Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review

Recent advances in point of care testing for COVID-19 detection

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ABSTRACT

The World Health Organizations declaration of the COVID-19 pandemic was a milestone for the scientific community. The high transmission rate and the huge number of deaths, along with the lack of knowledge about the virus and the evolution of the disease, stimulated a relentless search for diagnostic tests, treatments, and vaccines. The main challenges were the differential diagnosis of COVID-19 and the development of specific, rapid, and sensitive tests that could reach all people. RT-PCR remains the gold standard for diagnosing COVID-19. However, new methods, such as other molecular techniques and immunoassays emerged. Also, the need for accessible tests with quick results boosted the development of point of care tests (POCT) that are fast, and automated, with high precision and accuracy. This assay reduces the dependence on laboratory conditions and mass testing of the population, dispersing the pressure regarding screening and detection. This review summarizes the advances in the diagnostic field since the pandemic started, emphasizing various laboratory techniques for detecting COVID-19. We reviewed the main existing diagnostic methods, as well as POCT under development, starting with RT-PCR detection, but also exploring other nucleic acid techniques, such as digital PCR, loop-mediated isothermal amplification-based assay (RT-LAMP), clustered regularly interspaced short palindromic repeats (CRISPR), and next-generation sequencing (NGS), and immunoassay tests, and nanoparticle-based biosensors, developed as portable instruments for the rapid standard diagnosis of COVID-19.

1. Introduction

Coronaviruses (CoV) are enveloped, large, positive-sense single-stranded RNA viruses, ranging from 26 to 32 kilobases in length, belonging to the Coronavirinae subfamily of the Coronaviridae family. The Coronavirinae subfamily is divided into four major genera: Alpha and Beta coronavirus, which infect mammals primarily; Gamma and Delta coronavirus, which infect mostly birds. Currently, there are seven human Coronaviruses (CoV) identified that may cause respiratory, gastrointestinal, and liver infection, and neurological diseases. New coronaviruses strains appear to emerge periodically in humans, mainly due to the high prevalence and wide distribution of coronaviruses, the large genetic diversity and frequent recombination of their genomes, and the increase of human-animal interface activities [1,2].

Abbreviations: **ACE2**, Angiotensin-converting enzyme II; **ANVISA**, Brazilian National Health Surveillance Agency; **AuNIs**, Gold nanoislands; **cdPCR**, Chip-based; **CLIA**, Chemiluminescent immunoassays; **CoV**, Coronaviruses; **COVID-19**, Coronavirus Disease 2019; **CRISPR**, clustered regularly interspaced short palindromic repeats; **ddPCR**, Droplet-based; **dPCR**, Digital PCR; **dsDNA**, Double-stranded DNA; **ELISA**, Enzyme-linked immunosorbent assay; **endoRNase**, RNA-guided endoribonuclease; **FET**, Field-effect transistor; **IC**, Lateral-flow immunochromatography; **ICT**, immunochromatographic tests; **LFB**, lateral flow biosensor; **LSPR**, Localized surface plasmon resonance; **mdPCR**, Microfluidic digital PCR; **MERS**, Middle East respiratory syndrome; **mRT-LAMP**, Multiplex reverse transcription loop-mediated isothermal amplification; **NAAT**, Nucleic acid amplification tests; **NGS**, Next-generation sequencing; **NSP1** —**NSP16**, 16 nonstructural proteins; **POCT**, Point of care tests; **RT-LAMP**, Reverse transcription loop-mediated isothermal amplification; **RT-PCR**, Real-Time Reverse Transcription Polymerase Chain Reaction; **RT-RAA**, Reverse transcription–recombinase aided amplification; **RT-RPA**, Reverse transcription-recombinase polymerase amplification; **SARS**, Severe acute respiratory syndrome; **SARS-CoV-2**, Severe acute respiratory syndrome coronavirus 2; **sgRNA**, Single guide RNA; **SPIAs**, Solid-phase immunoassays; **ssRNA**, Single-stranded RNA.

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In this century, the CoV has become one of the major pathogens of emerging respiratory disease epidemics such as the severe acute respiratory syndrome (SARS) in 2002 and the Middle East respiratory syndrome (MERS) in 2012. In December 2019, the SARS-CoV-2 was identified as a novel coronavirus, from cases of pneumonia diagnosed in Wuhan, Hubei Province, China, that later was established as Coronavirus Disease 2019 (COVID-19). In March 2020, the COVID-19 outbreak received recognition as a pandemic by the World Health Organization (WHO). To date, COVID-19 has already affected more than 512 million people, leading to around 6.2 million deaths [3–5].

The clinical presentation of COVID-19 is extremely diverse, especially since new mutations arise, ranging from asymptomatic status to SARS and multiple organ failure. Common symptoms include fever, dry cough, sore throat, shortness of breath, fatigue, myalgia, nausea/vomiting or diarrhea, headache, weakness, and runny nose. Anosmia or ageusia may be the only presenting symptom in approximately 3% of individuals with COVID-19 [5,6]. Respiratory droplet transmission is the main route of infection, and it can also be transmitted through aerial droplets and contact. Moreover, asymptomatic cases play a critical role in the transmission process. To date, although some drugs have been approved for the treatment of COVID-19, there is no consensus on an effective therapy regimen that might be undertaken on large scale for the population. Although the vaccination campaign is advanced, the key management of COVID-19 patients includes early diagnosis, immediate patient isolation, and protective conditions to prevent the infection [1,7,8].

Identifying the asymptomatic cases remains the main challenge preventing the dissemination of SARS-CoV-2 infection. Early diagnosis can prevent the virus from spreading and control the appearance of possible new waves of COVID-19. Moreover, one of the most important aspects of effective treatment in a pandemic is the early and rapid detection which can significantly improve a patient's prognosis. Therefore, efforts have been made to improve the diagnostic techniques already used and to develop fast, specific, and sensitive new methods to detect SARS-CoV-2, especially in point-of-care tests [4,8–11]. Point-of-care testing (POCT) are used to diagnose patients without sending samples to centralized facilities, thereby enabling communities without laboratory infrastructure to detect infected patients. Besides being cost-effective, POCT enabled population-wide mass screening with shorter response times [12,13]. Although different types of POC devices have been authorized in various countries for emergency use, many novel biosensing strategies and designs still seek validation and efforts are still being made in order to develop new devices with different techniques [13].

In this review, we have focused on the advances in the diagnosis field since the pandemic started, emphasizing laboratory-based techniques for detecting COVID-19. We reviewed the main existing diagnostic methods, as well as POCT under development, starting with RT-PCR detection, but also exploring other nucleic acid techniques, immunoassay tests, and nanoparticle-based tests, developed as portable instruments for the rapid standard diagnosis of COVID-19.

2. SARS-CoV-2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are enveloped viruses with a positive single-stranded RNA genome. SARS-CoV-2 viral particles (Fig. 1) are spherical to pleomorphic with an average diameter ranging from 65 to 125 nm. Inside the particle, the viral RNA, with about 30 K nucleotides, is tightly curled and coated by the nucleocapsid (N) protein. The genome encodes four structural proteins including Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N) proteins, six accessory proteins with open reading frames (ORF), and 16 nonstructural proteins (NSP1 −NSP16). Three glycoproteins, called spike (S), membrane (M), and envelope (E), are embedded in the lipid bilayer. SARS-CoV-2 shares 79 % genome sequence identity with SARS-CoV and 50 % with MERS-CoV [14–17].

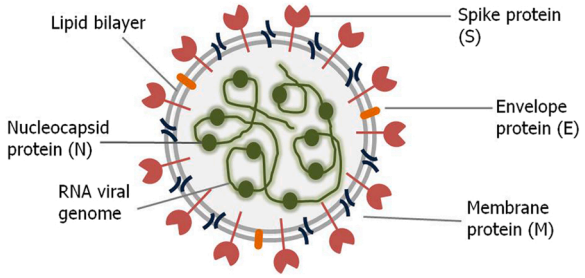

Fig. 1. Schematic representation of SARS-CoV-2 virus structure. The virus has four structural proteins, S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins. The N protein holds the RNA genome while the S, E, and M proteins together create the viral envelope.

The M protein is the most abundant viral protein present in the viral particle, providing a definite shape to the envelope. It binds to the nucleocapsid and acts as a central organizer of coronavirus assembly. The coronavirus E protein is the smallest of the major structural proteins. It plays a multifunctional role in the pathogenesis, assembly, and release. It is a small integral membrane polypeptide (ranging from 8.4 to 12 kDa) and functions as an ion channel. The inactivation or absence of this protein is related to the altered virulence of coronaviruses due to changes in morphology and tropism. The N protein of coronavirus is multipurpose. Among several functions, it plays a role in complex formation with the viral genome, facilitates M protein interaction needed during virion assembly, and enhances the transcription efficiency of the virus [18–20]. Coronavirus S protein is a large, multifunctional class I viral transmembrane fusion glycoprotein, divided into two functionally distinct parts (S1 and S2). The S proteins reside on the virion surface, giving the virus a crown-like appearance. Functionally these proteins are required for the entry of the infectious virion particles into the host cell through interaction with various cellular receptors. The exposed surface contains the receptor-binding domains that specifically engage the host cell receptors, thereby determining virus cell tropism and pathogenicity. The transmembrane S2 domain contains a heptad repeat region and the fusion peptide, which mediate the fusion of viral and cellular membranes upon extensive conformational rearrangements [18,21,22].

