



Current status, advances, challenges and perspectives on biosensors for COVID-19 diagnosis in resource-limited settings

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ABSTRACT

As the COVID-19 pandemic has profoundly impacted human life, prompt diagnostic tests are becoming an essential part of the social activities. However, the expensive and time-consuming laboratory-based traditional methods do not suffice the enormous needs for massive number of tests, especially in resource-limited settings. Therefore, more affordable, rapid, sensitive and specific field-practical diagnostic devices play an important role in the fight against the disease. In this review, we present the current status and advances in the biosensing technologies for diagnosing COVID-19, ranging from commercial achievements to research developments. Starting from a brief introduction to the disease biomarkers, this review summarizes the working principles of the biosensing technologies, followed by a review of the commercial products and research advances in academia. We recapitulate the literatures with a wide scope of bio/marker detections, embracing nucleic acids, viral proteins, human immune responses, and other potential bio/markers. Further, the challenges and perspectives for their employment in future point-of-care applications are discussed, with an extended appraisal on the practical strategies to enlarge the testing capability without high cost. This critical review provides a comprehensive insight into the diagnostic tools for COVID-19 and will encourage the industry and academia in the field of diagnostic biosensing for future evolution to large-scale point-of-care screening of COVID-19.

1. Introduction

The novel coronavirus disease (COVID-19) pandemic has swept across the globe and resulted in hundreds of thousands of deaths and tens of millions of infections (Fig. 1). The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has profoundly impacted the world's public health, economic activities, and social life. The World Health Organization (WHO) has issued multiple severe alerts emphasizing the urgent need and importance of prompt diagnostic tests for COVID-19 with a strong emphasis on "test, test, and test" [1]. The aim is to test every suspected case and find each infected individual with developed, mild, or even no symptoms because of the significant role of asymptomatic individuals in SARS-CoV-2 transmission [2]. Adequate tests and screenings initiate the efficient isolation and proper treatment of infected individuals to break the COVID-19 transmission chain, which is essential to prevent the spread of this contagion. It has been many months since the COVID-19 was first reported, yet reports of shortages of test supplies for COVID-19 diagnosis are still all over the globe, especially in countries with limited resources [3–7]. The lack of sufficient testing leads to the underlying problem of allowing the persons with

mild or no symptoms to communicate the virus to susceptible groups, such as seniors and juveniles with pre-existing medical conditions and individuals in low-resource areas [8,9]. Other distressing outcomes can be the overcrowding of the hospitals creating a run-on of the healthcare resources, and even the collapse of the healthcare system, which may aggravate regular patients' access to the hospitals.

The ideal diagnostic devices for such a highly transmissible disease demand the sensing technologies be affordable (low cost and low requirement for personnel and instrument), sensitive, specific (low rates of false positives and false negatives), rapid (preferably in minutes or an hour), equipment-free (no expensive equipment or with a portable device) and deliverable (accessible to the end-users)—summarized as the ASSURED criteria [10,11] for point-of-care (POC) use. However, there are a series of hurdles for the diagnostic tests to meet the above criteria, ranging from the technology aspect to the manufacturing factors. Technology-wise, traditional methods, such as quantitative reverse transcription polymerase chain reaction (qRT-PCR), are expensive and time-consuming. Manufacturing- and supply chain-wise, the insufficient supply of high-quality reagents, test machines, and assembly factories significantly limited the testing capabilities. According to one report, as of

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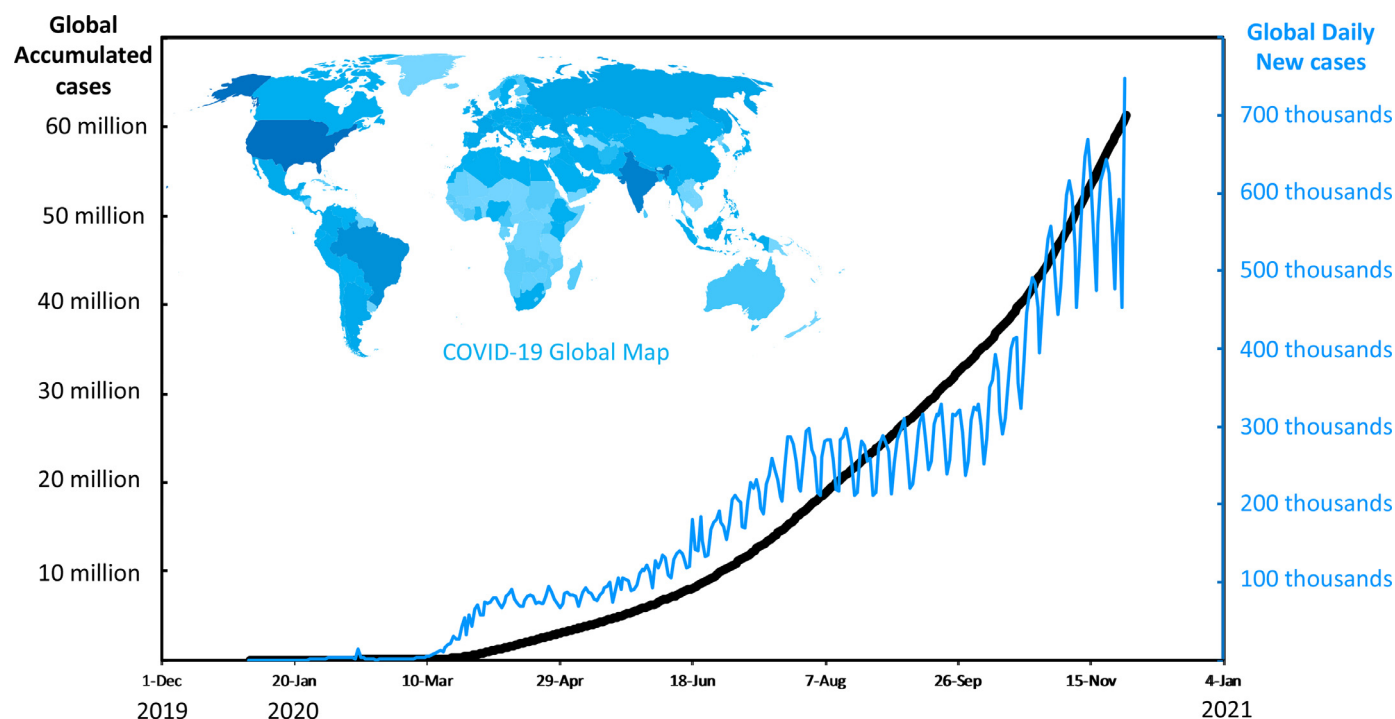


