgG was applied after the use of serum. It shows an optical density of $>1{\cdot}4$ for nucleoprotein and receptor binding domain antigens of SARS-CoV-2, with viral neutralization in titers up to 1/320 [35].

2.3.2. RADT and lateral flow immunoassay

Rapid antibody detection tests (RADT) are suitable for SARS-CoV-2 detection because IgM is produced in the body after 3–6 days and provides the first line of defense [36]. Many tests are developed using a different methodology to detect antibodies, mainly IgM. In a study, two rapid antibody tests, Anhui and Clungene, were compared with PCR. Anhui and Clungene tests have 33.3% and 72.85%, sensitivity and 35.48%, and 85.02%specificity, respectively. They concluded that these tests fail to detect anti-IgM and anti-IgG antibodies against SARS-CoV-2019 and can not be used for its detection [37].

2.3.3. Lateral flow immune assay

These tests are simple, quick, and suitable for point-of-care detection, based on antibody detection in the serum of patients. These antibodies can reveal the stage of infection of a patient. Li et al., 2020 have developed a lateral flow immunoassay for point of care detection based on the detection of IgM and IgG antibodies against the COVID-19 virus in human serum. It takes only 15 min. It involves the use of a plastic strip attached to different platforms or pads such as sample pad, conjugate pad, absorbent pad, and Nitrocellulose membrane. Antibodies were applied at separate lines like anti-human-IgM at the M line, anti-human-IgG at the G line, and anti-rabbit-IgG at the C line or control line. Then AuNP-COVID-19 recombinant antigen conjugate and AuNP-rabbit-IgG were applied to the conjugate pad. Then blood sample was used and got

specificity and sensitivity of 90.63% and 88.66%, respectively. The Control line and G and M lines show a positive case for SARS-CoV-2 [38].

Another study developed colloidal gold nanoparticle-based lateral-flow (AuNP-LF) assay for IgM antibody detection. It is an indirect immunochromatography-based method in which (SARS-CoV-2NP) nucleoprotein was coated on the membrane. Antihuman IgM conjugated with gold nanoparticles was used as a reporter for assay validation. Serum was used to test the sensitivity and specificity of 100 and 93.3%, respectively. It takes only 15 min and requires only 15–20 μl of the sample [39]. Lanthanide-doped nanoparticles were used for lateral flow immunoassay development. In this method, recombinant nucleocapsid and phosphoprotein of COVID 19 were assembled onto a nitrocellulose strip to bind with IgG antibody in serum. Lanthanide-doped polystyrene nanoparticles (LNPs) labeled mouse anti-human IgG antibody was used as reported. It took 10 min for the detection of anti-SARS-CoV-2 IgG in serum [40].

2.3.4. Luminescent immunoassay

This method lowers the detection limits for antibody-based reagents known as Luminescent immunoassays. It involves the creation of a magnetic chemiluminescence enzyme immunoassay based on peptides for COVID-19 diagnosis. Diazyme Laboratories, Inc. (San Diego, California) declared the availability of two novel, fully automated serological tests for SARS-CoV-2 that were applied successfully on an automated Diazyme DZ-lite 3000 Plus chemiluminescence analyzer. Here, IgG and IgM are detected by a peptide-based luminescent immunoassay. The cut-off value of the assay was analyzed from the sera of infected and healthy individuals for other pathogens except for SARS-CoV-2. IgM and IgG are detected in the sera to evaluate the assay performance of confirmed patients. The positive rate of IgM and IgG was 57.2% and 71.4%, respectively. Hence, the diagnostic accuracy of COVID-19 might be enhanced by combining RT-PCR and immunoassay [41].

2.4. CRISPR

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based assays use a family of nucleic acid sequences which is present in prokaryotes. CRISPR-associated enzymes named Cas 12, Cas9, and Cas 13 are used to recognize and cut these sequences. Cas12 and Cas13 families' certain enzymes specifically cut viral RNA sequences (The McGovern Institute for Brain Research, Massachusetts Institute of Technology). A method developed by SHERLOCK biosciences is based on the use of Cas13, which excises reporter RNA sequences when activated by SARS-CoV-2 specific guide RNA [42]. Mammoth biosciences used the Cas12 family enzyme to cleave reporter RNA to detect specific sequences of N and E genes. The cleavage is then followed by isothermal amplification of the target, and the fluorophore is used for a visual readout of the results [43]. SARS-CoV-2 presence detection involves paper strips without specificity and sensitivity loss by these CRISPR techniques. These are less time-consuming and low-cost tests.