The envelope spike protein of the SARS-CoV-2 recognizes the human angiotensin-converting enzyme II (ACE2) as an entry receptor, and preferentially infects lung epithelial cells. The receptor-binding domain of the spike protein latches onto the ACE2 receptor, and then the host TMPRSS2 protease cleaves the spike protein to expose fusion peptides that are in turn able to fuse the viral and cell membranes. Once the SARS-CoV-2 enters a human cell, the virion releases its RNA in the cytoplasm. Translation and replication occur, and new virions are then released from the cell through exocytosis, as briefly schematized in Fig. 2 [23,24]. The structural proteins constitute the mature virion, whereas the nonstructural proteins of SARS-CoV-2 are indispensable for viral replication and transcription. The substitution, deletion, and insertion of amino acid sites, in spike protein and the ORF of SARS-CoV-2, led to many virus variants. These mutations may alter the virus biological characteristics, including increasing transmissibility and generating immune escape from innate or acquired immune responses. During the COVID-19 pandemic, genetic variants of SARS-COV-2 began emerging and spreading around the world. Among them can be mentioned B.1.1.7 (alpha), B.1.1.529 (omicron), B.1.351 (beta), B.1.617.2 (delta), and P.1 (gamma) [17,25].

The emergence of the alpha, beta, and delta SARS-CoV-2 variants were associated with new waves of infections. In the early stages of the pandemic, the delta variant become the globally dominant variant mostly due to its increased transmissibility and its ability to escape from natural immunity. The omicron variant, on the other hand, is the most heavily mutated variant among all the variants of concern so far. The omicron variable is able to spread way more easily from person to

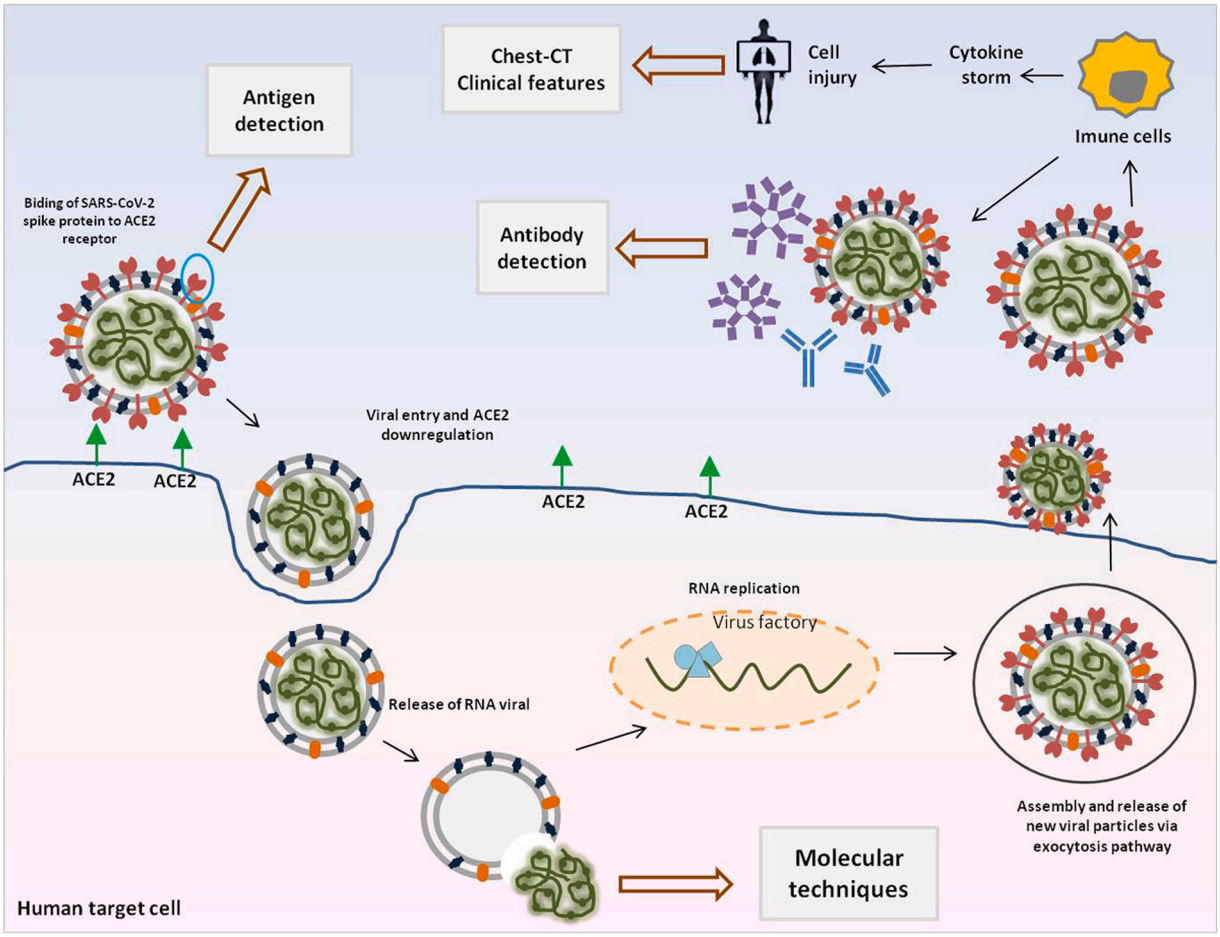

Fig. 2. SARS-CoV-2 virology (simplified, not to scale) and targets for diagnostic methods. SARS-CoV-2 binds to ACE2 receptor in human target cells and subsequently is internalized by endocytosis. Finally, the viral RNA is released for replication and translation by the host cell machinery and further assembly and exocytosis of new viral particles. The main diagnostic targets will depend on virology phase and they could be: molecular techniques, antibody or antigen detection and chest-ct and clinical features.

person and rapidly became the catalyst for the fourth wave of the COVID-19 outbreak. The omicron variant emerged at a time when vaccine immunity was increasing in the world, reducing disease severity and number of deaths [26,27].

Once the infection is clinically manifested, viremia has already occurred. As the disease progresses, the virus affects other organs that mainly express ACE2 receptors (e.g., the heart and its blood vessels, the kidneys, and the gastrointestinal tract). Thus, further disease progression and systemic organ damage in patients with severe pulmonary symptoms tend to occur. Cytokine storms, characterized by strong inflammatory responses because of immunological threats, likely contribute to acute respiratory distress syndrome and systemic organ dysfunction [28]. Delayed release of cytokines and chemokines occurs in respiratory epithelial cells, dendritic cells (DCs), and macrophages at the early stage of SARS-CoV-2 infection. Later, the cells secrete low levels of the antiviral factors interferons (IFNs) and high levels of proinflammatory cytokines and chemokines. Delayed release of IFNs in the early stages of the infection hinders the body's antiviral response. Afterward, the rapidly increased cytokines and chemokines attract many inflammatory cells, such as neutrophils and monocytes, resulting in body damage [29].

Different diagnostic methods have been used since the beginning of the pandemic. Detection techniques leverage the identification of unique surface markers on the virus, as summarized in Fig. 2. The current methods used in diagnosis can identify the specific viral gene regions through nucleic acid amplification techniques [Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) and isothermal nucleic acid amplification], the antibodies produced by the immune system in response to the viral infection (serology/Immunoglobulin M (IgM)/Immunoglobulin G (IgG) tests), and the antigen testing by lateral flow assays. Furthermore, cell injury leading to organ dysfunction can be useful in clinical diagnosis as anamnesis or chest computed tomography (chest-CT) [29–31].

3. Molecular detection methods

Nucleic acid amplification tests (NAAT) are the most sensitive molecular assays. Generally, they are preferred to detect early viral infections since viremia is usually observed in the beginning of the course of the disease. The molecular tests developed to date are reverse transcriptase real-time PCR (RT-qPCR), digital PCR, loop-mediated isothermal amplification-based assay (RT-LAMP), clustered regularly interspaced short palindromic repeats (CRISPR), and next-generation sequencing (NGS). Table 1 summarizes the advantages and disadvantages of these molecular techniques [32–35].

Conventional NAAT begins with RNA extraction from respiratory specimens obtained by nasal swab, followed by purification, amplification, and detection. These steps usually take a long time and requires three reagent kits: one for the RNA extraction, one for cDNA synthesis (since SARS-CoV-2 is an RNA virus), and another for the amplification and detection of the target nucleic acid. Therefore, simplification of NAAT by removing the RNA extraction step is being quite explored.

Table 1
Summary of advantages and disadvantages of main used NAAT techniques.