Fig. 1. A background of the recent statistics of cumulative global confirmed cases of COVID-19 as of November 25, 2020. Data source from World Health Organization [14].

mid-July 2020 in the U.S., only slightly more than a third (37%) of nasal swab tests returned the results in 2 days with an average wait time of 4.1 days [12,13]. Therefore, the advances in the sensing technologies and strategies for diagnosing COVID-19 are critical in overcoming the hurdles. In this review, we focused on the recent developments in biosensing technologies for the diagnosis of COVID-19. We start with examination of the critical biomarkers of COVID-19 and potential bio/markers that are prospectively useful. Then, we review the current status and developments of the commercial and research biosensing devices for COVID-19 diagnosis in resource-limited settings. We review the detection technologies of the viral nucleic acids, viral particles, human immunoglobulins, and other potential bio/markers with an extended emphasis on their advances and challenges towards the ASSURED criteria for POC scenarios.

2. Biomarkers for COVID-19

The methods for diagnosing COVID-19 are classified by the bio/markers (i.e., the analytes) (Fig. 2). The first group is the SARS-CoV-2 RNA, since RNA is the decisive proof of the virus existence (Fig. 2a). qRT-PCR is the mainstream tool to detect viral RNA and is often combined with clinical routines such as chest computed tomography (CT) and hematology tests to identify COVID-19 patients. The second category is to detect the specific viral proteins of SARS-CoV-2 to recognize the existence of viral particles (Fig. 2b). Among the total of 29 predicted proteins, there are four structural proteins—spike (S), envelop (E), membrane (M), and nucleocapsid (N) [15,16]. The S glycoprotein is a trans-membrane protein responsible for the binding of SARS-CoV-2 virus to host cells by attachment with angiotensin-converting enzyme-2 (ACE-2) and facilitating its entry into the host cell [17–19]. The N protein is a structural protein bound to the RNA, involved in the viral replication cycle, the viral genome process, and the cellular response of host cells to infections [20]. The M protein is responsible for determining the shape of the virus, and the E protein involves the virus production and growth [21]. The third type detects the specific human immunoglobulins (antibodies) against the COVID-19 after the viral infection (Fig. 2c). These include, the specific human immunoglobulin M (IgM) and

immunoglobulin G (IgG) antibodies, which are the two primary biomarkers of interest for the indication of current and past infection [22,23]. Other biomarkers with potential clinical significance include the levels of interleukin-6 (IL-6), C-reactive protein (CRP), lactate dehydrogenase, D-dimer, high-sensitivity troponin I, renal markers, etc. [24,25]. Although it remains challenging to use their level to identify the severity of COVID-19 manifestations because most investigations are observational [26–28], studies showed that despite being non-specific, the above-mentioned species assist the diagnosis, treatment, and prognosis of COVID-19 [24,25]. A typical biosensing technology for other bio/markers based on sandwich immunoassay is shown in Fig. 2d. Table 1, Table 2 and Table 3 summarize the commercial and research diagnostic biosensing technologies for SARS-CoV-2 viral RNA, viral proteins and serology proteins, and other bio/markers, respectively.