2.5. Amplicon-based metagenomic sequencing

A dual approach using metagenomics sequencing and amplicon-based sequencing is being explored to identify SARS-CoV-2. The background microbiome in the patient is addressed by metagenomics sequencing. This helps identify the SARS-CoV-2 virus and other pathogens contributing to the severity of COVID-19 symptoms. Amplicon-based sequencing allows viral evolution, contact racing, and molecular epidemiology studies. Further checks on the sequence divergence are done by sequence-independent single primer amplification (SISPA), a part of the metagenomics approach. The recombination possibilities of the SARS-CoV-2 virus with other human coronavirus are done with the help of these dual approaches. Both techniques have applications for the development of vaccine and antiviral efficacy. Moore et al. (2020) used this dual approach for SARS-CoV-2 genome sequencing and the rest of the microbiota of the nasopharynx through swabs obtained from the COVID-19 infected individual by ISARIC 4C consortium. The group chooses 16 primer binding sites for SARS-CoV-2 genome conserved regions that amplify 1000 bp fragments with approx. 200 bp overlapping region in the amplicon-based system. Then 30 amplicons from cDNA were generated using these primer sets, which were later on sequenced using MinION [44].

2.7. Biosensor

Biosensors provide a fast tool for detection. Lots of sensors are developed for many diseases, including SARS-CoV-2. In a study, a field-effective transistor-based sensor was fabricated for detection. Graphene sheets of field-effect transistor (FET) and specific anti-SARS-CoV-2 antibodies were used for sensor fabrication. These biosensors can sense nearby modifications on their surface and provide a low-noise and ultrasensitive detection environment. From this perspective, these graphene-based FET technologies are striking for applications related to immunological diagnosis [47,48]. PBASE (1-pyrenebutyric acid *N*-hydroxysuccinimide ester) is an effective interface coupling agent used to immobilize the SARS-CoV-2 spike antibody onto the device. Seo et al., 2020 [49] also developed a fabricated FET biosensor based on the identification of the S-protein of SARS-CoV-2. As earlier studied, S-protein is an ideal transmembrane protein of the SARA-CoV-2. The developed surface was functionalized with graphene and then immobilized with specific antibodies of S1-protein via a cross-linking agent:1-pyrene butyric acid NHS- ester in PBS. This sensor detected S-protein 1 fg/mL in PBS and 100 fg/mL in clinical samples. The selectivity of this biosensor was also tested with MERS-CoV protein which indicated the best platform for S-protein detection. The designed FET-based biosensor was found to be highly sensitive toward COVID-19 diagnosis. The FET sensor detected clinical samples (LOD: 2.42×10^2 copies/mL) and SARS-CoV-2 in a culture medium with a 1.6 × 101 pfu/mL L.O.D. Hence, it was concluded that the FET sensor is a sensitive immunological and promising tool for SARS-CoV-2 detection that doesn't require any sample labeling and pretreatment.

A label-free 2D- FET-based biosensor for the specific detection of spike proteins of SARS-CoV-2 was created by Ref. [50]. In this work, the author developed a novel sensing platform using a monolayer of WSe2 crystals to functionalize SARS-CoV-2 antibodies with 11-mercaptoundecanoic acid (MUA) as a probe-linker. This biosensor has the potential to detect the S-protein concentrations down to 25 fg/mL. And also, results indicate that TMDC-based 2D-FET biosensor may serve as a selective and sensitive platform for the fast detection of infectious diseases.

Although we have studied many methods for SARS-CoV-2, some recent technological advancements developed smartphone-based diagnostic procedures, and these methods are more reliable and faster. Few eliminated the RNA isolation step, and some used Loop-mediated isothermal amplification (LAMP) in combination with smartphones to develop an affordable point of care (POC) diagnostic tool.