Diagnostic technique	Advantages	Disadvantages	Point of care?
RT-qPCR	1. Gold standard 2. Universal protocol	1. High costs of thermocyclers 2. Possible false negatives results 3. RNA extraction is required 4. Specialized technical training	1. Yes.
Digital RT-PCR	1. Absolute quantification 2. Higher precision, stabilility and sensitivity than RT-qPCR	1. High cost 2. Long operation process and not automated	No. Laboratory-based.
RT-LAMP	1. Colorimetric assays 2. High amplification rate 3. RNA extraction is not mandatory 4. Not requires high-end equipment	1. Very specific primer design 2. Optimize reaction conditions is not easy 3. Possible false negatives results	1. Yes. Results can be visualized by the naked eye or simple mobile phone cameras.
CRISPR	1. Sensitive, fast, specific 2. Colorimetric assay 3. Low-cost, portable, easy to use 4. Do not require complex devices	1. Few standardized assays are available 2. Complicated to manufacture	1. Yes. Results can be visualized by the naked eye, LED or UV lamps, or by observing the lateral flow strips.
NGS	1. Convenient, high sensitivity 2. Suitable for detecting samples with low viral load 3. Virus genome sequencing 4. Detection of new variants	1. Sophisticated instruments, increased cost 2. Trained person 3. RNA extraction is required	1. No. Laboratory based

Avoiding RNA extraction is advantageous, once skipping this step would provide rapid, high-throughput results with minimal hands-on time and less contamination [32–35].

3.1. Next-generation sequencing (NGS) based methods

The methods of next-generation sequencing vary in their technical mechanisms, but all share the basic defining features. This technique relies on samples being broken down into fragment libraries that are each amplified and sequenced independently, generating millions of fragment reads (small sequences) that can be pieced together to generate a readout of the genome [36]. NGS sequencing technologies have rapidly become the method of choice for various applications in virology, including the identification of novel viruses from metagenomic samples, the reconstruction of complete or nearly complete viral genome sequences, and the analysis of viral evolution [37]. Recent experience with emerging infectious diseases, such as SARS, MERS, Zika, and Ebola, has demonstrated that NGS technologies represent powerful tools for tracing the origins, spread, and transmission chains of outbreaks and monitoring the evolution of the etiological agents [36].

One of the most relevant advantages of NGS-based approaches is that full-length viral genomes can be reconstructed even for unknown or poorly characterized viruses, starting from culture-enriched viral preparations or directly from clinical samples [36]. This method plays an essential role in the early diagnosis and informs not only about the presence of the virus but also detects if the pathogen underwent genetic variants or not. It is not as quick as other methods in providing results, however, NGS-based technologies have aided in the rapid identification of emerging novel RNA viruses via RNA-Seq, such as the first appearance of SARS-CoV-2 and its novel variants [38].

In late January 2020, Lu et al. reported SARS-CoV-2 genomic data from nine patients presenting with pneumonia of unknown origin at three hospitals in Wuhan, China. Since then, several NGS-based COVID-19 kits were developed for the observation of genetic variants in the viral population. In addition to diagnosis, NGS data have also been using to aid in understanding the attributes, processes, and phylogenetics of the SARS-CoV-2 virus [37].

3.2. Reverse Transcriptase Real-Time PCR (RT-qPCR)

RT-qPCR is a gene amplification process that allows precise quantification of specific nucleic acids in a complex mixture by fluorescent detection. In general, RT-qPCR is the process of reverse RNA transcription into a complementary DNA (cDNA) and then designing primers and a fluorophore-quencher probe to amplify specific parts of the cDNA and obtain quantified results about the presence of the virus. This process first begins with the extraction of RNA from the upper or lower respiratory tract, followed by incubation with all necessary reagents, including primers, a fluorophore-probe, and reverse transcriptase. In RT-qPCR, the mixture is put in a thermocycler, which has a series of temperatures and time periods set up. In each cycle, the cleavage of the fluorophore-quencher probe results in a fluorescent signal which is detected by the thermocycler to give information on the process in a real-time manner [4,7,9,10,39–44]. The RT-qPCR is the gold standard for confirming COVID-19 in upper respiratory samples (nasopharyngeal and oropharyngeal secretion). Several RT-PCR protocols were released by WHO to provide a proper diagnosis, help testing populations and contribute to controlling the spread of the disease. The protocols distinguish from each other mainly in the gene target, as summarized in Table 2 [4,7,9,10].

There are disadvantages to these highly sensitive approaches. Specifically, the assays are performed by specially trained technicians and utilize costly reagents and thermocycling equipment. Perhaps of greater concern is that detection is dependent upon designing primer / probes that recognize a specific portion of the protein of interest. The occurrence of RT-qPCR false-positive results is associated with handling errors and cross-contamination of samples, while false-negative results are related to the incorrect sample collection, storage, and processing [39–44]. Conventional end-point RT-PCR could serve as an alternative for SARS-CoV-2 detection, mainly when RT-qPCR is unavailable. Junior et al., described a sensitive and specific protocol for detecting the SARS-CoV-2 E gene through one-step end-point RT-PCR (conventional RT-PCR) in order to increase the diagnostic coverage of COVID-19, mainly in developing countries. The performance of the RT-PCR was evaluated in a combination of two nasopharyngeal and one oropharyngeal swab samples, in a total of 43 samples, of which 10 and 33 were

Table 2
Summary of available RT-PCR protocols authorized by WHO for COVID-19 diagnosis.

Institute	Gene target
China CDC, China	ORF1ab, E and N
Institut Pasteur, France	Two targets in RdRP
US CDC, USA	N1, N2, RdRP
National Institute of Infectious Diseases, Japan	Pancorona and multiple targets, Spike protein
Charité , Germany	RdRP, E
HKU, Hong Kong SAR	ORF1b-nsp14, N
National Institute of Health, Thailand	N

previously identified as negative and positive, respectively. End-point RT-PCR detected 32/33 of positive samples. The developed RT-PCR platform may be a viable option for molecular detection of SARS-CoV-2 in laboratories without access to more highly specialized RT-qPCR equipment [44].

The current challenges of the qRT-PCR method include the use of fluorescent label binding to the source signal produced by the amplified DNA, which not only increases the cost of the instrument, but also the complexities. However, efforts have been directed toward miniaturizing PCR to make it an automated, high-throughput device that can be applied at point-of-use [45]. Gibani and co-workers (2020) developed a POCT RT-PCR platform, to provide true sample-to-answer multiplex RT-PCR diagnosis of SARS-CoV-2, without the need for any laboratory facilities and trained personnel. In this device, a nasopharyngeal or oropharyngeal swab is immediately inserted directly into the swab chamber of the sample preparation unit at the time of collection. Seven SARS-CoV-2 gene targets (rdrp1, rdrp2, e-gene, n-gene, n1, n2 and n3) are used as well as a human ribonuclease P (RNaseP) as sample adequacy control. The overall sensitivity of the POCT compared with laboratory-based testing was 94% with an overall specificity of 100 %. This platform, called CovidNudge, has been implemented in UK hospitals since 2020 [46].

At the moment, there are about 220 SARS-CoV-2 RT-PCR tests authorized by the FDA for emergency use. Tests are available to perform in laboratories certified that meet requirements to perform high and moderate complexity tests, or for patient care (without a prescription), which include "DTC" (for direct-to-consumer home collection tests) or "OTC" (for over-the-counter at-home tests). Table 3 summarizes the latest RT-PCR approved tests [47].

3.3. Digital PCR (dPCR)

Despite RT-qPCR being the current standard method for diagnosis of SARS-CoV-2 infection, due to low viral load in some patients, false-negative have been widely reported. Because recurrence and asymptomatic COVID-19 patients usually have a very low viral load, a more sensitive detection method is urgently needed to improve the accuracy in identifying SARS-CoV-2 infected patients, to effectively prevent the virus transmission [13,48–50]. To achieve this goal, digital PCR (dPCR) has been developed worldwide. The principle of dPCR is to partition the reaction mixture into many sub-reactions prior to amplification. The original numbers are determined by counting the partition showing negative and positive reactions. This method does not require a standard curve or reference genes and is more resistant to interference factors such as specific template amplification inhibitors, which can reduce the false-negative results. The dPCR method can be classified into three types based on liquid separation: droplet-based (ddPCR), chip-based (cdPCR), and microfluidic digital PCR (mdPCR). dPCR can be used to quantify a low viral load, monitor the virus in the environment, evaluate anti-SARS-CoV-2 drugs, and detect genetic variants. Importantly, dPCR could be considered a POCT, being a more convenient and faster test, making possible a quicker diagnosis. However, it requires more sophisticated equipment, limiting its use more broadly. The commercially available ddPCR kits are intended for use by qualified clinical laboratory personnel specifically trained and instructed in ddPCR techniques and in vitro diagnostic procedures [13,48–50].

Kock and coworkers (2020) developed a sensitive one-step droplet digital RT-PCR (RT-ddPCR) multiplex assay for simultaneous detection of multiple SARS-CoV-2 genes N (N1 + N2), E, and RdRp, including the detection of patient-derived mRNA of a housekeeping gene to assure sample and assay quality, along with to enable quantification of viral RNA. Results showed that RT-ddPCR was more sensitive than the gold standard RT-qPCR in the clinical setting. As ddPCR enables absolute quantification, not only the viral RNA can be quantified, but also the mRNA from endogenous genes, which can be used to set validity criteria and to ensure reliable analysis [49]. Expanding on this paradigm, Dong and coworkers (2021) developed one-step reverse transcription digital PCR (RT-dPCR) method to detect the ORF1ab, protein N and E gene of SARS-CoV-2. They compared RT-qPCR and RT-dPCR on 196 clinical samples and found that RT-dPCR can significantly improve the sensitivity and diagnostic accuracy of COVID-19 [51].