3. Detection of the viral gene

3.1. qRT-PCR detection of viral RNA

qRT-PCR is a nucleic acid amplification assay that has been implemented as a gold-standard protocol to detect SARS-CoV-2 viral genes, including the RdRP gene, nucleocapsid gene (N gene), envelope gene (E gene), spike protein gene (S gene), and ORF1ab gene [29]. The qRT-PCR involves the following steps to perform the test (Fig. 3a & b). Firstly, the collected sample is mixed with a cell lysis solution and the viral RNA is extracted. The extracted RNA is then mixed with master mix solution containing forward and reverse primers, fluorophore-quencher probes, and a mixture of reaction enzymes, including reverse transcriptase and polymerase in nuclease-free water and is loaded to the thermocycler with a programmed thermal incubation process. The thermal cycling condition and enzymes allow the conversion of RNA to the complementary DNA (cDNA) by reverse transcription, followed by the amplification of cDNA. As the fluorophore-quencher probe strands bind to the target sequence of SARS-CoV-2, the polymerase cleaves the probe. It releases the reporter dye to generate the increased fluorescent signals monitored continuously at each amplification cycle. Positive controls (sample with known SARS-CoV-2 RNA) and negative controls (sample without viral

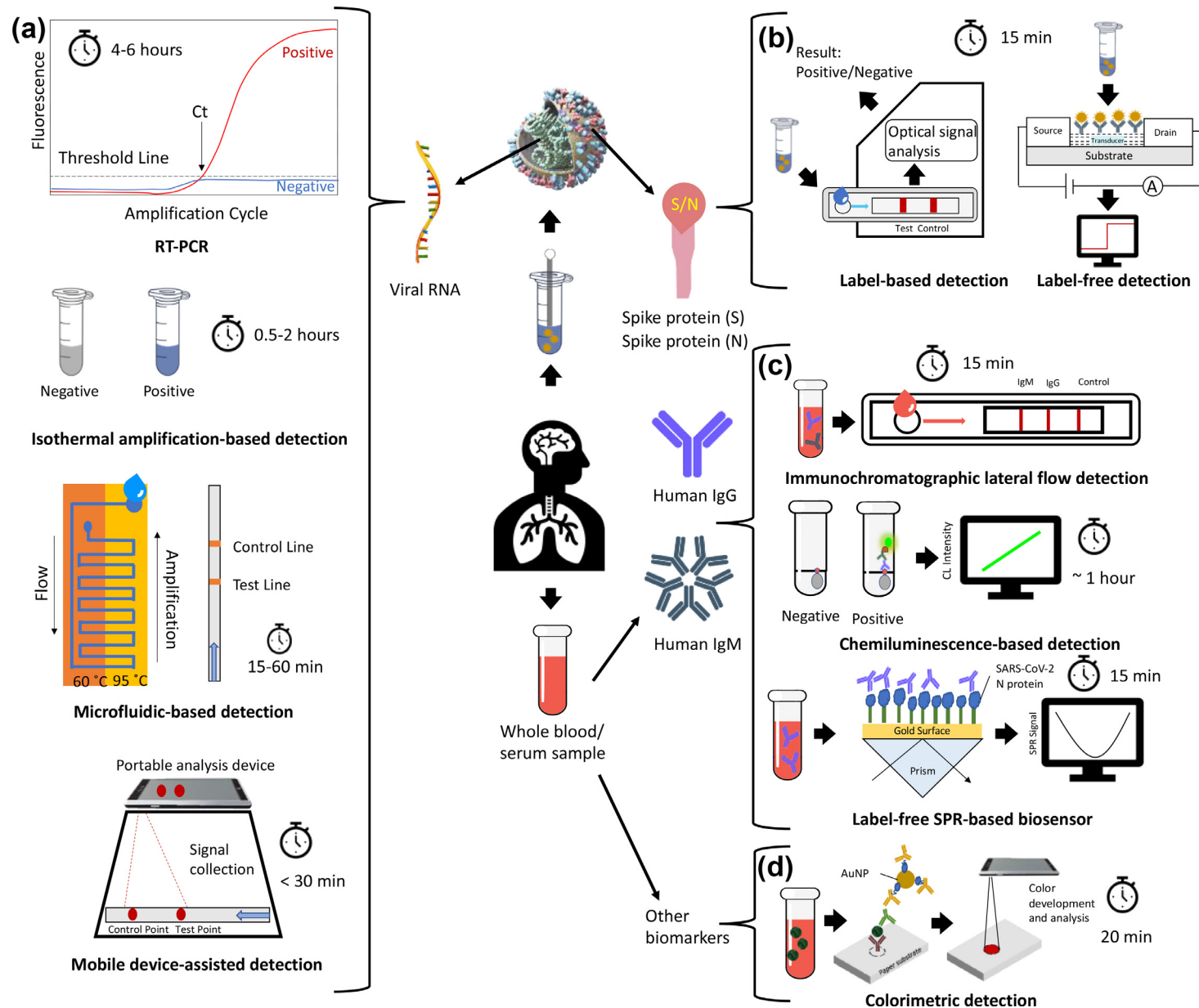


Fig. 2. A general description of the epitomized current biosensing technologies and approximate analysis time for the diagnosis of COVID-19 based on detection of (a) viral RNA, (b) viral proteins and viral particles, (c) serology tests for IgG/IgM and (d) other bio/markers.

Table 1

Summary of the reported POC-styled diagnostic tools for COVID-19 by detecting viral RNA. Source: US Food & Drug Administration, unless referred otherwise.