However, using (LAMP) loop-mediated isothermal amplification involves false positive results. Scientists altered the protocol conditions for cDNA synthesis and viral RNA stability to increase sensitivity and overcome faulty positive results. They performed a two-tailed Mantel-Haenszel test by using a primer with no templates and another with templates (classified as non-conjugate and conjugate primers) to check the sensitivity and specificity of the smartphone-based loop-mediated isothermal assay. For comparison limit of detection (LOD) of the CDC 2019-nCoV RT-qPCR diagnostic test was used, and the resulted value of the smartphone-based loop-mediated isothermal technique is similar to CDC 2019-nCoV RT-qPCR (one thousand copies per ml). SARS-CoV-2 possesses many varients all over the world, including.

1. B.1.617.2 (Delta) in India
2. B.1.526 (Iota) in New York
3. P.1 (Gamma) in Brazil

4. B.1.429 (Epsilon) in New York
5. B.1.1.7 (Alpha) in the UK

SARS-CoV-2 primers are amplified by using the smartphone-based diagnostic assay that eventually amplifies SARS-CoV-2 early variants from Hong Kong and the USA. Still, in other variants like MERS-Cov, HCoV-NL63, SARS-CoV-1 amplification was not observed, indicating the specificity of smartphone-based loop-mediated isothermal amplification [51].

A new framework was described for COVID-19 diagnosis using smartphone sensors. Today's smartphones have memory space, computation-rich processors, and many sensors like a color sensor, microphone, cameras, proximity, inertial sensors, temperature sensor, wireless chipsets/sensors, and humidity-sensor. This designed framework identifies viral pneumonia by sensing signals and scanning CT images. It takes less time in comparison to an expert radiologist to diagnose COVID-19. Radiologists can use this to track disease grade development and treatment evaluation. This framework is fabricated as a set of layers. The first layer of functionalities reads the data from all sensors. For instance, interpretation of the CT scan images-videos of the lung, voice recording through a microphone in case of cough, and temperature sensing while touching the screen of a smartphone. The second layer is designed to configure the smartphone sensors involving image size, reading intervals, timer resolution, buffers' size, etc. The 3rdlayer of this framework gives the level of a calculated sign distinctly, and then the information is stored as a record in the next layer. The final layer was used for predicting the COVID-19 virus by applying machine learning techniques. All the recorded data nature, these machine learning techniques could be used. For instance, a convolution neural network (CNN) could be used for abnormal sub-image of CT scan images, followed by a recurrent neural network (RNN). Hence, the anticipated framework is expected to use all these sensors and algorithms in a single record [52]. Numerous deep learning algorithms have been applied for organization or identification purposes, such as RNN and CNN. CNN is a feed-forward neural network that is used for image recognition [53]. While with RNN is good for temporal data storing and gives input in the next layer and signal measurements [54,55].

In 2021, Zhao et al. [56] fabricated an electrochemical biosensor for point-of-care testing of SARS-CoV-2 via detecting RNA in a sample. This work used calixarene functionalized graphene oxide to develop an electrochemical biosensor for detecting SARS-CoV-2 with a LOD score of 200 copies/mL. From the view of POC testing, electrogenerated chemiluminescence signals were detected in less than 10sec using a smartphone. Due to ease of the process, and rapid and ultra-high sensitivity, the prepared biosensor provided a stable and effective approach for the COVID-19 pandemic. Another sensor based on electrochemiluminescence was developed by Ref. [57] to rapidly detect the RNA-dependent RNA polymerase (RdRp) gene of SARS-CoV-2. RdRp-gene plays a significant role in the diagnosis of COVID-19. In this work, the electrode surface was modified with DNA tetrahedron (DT) for the sensitive assay of RdRp-SARS-CoV-2 via an entropy-driven-reaction strategy. Further, this reaction strategy controlled enzyme reagents' use and made it easier for large-scale screening of COVID patients. This target-DNA was participated reaction performed on the DT to enable the immobilization of the Ru (bpy)3 2+ modified S3. In addition, for better sensitivity, the DT probe's tetrahedral structure is rigid, maximizing the ECL intensity and showing no cross-reactivity between ssDNA. This DT-based ECL sensor achieved significant specificity and high sensitivity for SARS-CoV-2 with a LOD score down to 2.67 fM. A novel label-free graphene oxide-graphene FET biosensor was designed to detect proteins of SARS-CoV-2. A layer of GO was applied to the graphene surface, enhancing the sensor's sensitivity. The sensor can detect viral proteins in 20 min with a L.O.D of ~8 fg/mL [58].