Park and coworkers (2021) tested RT-qPCR and droplet digital PCR (ddPCR) to detect low amounts of viral RNA. The study showed that the cycle threshold (CT) of the viral RNA by RT-qPCR significantly varied according to the sequences of the primer and probe sets with in vitro transcript (IVT) RNA or viral RNA as templates, whereas the copy number of the viral RNA by ddPCR was effectively quantified with iVT RNA, cultured viral RNA, and RNA from clinical samples. Regarding the clinical samples, the sensitivity of the ddPCR was determined to be equal to or more than that of the RT-qPCR. Moreover, the ddPCR assay is more suitable for determining the copy number of the reference materials. These findings suggest that the qPCR assay with the ddPCR defined reference materials could be used as a highly sensitive and compatible diagnostic method for viral RNA detection [50]. Sun and coworkers (2021) compared the sensitivity and specificity of dPCR with RT-qPCR on simulated and clinical sputum samples. The results showed that dPCR was more sensitive than qPCR, especially for samples with low viral load ($\leq$3 copies). In addition, dPCR had similar specificity as qPCR and could effectively distinguish other human coronaviruses and influenza viruses from SARS-CoV-2. More importantly, dPCR was more sensitive than qPCR in detecting the virus in the “negative” samples from recurrent COVID-19 patients [48].

Table 3
Latest RT-PCR approved tests for emergency use by FDA.

Entity	Attributes	Authorized settings
Helix OpCo LLC (dba Helix)	Real-time RT-PCR, Home Collection, Multiple Targets	H
SML GENETREE Co., Ltd.	Real-time RT-PCR, Multiple Targets	H
Clinical Research Sequencing Platform (CRSP), LLC at the Broad Institute of MIT and Harvard	Real-time RT-PCR, Home Collection, Pooled Serial Screening - Swab, Multiple Targets	H
BioFire Defense, LLC	RT, Nested multiplex PCR, Pooling, Saliva, Multiple Targets	H,M
MiraDx	Real-time RT-PCR, Multiple Targets	H
UCSD BCG EXCITE Lab	Real-time RT-PCR, Home Collection, Screening, Multiple Targets	H
Nexus Medical Labs, LLC	Real-time RT-PCR, Home Collection, Multiple Targets	H
Laboratory Corporation of America (Labcorp)	Direct to Consumer (DTC), Real-time RT-PCR, Multi-analyte, Home Collection, Single Target	H
Cepheid	Real-time RT-PCR, Screening, Multiple Targets	H,M,W
LGC, Biosearch Technologies	Real-Time and End-Point RT-PCR, Multiple Targets	H

Abbreviations: H: Laboratories certified that meet requirements to perform high complexity tests; M: Laboratories certified that meet requirements to perform moderate complexity tests; W: Patient care settings operating under a CLIA Certificate of Waiver.

3.4. RT-LAMP

The loop-mediated isothermal amplification (LAMP) technique has proven to be a rapid molecular biology analytical tool for detecting the viral target and a reverse transcription step is required (RT-LAMP). The technology relies on amplifying the target nucleic acid in a single-step based on the PCR method, at a constant temperature (usually 60ºC), offering an effective platform for diagnosing viral diseases. The LAMP

reaction has three steps: initiation, cycling amplification, and elongation. In this technique, 4–6 specifically designed primers are used to detect distinct nucleic acid sequences. Moreover, there is no requirement for initial template denaturation and the reaction time is minimized by up to 30 min using strand-displacement polymerases. RT-LAMP allows visualization of amplified products via either fluorescence, under a UV lamp or color with the naked eye. For a colorimetric-based analysis, the reaction mixture is often added with hydroxynepthol blue (HNB) before amplification [4,32,40]. The approach is simple to operate, easy to visualize for detection, and has less background signal. In addition to being fast, specific, and sensitive, the method does not require skilled personal or high-end equipment. LAMP-based protocols enable the efficient amplification of nucleic acids at a single point temperature. This feature makes it a strong contender for direct field applications, since incorporating the thermal cycling steps in PCR assays has traditionally been a significant limitation for point-of-care devices [35]. The drawbacks to RT-LAMP are the challenges of optimizing primers and reaction conditions [4,12,40].

In the field of rapid detection and POCT, the Abbott ID Now™ COVID-19 test assay can detect antigens in < 15 min. This molecular POCT was the first to use isothermal nucleic-acid amplification technology to specifically detect SARS-CoV-2 RNA by fluorescence. The added advantage of this technique is its portability and light weightiness, which enable its smooth transport to different locations. The main components are the reaction tube (which contains the reagents required for amplification of SARS-CoV-2, as well as an internal control), and the device. The templates (similar to primers) designed to target SARS-CoV-2 RNA amplify a unique region of the RdRp segment. Fluorescently-labeled molecular beacons are used to specifically identify each of the amplified RNA targets. To perform the assay, the sample and the reaction tube are inserted into the ID NOW Instrument: amplification, heating, mixing, and detection are provided by the instrument, making the test fast and easy to perform [30].

Colorimetric RT-LAMP assays have been drawing attention to detecting DNA produced by the reaction. One possibility is using a pH indicator (phenol red, crystal violet) and running the reaction in a weakly buffered environment. As the chain reaction proceeds, the pH is lowered, which results in a visible color change from red to yellow making it an appealing assay for point-of-care diagnosis. The results can be visualized by the naked eye or simply mobile phone cameras, copy machines, office scanners, or plate scanners with spectrophotometric quantification can also be used [52,53]. Thi and coworkers (2020) developed a two-color RT-LAMP assay protocol for detecting SARS-CoV-2 viral RNA using a primer set specific for the N gene, on RNA samples isolated from 768 pharyngeal swab specimens. Results showed that the RT-LAMP assay reliably detected SARS-CoV-2 RNA with an RT-qPCR cycle threshold (CT) up to 30, with a sensitivity of 97.5 % and a specificity of 99.7% [53].

In a parallel platform, Nawattanapaiboon and coworkers (2020) developed a visual diagnostic platform methodology for SARS-CoV-2 based on colorimetric RT-LAMP with levels of sensitivity and specificity comparable to that of commercial qRT-PCR assays. In the assay, the primers were designed to target a conserved region of the RNA-dependent RNA polymerase gene (RdRp) and the diagnostic performance of the developed RT-LAMP assay was evaluated in 2120 clinical specimens and results revealed high sensitivity and specificity of 95.74 % and 99.95 %, respectively [52]. Alves and coworkers (2021) developed a pH-dependent colorimetric reverse transcription loop-mediated isothermal amplification (RT-LAMP) method for SARS-CoV2 detection. The method revealed a limit of detection of 19.3 ± 2.7 viral genomic copies/μL when using RNA-extracted samples obtained from nasopharyngeal swabs collected in guanidine-containing viral transport medium. The test, called OMNILAMP, proved to be sensitive and specific, and comparable with RT-PCR. The method meets POCT requirements and has already received authorization from the National Health Surveillance Agency (ANVISA) for clinical use in Brazil [54].

RNA isolation is time-consuming, costly, and depends on reagents with potentially limited supply during a pandemic. Alternative, noncommercial solutions for RNA isolation, e.g., using silica gel matrix or magnetic beads, require specialized knowledge and cannot be implemented easily for point-of-care or decentralized screening [53]. In this sense, Mautner and coworkers (2020), developed an RT-LAMP-based method to detect SARS-CoV-2 genes ORF8 and N directly from pharyngeal swab samples, in the absence of time-consuming and laborious RNA extraction. The assay is sensitive and highly specific for SARS-CoV-2 detection, showing no cross-reactivity when tested on 20 other respiratory pathogens. The assay is 12 times faster and 10 times cheaper than routine reverse RT-qPCR. The proposed test costs less than 2 euros per reaction and the device is about 10,000 euros, which is about seven times cheaper than a routinely used real-time thermal cycler for RT-qPCR tests [55].

Garneret and coworkers (2021), developed a portable and low-cost molecular test, that combines RNA extraction, RT-LAMP, and naked eye visualization capability on the same device. The performance was evaluated in nasopharyngeal swabs samples. Results indicate that, with minimal equipment, which can extract, wash, elute, reverse-transcribe, amplify, and measure the kinetics, with a limit of detection (LoD) comparable to the gold standard real-time RT-PCR, i.e. one genome copy per microliter of a clinical sample, and a specificity of 100% [56]. In separate studies, Taki and coworkers (2020) compared the utility of saliva and nasopharyngeal swab samples to detect SARS-CoV-2 by a novel RT-LAMP method since saliva is a non-invasive specimen more suitable for mass screening. Their results showed that RT-LAMP detects SARS-CoV-2 as effectively as PCR. The efficacy of nasopharyngeal swab and saliva is equivalent to detecting SARS-CoV-2, but RNA extraction process is essential for better detection of SARS-CoV-2 particularly in saliva [57].

Given saliva is less invasive. Lali et al., (2021) developed a rapid colorimetric assay using RT-LAMP optimized on human saliva samples without an RNA purification step. They developed a saliva pretreatment protocol to enable analytically sensitive viral detection. They demonstrated that the pretreatment protocol allowed analytically sensitive extraction-free detection of SARS-CoV-2 from saliva samples by colorimetric RT-LAMP or RT-qPCR. RT-LAMP assay had a limit of detection of 59 particle copies per reaction, with specificity and sensitivity of 100% and 85%, respectively. [58].