Developer	Product	Instrument	Sample source	Target	Sensing method	Assay time	Lowest LOD reported	Comment
Nucleic acid detection								
Yale School of Public Health, Department of Epidemiology of Microbial Diseases	SalivaDirect	RT-qPCR system	Saliva	N1 gene	qRT-PCR	~ 2 h	6-12 copies/μL	Nucleic acid extraction done with a simple proteinase K and heat treatment step
Pro-Lab Diagnostics	Pro-AmpRT SARS-CoV-2 Test	Genie HT instrument	Swabs	ORF1ab gene	LAMP	Amplification time <16 min	125 copies/swab	Isothermal amplification
Ethos Laboratories	Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay	Veriti 96 Fast Thermal Cycler; MALDI-TOF mass spectrometer	Non-invasive nasal swab	N gene, ORF1 gene, and ORF1ab gene	RT-PCR and mass spectrometer	> 7 h	1 TCID ₅₀ /mL	At-home sampling
Clinical Reference Laboratory, Inc.	CRL Rapid Response	BioRad CFX-96 TOUCH Thermal Cycler	Saliva	RdRp gene	qRT-PCR	N/A	250 copies/μL	At-home sampling
Atila BioSystems, Inc.	iAMP COVID-19 Detection Kit	Biorad CFX96 Real-Time System	Nasal, nasopharyngeal, and oropharyngeal swabs	N gene, ORF1ab gene	LAMP	< 1.5 h	4000 copies /mL	N/A
Abbott Molecular Inc.	Alinity m SARS-CoV-2 assay	Abbott m2000sp and Abbott m2000rt	Nasal, nasopharyngeal and oropharyngeal swabs; bronchoalveolar lavage fluid	RdRp gene, N gene	RT-PCR	N/A	100 virus copies/mL	N/A
Abbott Diagnostics Scarborough, Inc.	ID NOW COVID-19	ID NOW instrument	Throat, nasal, nasopharyngeal swab	RdRp gene	NEAR	< 13 min	125 genome equivalent/mL	Product storage at 2-30 °C. Portable device.
BioFire Defense, LLC	BioFire COVID-19 Test	FilmArray 2.0 system	N/A	N/A	N/A	50 min	330 genome copies/mL	Automated system for nucleic acid extraction, amplification and detection
Cepheid	Xpert Xpress SARS-CoV-2 test	GeneXpert Xpress System	Nasopharyngeal, nasal, or mid-turbinate swab	N/A	real-time RT-PCR	N/A	250 copies/mL	Automated system for nucleic acid extraction, amplification and detection. 2-28°C storage
Cue Health Inc.	Cue COVID-19 Test	Cue COVID-19 Test Cartridge Pack REF C1018	Nasal sample	N gene	isothermal amplification	25 min	1.3 copies genome/μL	Automatic heating, mixing, amplification, and detection in a cartridge. Cue Health App
Mesa Biotech Inc.	Accula SARS-Cov-2 Test	Accula SARS-CoV-2 Test Cassette	Nasal swab	N gene	N/A	30 min	100 copies/reaction	Test cassette. Storage at room temp. Easy readable results
[36]	N/A	PCR fluorometer	Pseudovirus samples	ORF1b gene, N gene	RT-PCR	~ 30 min	10 copies of pseudovirus	Magnetic NP-based RNA extraction
[55]	N/A	N/A	Nasopharyngeal swab	N gene	RT-LAMP	< 35 min	1.0 × 10 ¹ copies/μL	Simple turbidity results
[57]	N/A	N/A	N/A	Synthesized ORF1ab gene	RT-LAMP	> 20 min	10 copies/μL	Isothermal amplification, simple colorimetric readout
[60]	N/A	N/A	N/A	Synthetic ORF1ab gene	Penn-LAMP	< 1 h	7 copies/reaction	Isothermal reaction, high sensitivity,
[65]	N/A	Portable test strip	N/A	ORF1ab and S gene	SHERLOCK	< 1 h	10 copies/μL	POC paper stick, easy colorimetric readout
[81]	N/A	Mobile unit	Throat swab, sputum, and nasopharyngeal samples	ORF3a and E gene	RT-LAMP	< 2 h	N/A	Van-sized unit; suitable for quarantine camp
[82]	N/A	Customized instrument	Nasopharyngeal swabs	RdRp gene	RT-LAMP	~ 30 min	10 ² copies/reaction	Eye-observable and artificial intelligence-assisted color readout; gel electrophoresis double-check
[83]	N/A	PCR fluorometer	Clinical sample	RdRp gene	RT-PCR	< 30 min	11.2 copies/reaction	High sensitivity and specificity