2.6. Virus culture and electron microscopy

According to a study, lower and upper respiratory tract secretion samples from infected COVID-19 patients were inoculated upon cells for virus isolation. Viral isolation and replication were then confirmed with the help of gene detection, cytopathic effects, and electron microscopy. Biosafety level-3 was used for the viral isolation procedure of SARS-CoV-2. For further viral identification, electron microscopy and complete genome sequencing were employed. The study of the cytopathic effects observed the replication of the virus in Vero cells. The results of whole genome sequencing showed that the viral genome possesses more than 99.9% sequence homology with other samples of SARS-CoV-2 isolated from other countries, such as China. SARS-CoV and MERS-CoV virus shows 77.5% and 50% sequence homology with SARS-CoV-2. The full-length genome sequencing exhibited that the formerly described SARS-CoV-2 virus is present in the same cluster with a homology of more than 99.5% with other extracted SARS-CoV-2 viruses. Electron microscopy was used for coronavirus-specific morphology identification in virus-infected Vero cells. On the 4th day after inoculation, 80% of harvested cells show cytopathic changes. Real-time (RT-PCR) was done with the extracted RNA from cultured cells for viral replication confirmation. The observed cycle threshold (Ct) values were 18.26 for oropharyngeal and 14.40 for nasopharyngeal samples. These values were lower than the Ct value of 21.85 and 20.85 in the pre-inoculated samples. Researchers observed 3.9×10^8 and 7.6×10^8 copy/ml viral copies before inoculation and then increased up to 10–70 fold to 4.2×10^9 and 5.4×10^{10} respectively, in the supernatant of cell culture. The viral structure was observed with electron microscopy after 3-days of inoculation. The observed morphology was coronavirus specific. The size of the virus was also observed in a broad range of cell organelles and ranges between 70 and 90 nm [45]. Electron microscopy also reveals the outer covering of the virus, which is made up of the endoplasmic reticulum of infected cells in a vacuole of the Golgi body after entering the kidney cell [46].

2.7.1. Aptamer based sensor

Aptamers are the specific agents designed by the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [59]. They can bind to various analytes for their detection, including protein, carbohydrate, DNA, RNA, and other molecules. Aptamers can be a piece of protein or DNA, or RNA. It binds specifically with its substrate and provides specificity, referred to as an alternative for antibodies due to its cost-friendly and higher specificity nature. Due to broader specificity, different SELEX variant is required for specific analytes, including capillary SELEX, immuno-SELEX, and capture SELEX [60]. designed a multifunctional aptamer-based plasmonic platform for the detection of SARS-CoV-2. This sensor was based on a dual-functional plasmon, a union of localized surface plasmon resonance and plasmonic photothermal. This sensing transduction offers a capable and alternate option for COVID-19 diagnosis. The 2D gold nano-islands (AuNIs) are attached with a complementary ssDNA sequence for binding with a specific sequence of SARS-CoV-2 virus in the sample. Thermoplasmonic heat improves sensing on the AuNIs chips after illumination at their proper plasmonic resonance frequency. This heat efficiently increases the *in-situ* hybridization temperature so that the two complementary gene sequences can be discriminated accurately. This dual LSPR biosensor possesses a LOD of 0.22 pM and permits the exact sensing of a particular target in a mixture of multigene. In this study, the author used a signal enhancement platform mainly comprised of localized surface plasmon resonance (LSPR) and plasmonic photothermal therapy (PPTT). They used gold nano-islands (AuNIs) as a receptor for the detection, functionalized with cDNA sequences via a hybridization process. The fabricated biosensor was found sensitive for sensing of RdRp-gene. In diagnostics, this research attains attention to thermoplasmonic enhancement and its

application in viral disease diagnosis and nucleic acid tests [60].