3.5. CRISPR

Recently, clustered regularly interspaced short palindromic repeats (CRISPR) have received substantial attention for nucleic acid detection due to its simplicity, speed, high sensitivity, and specificity. CRISPR are the DNA sequences found in bacteria and archaea that have been extensively used in gene editing experiments. They play an important role in antiviral defense as the sequences are derived from bacteriophages that have previously infected bacteria [59,60]. CRISPR-based methods utilize enzymes with nonspecific DNase or RNase activity, such as Cas9, Cas12, or Cas13, from the bacterial immune system, along with guide RNAs to direct enzyme binding to specific target areas on pathogenic DNA or RNA sequences [61].

There are two components in the CRISPR detection: first, the CRISPR-RNA complex will cut the target region, which activates the next step, collateral cleavage of the surrounding nucleic acids. CRISPR Cas proteins have a unique collateral cleavage ability, enabling these tools to indiscriminately cleave surrounding nucleic acid once they bind to the target site. By introducing appropriate nucleic acid reporters (ssDNA, ssRNA, dsDNA), different detection signals (e.g., fluorescent, colorimetric, electrochemical) can be specifically registered [60]. Because of this collateral cleavage activity, CRISPR can be combined with isothermal nucleic acid amplification to simplify the detection method by visualizing the result of positive or negative samples with the naked eye, LED or UV lamps, or by observing the lateral flow strips. The

combination of isothermal amplification and CRISPR improves the sensitivity and specificity because the crRNA only binds to the target region [60].

The combined approach of CRISPR-associated proteins (Cas) and lateral flow chemistry provide the platform to develop definitive, highly specific, rapid, and cost-effective diagnostic kits. This technology has been used in the development of Zika virus, human papillomavirus, and Dengue virus molecular diagnostic kits and is explored by various workers across the world for SARS-CoV-2 diagnosis. Some notable examples of CRISPR-based assays for rapid diagnosis of SARS-CoV-2 are SHERLOCK, DETECTR, AIODCRISPR, CASdetec, ENHANCE, and FELUDA [38] RNA extraction from clinical samples is required for all CRISPR-based assays. Then, these methods differ from each other in the RNA amplification, Cas proteins, and detection signal. The differences among the techniques are summarized in Table 4 [38].

According to the organization of effector protein, the systems are divided into two major distinct classes, termed Class 1 and Class 2. Class 2 systems utilize single-protein effectors and are divided into several subtypes, including Cas9, Cas12, and Cas13. Cas9 recognizes and cleaves the specific double-stranded DNA (dsDNA) under the guidance of a single guide RNA (sgRNA). Cas12a is a programmable RNA-guided DNA nuclease without the requirement of trans-activating crRNA for crRNA maturation, and it recognizes a T-rich PAM for dsDNA cleavage. Cas13a is an RNA-guided endoribonuclease (endoRNase) that is programmed to recognize and degrade single-stranded RNA (ssRNA) targets carrying complementary sequences [62].

The RNA sample can be amplified using reverse transcription–recombinase aided amplification (RT-RAA), reverse transcription-recombinase polymerase amplification (RT-RPA), or RT-LAMP. RPA and LAMP conveniently eliminate the need for a labor-intensive, temperature-sensitive PCR method because these techniques operate under isothermal conditions. In addition, detection by lateral flow, UV or LED lamps could be employed, which makes easier the development of fast and inexpensive POCT [59].

Since CRISPR-based diagnostic methods can be performed with simple equipment, without requiring extensive technical expertise, they may be used outside centralized laboratories, including airports, clinics, and resource-limited settings. These methods are fast, low-cost, portable, easy to use, highly sensitive and specific, and do not require complex devices [59]. To this end, Sun and coworkers (2021) developed a one-tube detection platform based on RT-RPA DETECTR technology, termed OR-DETECTR, to detect SARS-CoV-2 by lateral flow assay. Clinical samples were used to validate the platform and all results were compared to rRT-PCR. Results showed that the OR-DETECTR detection process could be completed in one tube, in approximately 50 min. This method can specifically detect SARS-CoV-2 from seven human coronaviruses and Influenza A (H1N1). Results of six samples from SARS-CoV-2 patients, eight samples from patients with fever but no SARS-CoV-2 infection, and one mixed sample from 40 negative controls showed that OR-DETECTR could detect SARS-CoV-2 with the detection limit of 2.5 copies/μL input, and it is 100% consistent with rRT-PCR [25].

Fozouni and coworkers (2021) developed an amplification-free CRISPR-Cas13a assay for direct detection of SARS-CoV-2 from nasal swab RNA that can be read with a mobile phone. The assay achieved ~100 copies/mL sensitivity in under 30 min of measurement time and accurately detected pre-extracted RNA from a set of positive clinical samples in under 5 min. The fluorescence was measured with a mobile phone camera in a compact device that includes low-cost laser illumination and collection optics. This approach has the potential to enable a fast, accurate, portable, and low-cost option for point-of-care SARS-CoV-2 screening [63].

Puig and coworkers (2021) described the development of a low-cost, self-contained, POCT called miSHERLOCK (minimally instrumented SHERLOCK) capable of concurrent universal detection of SARS-CoV-2 as well as specific detection of the B.1.1.7, B.1.351, or P.1 variants. miSHERLOCK combines instrument-free, built-in sample preparation from saliva, room temperature stable reagents, battery-powered incubation, and simple visual and mobile phone–enabled output interpretation with a LoD) that matches U.S. Centers for Disease Control and Prevention (CDC) RT-qPCR assays for SARS-CoV-2 of 1000 copies (cp)/mL [64]. Wang and coworkers (2021) proposed a one-pot visual SARS-CoV-2 detection system named opvCRISPR by integrating RT-LAMP and Cas12a cleavage in a single reaction system. The opvCRISPR enabled detection at the nearly single-molecule level in 45 min. The method was validated with 50 SARS-CoV-2 potentially infected clinical samples. The opvCRISPR diagnostic results provide 100% agreement with the CDC-approved RT-qPCR assay. The opvCRISPR showed great potential for SARS-CoV-2 detection in next-generation point-of-care molecular diagnostics [65].

Table 4
Summary of CRISPR-based assays being developed for the detection of SARS-CoV-2.

	Amplification	Cas	Detection	Viral target
SHERLOCK	RT-RPA	Cas13a	Fluorescence and lateral flow	S, N and Orf1ab gene
DETECTR	RT-LAMP	Cas12a	Lateral flow	N and E gene
AIOD-CRISPR	RT-RPA	Cas12a	UV or LED	N gene
ENHANCE	RT-LAMP	Cas12a	UV or LED	N gene
CASdetec	RT-RAA	Cas12b	Paper-based	RdRp gene
FELUDA	RT-RPA	Cas9a	Paper-based	Nsp8 and N gene

Abbreviations: SHERLOCK: Specific High-sensitivity Enzymatic Reporter unLOCKing; DETECTR: DNA Endonuclease Targeted CRISPR Trans Reporter; AIOD-CRISPR: All-inOne Dual CRISPR; CASdetec: CRISPR-Cas12b-mediated DNA detection; ENHANCE: Enhanced analysis of nucleic acids with crRNA (CRISPR RNA) extensions; FELUDA: FNCAS9 Editor-Linked Uniform Detection Assay.

4. Immunoassays

Immunoassays are diagnosis methods based on antigen-antibody bounds largely used to detect hundreds of biomolecules, including viral antigens (Ag) and antibodies (Ab) produced in response to viral infections. The method was described in the early 1960 s to detect insulin and thyrotoxin in human plasma [66,67]. However, it was refined across the decades with the development of monoclonal antibodies, dyes, amplification reactions, and detection systems [68].

Immunodiagnosis in virology is generally faster than the gold-standard methods, as molecular techniques, and is suitable for automation, POCT, and self-testing. In the COVID-19 pandemic, the rapid community spread of SARS-COV-2 and the needing to detect the viral infection easily in the population encourage the development of several immunoassay kits with different methodologies [69,70]. The solid-phase immunoassays (SPIAs) and lateral-flow immunochromatography (IC) are methodologies frequently used in COVID-19 diagnosis. SPIAs refer to the adsorption of an antigen (Ag) or antibody (Ab) on a solid phase such as a microplate or microparticles, and the positive reaction is detected by colorimetry in enzyme-linked immunosorbent assay (ELISA) or chemiluminescence in chemiluminescent immunoassays (CLIA). ELISA and CLIA are largely used in clinical routine due to the sensitivity and specificity performance and the full automation possibility. On the other hand, in the IC, Ab labeled with colloidal nanoparticles (Np) are adsorbed on the test strip and interact with the patient sample. The conjugate migrates by capillarity across the strip, reacting with the Ag or Ab of interest in the testing well (Fig. 3). Besides the poor sensitivity (percentage of true-positive tests in a populational sample) to low viral charge and Ab titles, IC has been the primary choice for POCT, self-test, and population triage for COVID-19 [69–71].