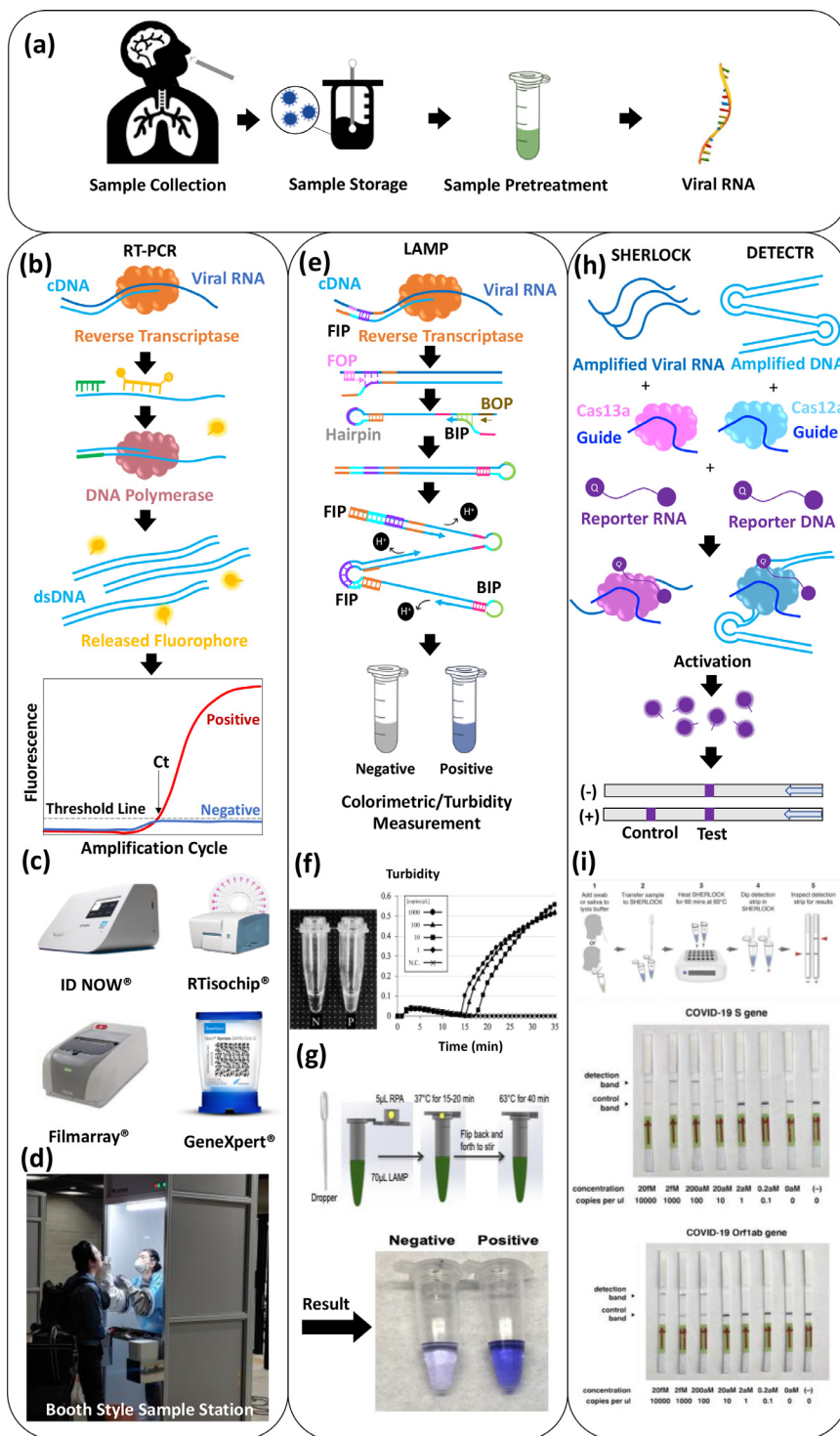


Fig. 3. An overview of biosensing technologies for the nucleic acid test of COVID-19 diagnosis. (a) Samples are collected and stored properly before testing. Sample pre-treatment usually include cell lysis and RNA extraction and in some detection technologies purification. (b) A schematic illustration of the protocol for RT-PCR test. Viral RNA is reverse transcript to cDNA. Then, for each thermal cycle, DNA polymerase synthesizes the dsDNA and releases the fluorophore from being quenched, thus expanding the fluorescence signal. (c) A few examples of commercial PCR test instruments developed for the COVID-19 diagnosis. (d) A booth-styled sample collection station in South Korea that is portable with flexible wheels (1 meter wide and long, 2.5 meters tall, with 230 kg weight), affordable than a negative-pressure tent. Reproduced from [66]. (e) A schematic illustration of the isothermal nucleic acid amplification technology. LAMP uses four customarily designed primers that contain the sequences complementary to six target RNA sequences. The forward inner primer (FIP) initiates the DNA synthesis, with the forward outer primer (FOP) displacing the synthesis afterwards. The reverse complementary sequence anneals with the downstream ssDNA to form a hairpin/loop structure. Similarly, the backward inner primer (BIP) and backward outer primer (BOP) form a loop at the opposite end. Multiple looping results in the cauliflower-like structure and releases protons that can be detected by colorimetric pH dyes, while some reported the turbidity as an alternative to color formation. (f) Kitagawa et al. developed a LAMP-based method to detect COVID-19 by simple turbidity measurement. Reproduced with permission from [55]. (g) El-Tholoth et al. developed the colorimetric and fluorescent detection of SARS-CoV-2. False green color of the medium is shown in the top half, while the true reaction mix shows blue color. Reproduced with permission from [60]. (h) An illustrative scheme of CRISPR-based technology for COVID-19 RNA detection. SHERLOCK uses the amplified RNAs to activate Cas13a RNase upon the binding of target RNA to release the fluorophores. DETECTR uses Cas 12 to cleave the report DNA and release the fluorophores. A lateral flow assay stick can be used to display the colorimetric readout. (i) Zhang et al. developed a protocol based on the CRISPR principle to detect the SARS-CoV-2 S gene and Orf1ab gene and visually presented the results using a lateral dipstick. Reproduced with permission from [65].