A dual-aptamer-based electrochemical biosensor comprised of a metallic organic framework for detecting SARS-CoV-2 nucleocapsid-protein was developed by Ref. [61]. In this work, the author designed a sandwich-type electrochemical detection platform composed of MIL-53 (A1) decorated with Au@Pt nanoparticles and enzymes to establish SARS-CoV-2 nucleoprotein. Primarily, the dual-thiol-modified aptamers (N48 and N61) were immobilized on the Au electrode surface as a recognition element to fascinate the nucleoprotein. Further, the nano-composite of Au@Pt/MIL-53 (A1) was labeled with HRP and hemi-n/DNAzyme as signal nanoprobe. The constructed nanoprobe was successfully employed to develop the aptasensor signals via the co-catalysis reaction of HQ/H_2O_2. This dual-aptamer-based biosensor showed a broad linear range of 0.025–50 ng/mL with a LOD score of 8.33 pg/mL. And also, this aptasensor has achieved higher sensitivity, selectivity, and stability with an excellent potential range for early diagnosis of COVID-19. Another study reported a fast and sensitive impedimetric biosensor for detecting SARS-CoV-2 S-protein. In this work, the author designed an aptamer-based -AuNPs modified screen-printed carbon electrode platform to target the RBD of SARS-CoV-2. For the first time, this platform was identified by electromagnetic spectrum between 770 and 1910 $cm^{-1,}$ which signifies the abundance of S-protein homogeneously spread on the sensing probe. This biosensor has great potential to detect S-protein with a detection limit of 1.30 pM (66 pg/mL), in contrast to the detection limit of PCR for the same analyte [62].

A fast, less expensive, portable, and sensitive aptasensor to detect nucleocapsid protein was successfully designed by scientists [63]. In this work, they have used the CRISPR/Cas12a-derived strategy in which the gold electrode sensing interface was functionalized with immobilization of MB-labeled poly-adenines DNA-sequence. An aptamer-based probe was created along with an activator strand. In the presence of nucleo-capsid protein, aptamer hybridizes with it, and CRISPER/Cas12a release the MB labeled DNA sequences, which decreases the current of differential pulse voltammetry. This CRISPR/Cas12a-based aptasensor can detect a L.O.D of 16.5 pg/mL of nucleocapsid protein.

2.7.2. Immunosensor

Immunosensors are a promising area of excellent specificity and sensitivity within a short time. These are based on the binding of antigen with a particular antibody. Several sensors were developed by researchers for the detection of several pathogens. Likewise, many sensors were also developed to detect the COVID-19 virus. A digital, Gr-FET sensor was designed without any labeling to detect the spike protein S1 of COVID-19 and to assess neutralizing antibodies that can obstruct corona-virus from binding and affecting healthy cells. In this work, the graphene surface was functionalized with angiotensin-converting enzyme 2 or with an antibody against spike S1 subunit as intact membrane protein with higher affinity, which mainly binds with the RBD domain of S1-protein.

Moreover, based on detection, the RBD-domain of S1-protein holds a slight positive charge with graphene surface receptors which can alter the resistance in Gr-FET. The modified- Gr-FET sensor has a detection limit of 0.2 pM with better sensitivity. Gr-FET immunosensor was entirely based on surface potential variations affecting electrical signal response [64].