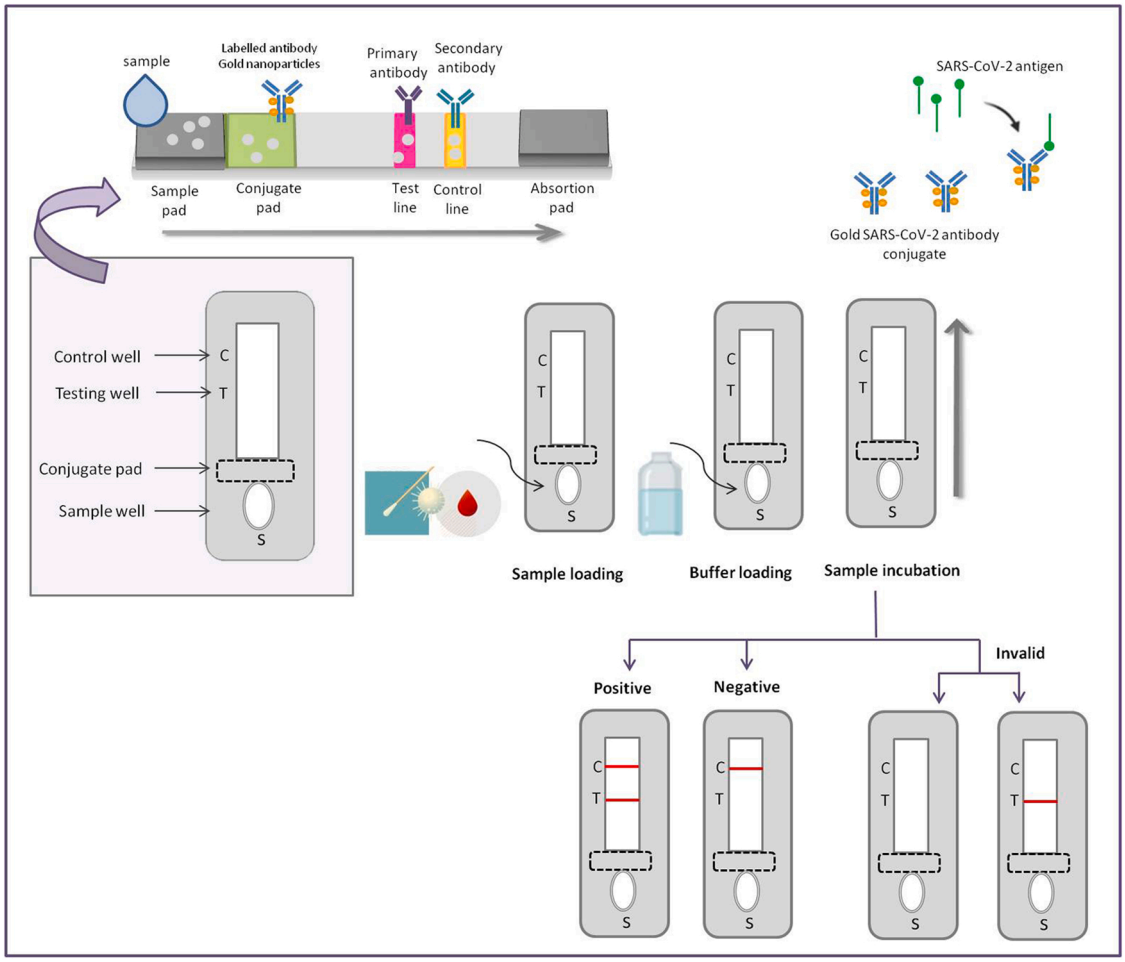

Fig. 3. Lateral flow immunoassay for COVID-19 detection (LFA). A simple LFA test strip consists of a sample pad, a conjugate pad, test line, control line, and an absorption pad. The sample containing the target analyte is absorbed by the sample pad and it moves toward the conjugate pad. The analyte can interacts with a specific antibody or antigen (labeled with a colored molecule) and forms a mobile conjugate which flows onto the nitrocellulose membrane. The conjugates which are complementary to the immobilized bioreceptors on the test and the control lines get captured. As a result, a change in the color of the lines can then be seen. The test is only valid if the control line is completely visible.

4.1. Antibodies-based immunoassays

In the early phase of the COVID-19 pandemic, the Ab-based tests were widely used to avoid RT-PCR false-negative results in hospitalized patients late in the disease course, when the viral charge decreased. This modality of immunoassays was also applied widely in epidemiological research, an important method to evaluate the virus spread in the population when the testing capacity was lacking to monitor asymptomatic cases and to analyze the infection prevalence (Silveira et al., 2020; Chen et al., 2021) [72,73]. The commercially available test detects immunoglobulins M (IgM), G (IgG), or both mainly in serum, plasma, or whole blood, produced in response to viral infection [70,74,75]. The median of seroconversion, occurs on day 10–13 after symptom onset for IgM and day 12–15 for IgG, with maximum seroconversion for IgM, IgG, and total Ab occurring after 2 weeks [76].

Zonneveld and colleagues, in 2021, validate six Ab-based tests, with different methodologies in comparison to RT-PCR tests. The overall sensitivity ranged between 70.6 % and 88.9 %, however, these values achieve 94.0 % at 10 days post symptoms onset. In similar studies, the mean sensitivity at 10 and 14 days post symptoms onset of commercially available immunoassay kits (ELISA, CLIA, and IC) was 62.7 % and 71.1 %, respectively. As expected, the sensitivity of tests is increased after 2 weeks after symptoms onset [74,77]. In addition, the specificity rate of these immunoassays was above 96%, indicating a high true-negative rate in patients with other respiratory diseases [74]. Performance of tested assays varied according to the methodology. Generally, ELISA and CLIA kits presented a better performance compared to IC tests. Therefore, despite contributing to detect the viral infection late in the course of the disease, the limited sensitivity in early SARS-CoV-2 infection limits the use of Ab-based immunoassays in COVID-19 diagnosis [70,74–77]. Additionally, another important use of Ab-based immunoassays is in vaccine development and the evaluation of its efficiency. It is important to note that as the vaccination rates increase in the population, antibody detection by commercially available methods such as IC should be interpreted with caution, as it may reflect the individual's vaccination status, rather than a response to the virus during acute infection [72,73]. On the other hand, the study of persistent serum neutralizing Ab (nAb) has gained importance. The nAb binding assays are SPIAs immunological methods to determine the capacity of nAb to prevent cell infection, by blockage of the virus binding site [78,79]. In convalescent or vaccinated individuals, the nAn dosage is important to understand the population protection half-life against SARS-CoV-2 infection, especially in the emergency of several variants of concern [80,81].

4.2. Antigen-based immunoassays

The poor availability of reagents, specialist staff, and facilities in many countries are drawbacks in performing molecular assays, thus, the development of Ag-based immunoassays to detect SARS-CoV-2 infection

was undertaken [82,83]. The Ag-based immunoassays detect viral nucleocapsid protein in nasopharyngeal secretion or saliva, reflecting active infection as RT-PCR [23,82,84]. There are hundreds of commercially available Ag-based tests, most of them using IC methodologies, however, SPIAs methodologies can also be employed. These kits are an alternative to fast population triage even in the early infection stage, controlling, and suppressing the spread of the virus [47,85]. Generally, the biggest disadvantage of Ag-based immunoassays is the lower sensitivity compared to molecular diagnostic tests. The clinical specimen, immunoassay methodology, and the number of viral copies in the sample could impact the assay performance [86,87]. Experiments comparing the PCR techniques and IC showed that the sensitivity of commercially available IC kits was 66.7–75,5 % for nasopharyngeal swabs, with a specificity of 94.9 % [88,89]. However, the test sensitivity can decrease to 23.1 % in saliva specimens [90]. Albert and collaborator (2021) described similar observation. In this study, a sensitivity of 79.6 % for IC kit was observed and this parameter was slightly increased in early clinical course patients. Nevertheless, the IC test sensitivity is lower in asymptomatic patients, limiting the use in close contact tracking [91].

5. Nanoparticle-based tests as new perspectives

Conventional detection methods used to diagnose COVID-19 have limitations, such as low sensitivity, a large time needed to announce results, high false-negative, and lack of specificity due to similarity with other viral diseases. Nanotechnology can be employed to overcome such drawbacks. Nanomaterials can be used as labels to achieve the significant enhancement of signals, high enough to be easily detectable [92,93].

Metallic (Au, Ag, and Cu) and magnetic nanoparticles, as well as polymeric nanoparticles and quantum dots, are among the main nanoparticles that have been implemented for coronavirus detection. These materials are characterized by colorimetric, electrochemical, fluorescence, and optical detection techniques [92]. Moreover, the size and shape of nanomaterials can be easily tailored, and, therefore, surface modification/immobilization with numerous biological species via covalent or non-covalent bonding is possible to enhance the biosensing characteristics in terms of low-detection limit (increased up to several orders of magnitude), high sensitivity, selectivity, and rapid response towards the sample analytes [93].

5.1. Biosensors

Nanobiosensors are devices in which the transducer is modified to capture the target element, convert the biological response into electrical signals, and quickly detect it with high accuracy. Nanobiosensors have the advantage of selectively detecting all types of analytes by combining the excellent electrical and optical properties of nanomaterials with biological or synthetic molecules used as receptors [94]. Table 5 summarizes some newly developed nanobiosensors for COVID-19 detection.