RNA) are required to determine the assay's background level and threshold cycle count (Ct). Ct refers to the number of amplification cycles required for the fluorescence signal to exceed the negative control level (Fig. 3b). A test result is positive when the fluorescence signal exceeds that of Ct [30,31]. However, due to the enormous growth of the epidemic and the surging needs for quick and affordable sensing devices, qRT-PCR does not satisfy the entire demands for testing since it requires 2–6 h for the assay, multiple reagents, centralized laboratories, and professionals. Thus, many POC-styled nucleic acid biosensing devices have been developed to detect SARS-CoV-2 with a shorter time, lower cost, and excellent sensitivities and specificities (Fig. 3c). For example, Abbott™ in the USA released the ID NOW® portable instrument

(~3 kg) and claimed to detect positive samples in 5 min and negative in 13 min [32]. Another example is the RTisoChip® developed by Capital-Bio™ to identify the SARS-CoV-2 from 5 other common respiratory viruses in 1.5 h [33]. Xpert Xpress® from Cepheid™ and Filmarray® from BioFire™, use microfluidics to integrate sample preparation, nucleic acid amplification, and signal detection in one detection kit for fully automated analysis [34,35]. On the other hand, many lab-scaled biosensors are developed towards fast, affordable, sensitive, and specific viral RNA detection. For instance, Zhao et al. developed a method using magnetic beads coated with carboxylic groups for fast viral RNA extraction within 30 min, incorporating the virus lysis and RNA binding in one step. They claimed to obtain a 10-copies sensitivity over a 5-log

concentration range of SARS-CoV-2 viral RNA [36]. Merindol et al. reported that SARS-CoV-2 viral RNA detection without RNA extraction was possible if samples were stored in universal transport medium (UTM) medium, a standard medium for swab sample storage, or molecular grade water when there is a shortage of the standard medium [37,38]. The above-mentioned diagnostic devices for detecting the viral gene are summarized in Table 1.

Despite great efforts from academia and industry, the current technologies and commercial products still have limitations. For example, while the ID NOW® instrument claimed to have test results within 13 min, the time and labor required for sample inactivation, initiation of the instrument, program selection, and sample and reagents loading were not accounted for, thus reducing the workload capability. The detection limit was claimed to be 125 genome equivalent/mL [32] whereas studies showed much higher LOD of 20,000 copies/mL [39,40]. Other commercial products such as Xpert Xpress and ePlex gave lower LODs and higher sensitivities but required much longer assay time [40]. Furthermore, the reagents and certified biosafety labs required can be unaffordable for resource-limited settings.

Currently, since RT-PCR is the gold-standard method for viral presence determination, a range of sensing strategies and tactics were used to facilitate the process. For example, in South Korea, the photo booth-styled sample collection stations are cost-effective in collecting massive numbers of samples and reducing the risk of infections of healthcare workers [41] (Fig. 3d). Furthermore, as many samples await tests, pooled samples can be employed to reduce the labor in areas with low infection rate. As reported, pooling of up to 30 samples increased the testing capacity with sufficient diagnostic accuracy by comparing the Ct values of positive pooled samples and positive individual samples [42]. If a mixture of individual samples (pool sample) was tested negative, all the individual samples were considered negative, while only positive pool samples required new tests for each person in the pool to identify the positive individual(s). Such a tactic was employed in China [43], USA [44,45], Israel [46], Germany [47], India [48], New Zealand [49], South Korea [50], etc. For example, in May 2020, Wuhan city conducted a 6-million-sized screening to pinpoint the potential virus carriers in 10 days [51]. Briefly concluding, the widespread nucleic acid testing and screening are efficient in providing accurate and abundant diagnostic results and subsequent measures, including quarantines and medical therapies. However, in resource-limited settings, the long-established qRT-PCR test that requires proper sampling (since different samples from nasal swab, nasopharyngeal swab, throat swab, saliva, and alveolar lavage fluid, etc., contain different viral loadings), pretreatment of samples (lysis of cells, RNA extraction), thermal cycling (usually a few hours), multiple reagents (lysis buffer, reverse transcription, and amplification enzymes, fluorescence) to operate the test, and limited certified lab set-up and trained personnel, makes a PCR test to be unaffordable and time-consuming.

3.2. Isothermal detection of RNA

Since the conventional qRT-PCR technology requires trained personnel, high demand for testing ingredients, and long assay time, the widespread use of PCR to diagnose COVID-19 is limited in resource-restrained areas. As an alternative, the isothermal nucleic acid amplification technologies dispel the thermal cycling and high equipment cost. The isothermal amplification methods include loop-mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), helicase dependent amplification (HDA), rolling circle amplification (RCA), nicking enzyme amplification reaction (NEAR) and strand displacement amplification (SDA). Among all, LAMP technology (Fig. 3e) is a one-step DNA amplification carried out in a mixture of DNA polymerase, reaction buffer, primers, DNA template, and a fluorescent dye. LAMP utilizes the strand-displacing DNA polymerase and four specifically designed primers (forward inner primer, FIP; forward outer primer, FOP; backward inner primer, BIP; and backward outer primer,