A portable sensor was developed by Ref. [65] as eCovSens for diagnosing spike protein (S-protein). They used SARS-CoV-2 specific monoclonal antibodies immobilized on the screen-printed carbon electrodes. The developed biosensor was highly sensitive for detecting S-protein with a LOD score of 10 fM in buffer and 90 fM in saliva. eCovSens was portable, rapid, and a non-invasive tool for COVID-19 diagnosis as it provides the results within 10–30sec. A novel electrochemical biosensor using anti-spike antibodies attached to screen-printed electrode was used for fast detection of S-protein developed by Ref. [66]. SPE was further functionalized with a 1-pyrene butyric acid *N*-hydroxysuccinimide ester (PBASE) linker that was attached with antibodies. This biosensor could detect 5.5*105 PFU/Ml that has reached a low LOD score of 260 nM (20 μg/mL) in 45min contrary to PCR. Another portable sensor was created by Ref. [67] to detect the S1-antigen of the SARS-CoV-2 virus based on a bio-recognition assay. The strategy was based on the chemical bonding of protein to the antibody surface, which significantly changed evaluated bioelectric properties. In this work, the author developed a membrane-engineered kidney cell modified with SARS-CoV-2 spike S1-antibody via electrical insertion method to detect S1-antigen on SPGE's coated by polydimethylsiloxane (PDMS) layer. The change in membrane potential was measured by antibody-antigen interaction. The fabricated potentiometric-biosensor was used for monitoring COVID-19 as it showed early detection of a specific protein within 3min with a linear range of 10fg-1μg/mL, LOD score of 1 fg/mL, and no cross-reactivity. An electrochemical biosensor composed of a highly uniform gold microcuboid pattern to strengthen the conductivity was developed by Ref. [68]. In this work, the author used anti-COVID antibodies as a probe for the identification of COVID-19 characterized with cyclic voltammetry and square wave voltammetry techniques. This biosensor has achieved a wide concentration range of 5 nmol/L to 100 pmol/L with a low LOD score of 276 fmol/L. Moreover, this biosensor could be used in an actual human sample of a nasal swab so that it becomes easy to detect COVID-19 without needing sample preparations. The results indicate that the SWV technique provides high sensitivity and efficacy in detecting COVID-19 in an actual human sample [69]. designed a novel label-free DNA biosensor based on capacitance evaluation to detect the SARS-CoV-2 RdRp gene. This biosensor was comprised of crossed-platinum/titanium electrodes on the glass substrate to analyze the hybridization of target DNA with probe DNA. The prepared kit revealed significant sensitivity for detecting the SARS-CoV-2-RdRp gene with a detection limit of 0.843 nF/nM for a one-step hybridization.

2.7.3. Nanomaterials based sensor

A colorimetric biosensor for the identification of nucleoprotein of SARS-CoV-2 was constructed by Ref. [70]. This study used gold nanoparticles (AuNPs) treated with DNA anti-sense oligonucleotides (ASOs) modified with a thiol group for specific detection of SARS-CoV-2. In the presence of RNA, agglomeration will happen in the suspension and give a visual color change after the complementary binding of sample RNA with DNA. This specific biosensor for SARS-CoV-2 nucleoprotein results in 10min with a LOD score of 0.18 ng/μL [71]. reported an antifouling genosensor based on PANI-nanowires and synthesized peptides for the specific identification of N-gene for SARS-CoV-2. In this work, the chemistry of the sensing platform was based on a biotin-streptavidin affinity system. The covalent immobilization of the probe can affect the zwitterion property of the peptide. Therefore, biotin-labeled probes were directly applied onto the peptide-PANI nanowires film, forming a susceptible and specific electrochemical sensing platform for diagnosing COVID-19. Due to the excellent antifouling properties of PANI-nanowires, the developed genosensor could identify nucleic acid with a wide linear range, i.e., 10^{-14} to 10^{-9} M, and showed a low LOD score of 3.5 fM.

Another sensor was developed, which is a cost-effective and reliable biosensing system for the detection of the receptor-binding domain (RBD) of SARS-CoV-2. Au-nanoparticles-capped 11-mercaptoundecanoic acid (AuNPs@MUA) modified ITO electrode was used via EDC-NHS. AuNPs@MUA provides a stable and reproducible excellent biocompatible surface for this novel sensing system. And fabricated biosensor was characterized by EIS and CV using Ferro-Ferri as a redox probe. This protocol gives a reliable biosensing platform and a high sensitivity of 0.238 kohmpg/mL/cm. The impedimetric immunosensor has achieved a wide linear range of 0.002–100 pg/mL with a low LOD score of 0.577 fg/mL and was successfully applied for RBD detection in nasal samples [72]. In 2022, Galvez et al. synthesized gold nanomaterials and carbon nanodots to develop an electrochemiluminescence-based DNA biosensor. The synergic effect of both nanomaterials enhances the