Biosensors are highly sensitive, cost-effective, and most suitable for diagnosing the disease, besides being an alternative tool for the POCT since they are ideal for providing continuous and real-time detection [95]. These devices primarily comprise three components: (a) the detector for perceiving the stimulus; (b) the transducer for converting the stimulus into a measurable signal; and (c) an output system that can

Table 5
Methods and properties of biosensors being used for the detection of COVID-19.

Nanomaterial	Target	Detection method	Type of sample	Remarks	Ref
Gold nanoparticles capped with antisense oligonucleotides	N-gene	Colorimetric	Nasal swab, nasopharyngeal swab, and oropharyngeal swab	Integrates nucleic acid (NA) amplification and plasmonic sensing for point-of-care detection	Alafeef et al. [108]
Carbon electrodes coated with gold nanoparticles	N-protein	Voltammetric	Nasopharyngeal	The electrodes were functionalized using 11-mercaptoundecanoic acid, which was used for the immobilization of an antibody against SARS-CoV-2 nucleocapsid protein	Eissa et al. [109]
Gold nanoislands functionalized with complementary DNA receptors	ORF1ab-COVID, and E genes from SARS-CoV-2	Localized surface plasmon resonance	SARS-CoV-2 Viral Sequences.	dual-functional plasmonic biosensor combining the plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR)	Qiu et al. [104]
Gold nanoparticles, capped with suitably designed thiol-modified antisense oligonucleotides (ASOs)	N-gene	Colorimetric	Oropharyngeal swab	The thiol-modified ASO-capped AuNPs agglomerate selectively in the presence of its target RNA sequence of SARS-CoV-2 and demonstrate a change in its surface plasmon resonance.	Moitra et al. [102]
Gold nanoparticles	Oligo probe	Colorimetric	Nasopharyngeal samples	Nanomaterial-based optical sensing platform to detect RNA-dependent RNA polymerase (RdRp) gene of SARS-CoV-2, where the formation of oligo probe-target hybrid led to salt-induced aggregation and change in gold-colloid color from pink to blue visibility range	Kumar et al. [110]
Gold rabbit IgG conjugate	IgM and IgG antibodies	Colorimetric	Human blood sample	IgG-IgM combined antibody test using lateral flow immune assay	Li et al. [111]
Graphene	SARS-CoV-2 antigen protein	Field-effect transistor (FET)	Nasopharyngeal swab	The sensor was produced by coating graphene sheets of the FET with a specific antibody against SARS-CoV-2 spike protein	Seo et al. [105]
Gold nanoparticles	Spike protein	Amperometric	Saliva	Fluorine doped tin oxide electrode (FTO) with gold nanoparticle (AuNPs) and immobilized with nCovid-19 monoclonal antibody (nCovid-19Ab) to measure a change in the electrical conductivity	Mahari et al. [112]
Polymeric nanoparticle coated with dye streptavidin	ORF1ab and N-gene	Colorimetric lateral flow biosensor	Oropharyngeal swab	Multiplex reverse transcription loop-mediated isothermal amplification (mRT-LAMP) coupled with a nanoparticle-based lateral flow biosensor (LFB) assay	Zhu et al. [107]
Lanthanide-doped polystyrene nanoparticles	IgG human antibody	Lateral flow immunoassay based on fluorescence biosensing	Human serum sample	A recombinant nucleocapsid phosphoprotein of SARS-CoV-2 was dispensed onto a nitrocellulose membrane to capture specific IgG. Mouse anti-human IgG antibody was labeled with self-assembled LNPs that served as a fluorescent reporter.	Chen et al. [113]

amplify and display the result in an appropriate form [96]. The principal structural proteins in SARS-CoV-2 that may be suitable targets for viral detection are spike (S) protein, membrane (M) protein, envelope (E) protein, and nucleocapsid (N) protein [97,98]. In these applications, the target molecule attaches to the bioreceptor to detect a biological molecule by a particular reaction. Then, the transducer with integrated nanostructures converts the detection into an electrical signal determined by the detector. The schematic diagram of different analytes, bioreceptors for biorecognition elements, and transducers with integrated nanomaterials used for biosensing, as parts of a typical nanobiosensor for respiratory viruses, are presented in Fig. 4 [99].

Different types of nanomaterials can be used in biosensors to increase their selectivity and accuracy. Carbon-based, metal-based (gold, silver), and quantum dots are the preferred materials. Carbon nanotubes have electrical conductivity, chemical stability, high surface area, fast heterogeneous and long-range electron transfer, excellent biocompatibility, and mechanical strength. Gold nanoparticles (AuNPs) have been intensively studied in the development of nanoassays for two reasons: ease of electrostatic surface-decoration with various moieties such as antigens and antibodies and; surface plasmon resonance shift and color changes. AuNPs have been commonly used in colorimetric hybridization assays. They have unique properties like simple and rapid synthesis, large surface area, strong adsorption ability, and facile conjugation to biomolecules. Silver nanoparticles are the most used metallic nanoparticles, particularly in biological detection, especially because of their physicochemical properties, strong adsorption, and good electric conducting properties. Other nanomaterials such as fluorescent nanoclusters and quantum dots also exhibit excellent optical properties, thereby improving sensing and disease diagnosis [93,97,99,100].

Semiconductor-based nanobiosensors also have wide applications in detecting analytes because of their surface potential and tunable fluorescence properties. They have unique photophysical, optical, catalytic, and electronic properties. The most commonly used semiconductors in the application of nanobiosensors include zinc oxide (ZnO). Nanostructures such as nanorods, nanobelts, nanodisks, nanoparticles, nanosheets, nanoporous, and radial nanowire were synthesized from these semiconductors [97].

Optical biosensors measure the change in optical characteristics after the interaction of receptor and target. Optical biosensors have great attributes for their use as a transducer, such as high sensitivity, robustness, immunity to electromagnetic interference, having computable optical outputs, ease of translation into miniaturization, integration capabilities, portability, multiplexing capability, and providing concurrent detection of various targets. Highly effective optical biosensor-based detection of SARS-CoV-2 has been demonstrated with surface plasmon resonance and fluorescence [98,101]. Moitra and coworkers (2020) developed a colorimetric assay based on gold nanoparticles (AuNPs) that when capped with suitably designed thiol-modified antisense oligonucleotides (ASOs) specific for N-gene (nucleocapsid phosphoprotein) of SARS-CoV-2 (oropharyngeal swab), could be used for diagnosing positive COVID-19 cases within 10 min from the isolated RNA samples. The thiol-modified ASO-capped AuNPs agglomerate selectively in the presence of its target RNA sequence of SARS-CoV-2 and demonstrate a change in its surface plasmon resonance. The selectivity of the developed colorimetric assay was investigated towards the MERS-CoV viral RNA and the detection limit of 0.18 ng/μL for SARS-CoV-2 RNA was determined along with the dynamic range of 0.2–3 ng/μL [102].

The plasmonic biosensor has successfully shown high sensitivity, quickness, and trustworthiness for the diagnosis of COVID-19. The localized surface plasmon resonance (LSPR) technique offers many advantageous features such as fast detection, high sensitivity, and low sample/analysis volume. However, the approach is very expensive and there are still some limitations associated with mass transport [103]. Qiu et al. have developed a dual functional LSPR biosensor that showed high sensitivity to SARS-CoV-2 sequences even at a lower level of the detection limit of 0.22 pM and allowed precise detection of the specific target in a multigene mixture. In this approach, plasmonic photothermal and LSPR techniques were combined and two-dimensional gold nanoislands (AuNIs) were functionalized with complementary DNA receptors that can perform a sensitive detection of viral RNA. Using two different incidence angles, the plasmonic resonances of PPT and LSPR can be excited at two different wavelengths, which significantly enhances the sensing stability, sensitivity, and reliability [103,104].

An electrochemical sensor has the ability to measure changes in potential, conductivity, current, and impedance due to the recognition process happening on the sensing surface while the electrode material acts as the transducer. A FET-centered electrochemical sensor comprises

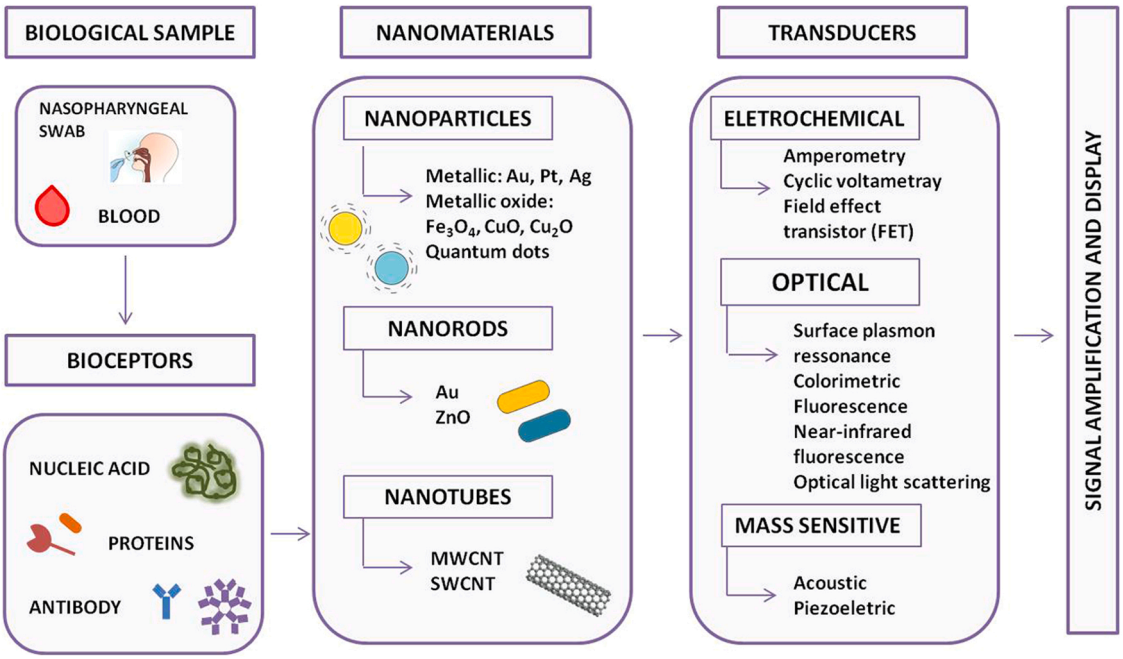

Fig. 4. Schematic diagram of different components of nanobiosensors.