BOP) that are complementary to the six target sequences. Since the dsDNA is in dynamic equilibrium at the temperature around 60 or 65 °C, starting from the 3' end of the target gene, FIP can anneal an initial complementary sequence to the target gene using the DNA polymerase with displacing activity. The initial FIP-ssDNA strand is then displaced by the DNA strand primed by FOP. Then, the released FIP-ssDNA contains a reversely complementary sequence at its 5' end, which binds to its downstream sequence of the displaced FIP-ssDNA and forms a hairpin/loop. The same loop formation applies to the BIP and BOP at the other end of the displaced ssDNA forming a FIP-ssDNA-BIP "dumbbell" structure for the subsequent exponential amplification process. Repeated cycles of priming and DNA strands synthesis result in a mixture of various structures of stem-loops and cauliflower-like products consisting of alternatively inverted repeats of the target sequence. During the amplification process, 10^9 copies can be accumulated within 1 h, while the release of hydrogen ions can be detected via pH indicator or dyes for the detection of the viral gene [52,53]. It requires shorter time (0.5–2 h) for the nucleic acid amplification, a constant temperature at 60–65 °C [54] and a simple turbidity or color measurement with the addition of dyes or pH indicators [30,55–57]. Further, LAMP can detect unpurified samples and therefore becomes a promising choice for POC-styled COVID-19 diagnosis [58].

Table 1 summarizes the commercial and academic development for isothermal detection of the viral RNA of SARS-CoV-2. Seasun Biomaterials, Inc. developed a commercial LAMP-based qualitative detection kit (AQ-TOP® COVID-19 Rapid Detection Kit) that utilized the dual-labeled peptide nucleic acid probes targeting at ORF1ab gene of SARS-CoV-2, and human RNase P gene as an internal control. This test required the reverse transcription and LAMP reaction at 60 °C. The amplification products incorporated the fluorescence probe to be monitored by a real-time PCR instrument [59]. Kitagawa et al. has evaluated a commercial LAMP-based biosensor kit for SARS-CoV-2 detection (Fig. 3f). The Loop-amp® 2019-SARS-CoV-2 RT-LAMP test kit from Eiken Chemical™, Japan, was evaluated with nasopharyngeal swab samples and compared with the traditional qRT-PCR method. The test kit required only 10 µL of RNA sample, and after amplification, the positive results were presented as the increase in solution turbidity, with a high agreement of 97.4% (74/76) with the qRT-PCR method and a lower detection limit of 1.0×10^1 copies/µL within 35 min [55]. Yu et al. developed an RT-LAMP-based biosensing kit for colorimetric and fluorescent readouts. They targeted the ORF1ab gene, used a set of 6 primers (two outer primers, two inner primers, and two loop primers) for the isothermal amplification and employed the pH-sensitive indicator for color development. With optimized reaction protocol, 20 min enabled the naked-eye-observable color change for a sample with 1000 copies/µL. They achieved a lowest detectable concentration of 10 copies/µL. Moreover, GeneFinder dye was employed to expand the sensing capability by promoting the fluorescence signals [57]. Recently, El-Tholoth and coworkers devised a licensed Penn-LAMP method for the detection of ORF1ab gene of SARS-CoV-2. As shown in Fig. 3g, a process of amplification at 38 °C in the cap of the test tube was conducted, in which the recombinase polymerase assisted the LAMP primers to target the viral gene. The amplification took 15–20 min and the reaction mixture was transferred to the test tube and mixed with the pre-loaded LAMP reagents. The LAMP reaction in the test tube took another 40 min at 63 °C to develop a colorimetric change with a low detection limit at 7 copies/reaction [60]. In conclusion, the LAMP technology demonstrates ~100-fold more sensitivity of viral RNA detection than RT-PCR [61] and reduces the complexity, the production footprint and cost. Simple turbidity or color measurement renders it simple signal output compared to qRT-PCR that requires a bulky instrument to monitor the fluorescence. The main challenge of RT-LAMP technology is to design the specific primers to ensure specificity. The complicated primer design and multiplicity may result in non-specific amplification and false results. Overall, RT-LAMP can be considered as a promising alternative for viral RNA detection for POC use.

3.3. Clustered regularly interspaced short palindromic repeats (CRISPR)-based detections of viral RNA

Another useful technique for developing the POC diagnostics for COVID-19 is the CRISPR technology. This gene-editing tool can perform molecular detection of multiple viral RNAs to diagnose various infectious diseases. Compared to qRT-PCR and RT-LAMP, CRISPR coupled with DNA amplification can detect as low as attomolar level of viral RNA within 30 min [62]. As shown in Fig. 3h, the general workflow uses a CRISPR-associated nuclease (Cas) to pinpoint the target gene sequence by firstly amplifying the target gene sequences, followed by CRISPR to cleave and activate the fluorescence probe and report the signal as fluorescence. To be more specific, after the sequence recognition guided by the guide RNA and the cleavage by the Cas, a site-specific DNA double strand break (DSB) is generated. Following the recognition and cleavage of the target sequence, Cas12a, Cas13 and Cas 14 show collateral and non-specific activities against single strand nucleic acids, which are utilized in the fabrication of biosensors of viral gene. The interesting feature of Cas12a and Cas13 is that upon the activation of the recognition and cleavage of Cas and target RNA/DNA, a non-specific degradation of other existing nucleic acids in its vicinity occurs [63]. Specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) and DNA endonuclease-targeted CRISPR trans reporter (DETECTR) are the two main CRISPR methods for sensitive and specific detection of SARS-CoV-2 gene. Both methods require the preliminary cleavage and degradation of neighboring ssRNA by the protein Cas 13, and ssDNA by Cas12a, for SHERLOCK and DETECTR, respectively [30].