electrochemiluminescence and makes a better sensing platform for SARS-CoV-2 detection. A thiolated DNA probe was attached to the gold nanomaterials, which hybridized with the virus DNA. Combining [Ru(bpy)3]2+/CDs with AuNMs changes the ECL. The developed sensor can detect 514 aM of the virus [73]. Heo et al., 2022 developed a fast, sensitive, and PCR-free detection for SARS-CoV-2. In this work, the author utilized nanocomposite and gold nano-flower to enhance the sensor's conductivity. Later on, reporter RNA (reRNA) molecules were treated with a redox probe-methylene blue and biotin at both ends and attached on electrode surface covered with streptavidin. The biosensor detected a LOD Score of 4.4×10^{-2} fg/mL and 8.1×10^{-2} fg/mL of ORF and S genes, respectively [74].

3. Future perspective and challenges

There is no specific antiviral treatment, the vaccines are under development, and in trials for COVID-19 are presently accessible. SARS-CoV-2 detection has become a world challenge in the current time because of its novelty. Therefore, the need for fast, reliable, low-cost, and widely distributed tools for diagnostic is increasing. A biosensor targeting virus antigen with rapid and ultrasensitive detection can be used in laboratories, clinics, at home, in traffic areas, and even at airports. Nanotechnology can be employed for ultrasensitive detection in biosensor fabrication. Using multiplex biosensors that use different biomarkers enhances the diagnosis accuracy. Direct result readout and machine learning based-signal processes need to be developed to improve biosensors' reproducibility and reliability. Though SARS-CoV-2 can be found in asymptomatic carriers, there is an urgent requirement for a biosensor that can be used at home and rapidly determine the positive and negative samples. Around 30–60% positivity was found in RT-PCR results in the initial stages [29]. The positivity percent depends majorly on the time of sample collection because positivity can be seen in samples collected in the early stages of symptoms. It has been observed that in countries with low or middle income [75], the healthcare system is not very rapid, so laboratories face many complications in molecular testing procedures. In such low-resource settings, there is a requirement for cheap, sensitive, and quick methods for timely diagnosis. Diagnosis of COVID-19 in early stages and asymptomatic patients will be the key to preventing the spread of this pandemic.

However, only detection will not be enough; effective vaccination is also required to diminish the effect of SARS-CoV-2; as in the early stages of infection, we are taking preventive measures, including frequent hand washing, PPE kits, and social distancing. Patients suffering from SARS-CoV-2 provided plasma therapy as passive immunotherapy [76] and antibiotics treatment at the early stage of infection to diminish SARS-CoV-2. After some advancement, vaccines are produced that possess the ability to form neutralizing antibodies inside the patient body. These are based on.

1. Spike proteins [77].
2. N-protein using recombinant DNA technology [78].
3. Whole viral antigen [79].

These vaccines are either live attenuated or inactivated, and no such drug has been made until now that kills SARS-CoV-2 entirely. By vaccination, we can attain Herd immunity and diminish the effect of SARS-CoV-2, but for complete eradication, such drugs are necessary that entirely kill the virus. Until then, we are only left with vaccination and early detection of the virus so that prevention can be done.

Funding info

One of the author (Ravina) wishes to acknowledge the award of senior research fellowship (SRF) of Indian Council of Medical Research (ICMR), New Delhi during the tenure of this work.

Author contribution

Ravina had the idea for the article, and performed the literature search and data analysis, Ravina, Subodh, Twinkle, Jagriti Narang, Manjeet drafted and Ashok Kumar, Hari Mohan critically revised the work.

Data availability

All the data used in this review article is present on freely available public domains. We provided references which have all the information to search those articles.

Animal research (Ethics)

Not applicable.

Consent to participate (Ethics)

Consent was taken from all authors (participants).

Consent to publish (Ethics)

Yes.

Declaration of competing interest

There is no conflict of interest between the authors.

Acknowledgments

Authors wish to acknowledge the award of a senior research fellowship (SRF) from the Indian Council of Medical Research (ICMR), New Delhi, during the tenure of this work.

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