a field-effect transistor (FET) as a sensing surface and a transducer component, which includes a dielectric layer operationalized with receptors that have selective affinity for the target analyte [101]. Seo and coworkers (2020) developed a FET-based biosensor for detecting SARS-CoV-2 in clinical specimens. The sensor was produced by coating graphene sheets of the FET with a specific antibody against SARS-CoV-2 spike protein. The nasopharyngeal swab samples were taken from infected patients. The FET biosensor detected SARS-CoV-2 spike protein with detection limits of 2.42×10^2 copies/mL in nasopharyngeal swab samples. This device was very sensitive and could detect a small amount of target instantaneously without showing any cross-reactivity with MERS-CoV antigen [104,105].

Emerging diagnostic tests for SARS-CoV-2, such as nanoparticle-based flow detection strips, have been developed to speed up detection and avoid the requirement to send samples to specialized facilities. Nanoparticles could also be the detection components in immunochromatographic tests (ICT), also known as lateral flow immunoassays, which are mainly applied to detect antigens or antibodies [14,99]. A typical ICT configuration for detection of SARS-CoV-2 antigens or IgG and IgM antibodies against SARS-CoV-2 consists of: a sample pad, where the sample and buffer are added; the conjugate pad containing the antibodies or antigens labeled with colloidal AuNP (diameter around 20–40 nm); the chromatographic strip, which is a porous polymer membrane, where the captured biomolecules are immobilized in the test line and a suitable antibody in the control line; and the liquid adsorbent pad.

The Au-labeled molecules bind to the antibodies or antigens present in the patient sample and are dragged through the chromatographic strip by capillary action, reaching the test and control lines, where they concentrate developing a color that can be seen with the naked eye. The absence of color in the test line indicates the absence of the target antibodies/antigens in the sample [106,107]. The sample is deposited onto the sample pad through a port and moves through the strip by capillary action. When it encounters the first line, antibodies labeled with gold nanoparticles bind to the target molecule in the sample. Then as the sample continues to move, the gold-labeled antibodies are bound by the capture antibodies in the lines. The gold-labeled antibodies that are in excess then move further along the strip and are captured at the control line. Even in the absence of the target molecule in the sample solution, the gold-labeled antibodies must be captured at the control line, making the control line for the validity of the test [106,107].

As introduced in the molecular technique section, the loop-mediated isothermal amplification (LAMP) method is considered a boon to the diagnostic world nowadays. The major disadvantage associated with this method is that amplified sequences cannot be seen with the naked eye. If the technique is coupled with some colorimetric sensing probe or device, then it will add much value to the approach. In efforts to surpass these limitations, a new method was developed in which RT-LAMP can be coupled with the biosensor design, as a transducer, based on the use of colored nanoparticles for visual detection [103]. Abbott ID Now™ manufactured a detection kit based on the loop-mediated isothermal amplification (LAMP) technique. It can detect COVID-19 within 5 min by taking samples from oral swabs, nasopharyngeal ones, nasal ones, etc. In this method, fluorescent molecular beacon probes are used to identify the amplicons, and the primers are used to identify the RNA-dependent RNA polymerase (RdRp) gene sequences. Food and Drug Administration—Emergency Use Authorization (FDA EUA) approved this kit as a commercial product [101].

Zhu and coworkers (2020) developed a mRT-LAMP coupled with a nanoparticle-based lateral flow biosensor (LFB) assay (mRT-LAMP-LFB) for the diagnosis of COVID-19. Two target sequences, including ORF1ab and the nucleoprotein gene (N), were simultaneously amplified in an isothermal reaction and visualized in one test step by a naked eye. Dye streptavidin-coated polymer nanoparticles were used as nanomaterial and samples were collected by oropharynx swabs. The limit of detection of COVID-19 mRT-LAMP-LFB was 12 copies (for each detection target) per reaction, and no cross-reactivity was generated from non-SARSCoV-2 templates. The analytical sensitivity of SARS-CoV-2 was 100 %, and the assay's specificity was also 100%. The total diagnostic test can be completed within 1 h from sample collection to the final result [107].

6. What have we learned so far?

The WHO's declaration of the COVID-19 pandemic was a milestone for the world scientific community. The high transmission rate and the huge number of deaths, along with the lack of knowledge about the virus and the evolution of the disease, stimulated a relentless search for diagnostic tests, treatments, and vaccines. The emergence of the pandemic contributed significantly to the boom observed in the diagnostics field. At first, only RT-PCR and chest CT were options for COVID-19 diagnosis. Serological tests, for the detection of IgG and IgM immunoglobulins, were the first authorized rapid tests, however, the lack of sensitivity and specificity of the tests have limited their use as screening tests. The main challenges were the differential diagnosis of COVID-19 and the development of specific, rapid, and sensitive tests that could reach all people. Other limiting factors included the definition of the biological material to be used (nasal and/or oropharyngeal swab, plasma, serum, or whole blood), the biological marker most likely to be detected, the type of methodology, and the ideal moment of infection for sample collection.

Currently, RT-PCR remains the gold standard for diagnosing COVID-19. However, new methods, such as other molecular techniques and immunoassays also started to be used in the clinic. Accessibility and affordability are some of the crucial variables that should be considered while devising any diagnostic method and easy acquisition of effective diagnostic test kits to all the affected countries is imperative during a pandemic. In this sense, the need for accessible tests with quick results boosted the development of POCT. POCT are fast, and automated, with high precision and accuracy. They can reduce the dependence on laboratory conditions and mass testing of the population, dispersing the pressure regarding screening and detection. At present, the POCT for detecting SARS-CoV-2 can be divided into three categories: nucleic acid testing, immunoassays, and biosensor testing. Nucleic acid testing are molecular techniques and the detection target is the viral RNA. RT-LAMP and CRISPR are the most studied methods. The results can be visualized by the naked eye, LED or UV lamps, or by observing the lateral flow strips. Abbott ID Now™ COVID-19 test was the first authorized by FDA to use isothermal nucleic-acid amplification technology to specifically detect SARS-CoV-2 RNA by fluorescence.

Immunoassays can be based on viral antigens or human antibodies anti-SARS-CoV-2. The solid-phase immunoassays (SPIAs) and lateral-flow immunochromatography (IC) are methodologies frequently used in COVID-19 diagnosis. Serological tests are not usually used to determine acute infection. Antigen-based direct-to-consumer at-home tests have gained space as a tool for case monitoring and social isolation. Today, there are approximately 50 direct-to-consumer or over-the-counter tests authorized for emergency use by the FDA.

Biosensors are devices in which the transducer is modified to capture the target element, convert the biological response into electrical signals, and quickly detect it with high accuracy. Nanobiosensors have gained attention since is possible to combine the advantages of nanoparticles with the diagnostic platform in these systems. Nanobiosensors are versatile systems in which different targets and detection methods can be employed. Several studies have been reported and many devices are under pre-clinical or clinical trials, but until now there is no commercial product with authorized use. With advanced dissemination of the vaccination and the number of deaths decreasing every day, the end of the pandemic is in sight. However, this does not mean that COVID-19 cases will disappear. An accurate diagnosis, along with vaccination, will remain the forerunner in the management of the virus. The high-sensitivity, high-accuracy, rapid, and cost-effective detection methods developed during the pandemic will be crucial to the early detection of patients and monitoring case numbers. Although many tests have been

approved by health agencies all over the world for use in the clinic, there is still a gap between scientific research results and commercial products, mainly regarding biosensors. The work is not done. More studies are still needed so that all new diagnostic methods reach the clinic. However, we can certainly say that the COVID-19 pandemic has opened new avenues in the area of infectious disease diagnosis.

CRediT authorship contribution statement

Renata Salgado Fernandes: Conceptualization, Data curation, Writing – original draft. **Juliana de Oliveira Silva:** Data curation, Writing – original draft, Writing - review & editing. **Karina Braga Gomes:** Writing – original draft, Writing – review & editing. **Ricardo Bentes Azevedo:** Writing – review & editing. **Danyelle M. Townsend:** Writing – review & editing. **Adriano de Paula Sabino:** Writing – review & editing. **Andre Luis Branco de Barros:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Data Availability

No data was used for the research described in the article.

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