Sherlock Biosciences, Inc. developed a commercial kit (Sherlock CRISPR SARS-CoV-2) based on the detection of fragments of the ORF1ab gene and N gene of the virus with the human RNase P POP7 gene as an internal control. The isothermal amplification and signal detection can be performed with a heat block, CRISPR reagents complex, fluorescence reporter, and a microtiter plate reader within an hour [64]. However, the commercial product requires bulky instruments to perform the test. To eliminate the need for cumbersome and expensive set-ups, Zhang et al. developed SHERLOCK-based biosensing kit to detect the ORF1ab and S genes of the synthetic SARS-CoV-2 viral RNA fragments with a simple colorimetric readout on a paper-based microfluidic stick, as is summarized in Table 1. The viral RNA was first extracted and isothermally amplified. Then upon the Cas13 protein cleavage, and the activation of the fluorescence reporter, a fluorescence signal was generated. They used a dipstick to perform the lateral flow assay of the reacted sample mix. As the mixture wicked through the paper stick, visible color formation indicated the presence of the viral genes (control band and detection band in Fig. 3i). They achieved a detection limit of 10 copies/ μL . The entire assay was carried out within one hour, requiring simple instruments (water bath, centrifuge, paper dipsticks, pipettes, and necessary PPE). However, such tests still need a certified lab and trained operators; thus, it is suitable for institutions with basic lab set-ups [65]. In conclusion, POC-styled CRISPR methods for SARS-CoV-2 detection show advantages of high sensitivity, high specificity, fast assay, and easy use of sensing device. The technology can be adapted to many other infectious diseases and integrated with lateral flow assays for easy readout.

3.4. Lab-on-Chip device for nucleic acid testing

Since the traditional benchtop tests usually require multiple professional set-up and operations involving human intervention, microfluidic-based biosensing technology that integrates multiple lab functions such as sample collection, sample pretreatment, sample incubation and reaction, signal transduction, and result output on a single device, i.e., lab-on-chip (LoC), is an encouraging add-on to the aforementioned technologies. A variety of materials have been used as the substrate for the microfluidic LoCs, ranging from silicon wafer, glass, polymeric solids (PMMA, PDMS, etc.) to paper-like substrates [67]. For viral RNA

detection, there have been a diversity of studies incorporating the miniaturized lab functions into a single LoC. One of the major challenges for the POC detection of nucleic acids is to develop an on-site and cost-effective nucleic acid extraction method [68–70]. Here we review a burgeoning list of the microfluidic-based biosensing strategies aiming to detect nucleic acids of pathogens that can be adapted for the POC diagnosis of SARS-CoV-2 and summarize them in Table 1.

For non-isothermal PCR tests, portable dual heating elements are employed to supply the thermal cycling, as shown in Fig. 4a–c. A popular configuration for dual-temperature was developed by Cao et al. (Fig. 4a), where two heat blocks maintained the dual-temperature regions for nucleic acid amplification, while the fluid traveled intermittently for thermal cycling [71]. Tachibana et al. developed a pump-free microfluidic chip to detect the DNA of the human genome, influenza virus, and *Escherichia coli* (Fig. 4b). The silicon/glass microchannels were oxidized to provide a hydrophilic surface for the self-propelled capillary force. As the chip was placed on a dual heater of 60 °C and 95 °C for thermal cycling, the sample was self-driven to complete the nucleic acid amplifications within 15 min in the optimized channels. Such microfluidic design with the built-in heater shrinks the size and reduces the cost of the nucleic acid amplification step for qRT-PCR [72]. Li et al. developed a continuous flow-PCR (CF-PCR) that achieved automated sample manipulation, rapid nucleic acid amplification, and on-site detections (Fig. 4c). The device consisted of a main chip embedded with a microfluidic channel for 35 amplification cycles, where two heat blocks underneath enabled the thermal cycling. The microfluidic sample was propelled by a deformed elastic film that was pressed by a stepping motor. For the on-site PCR test, a CCD camera was integrated to capture the fluorescence intensities. To eliminate false results, additional capillary electrophoresis was attached for a size-based identification of the amplicons. They were able to distinguish between different pathogens that induced gingivitis in minutes with a limit of detection at 125 CFU/ μL [73].

Paper-based microfluidics offer advantages of low-cost, portability, biodegradability, low sample volume requirements, intrinsic microfluidic by capillary force, and rapid analysis when combined with isothermal amplification of nucleic acids. Rodriguez et al. devised a paperfluidics-based diagnostic chip for the extraction, amplification and detection of nucleic acids from real clinical samples. As shown in Fig. 4d, the paperfluidics were made with polyethersulfone (PES) filter and adhesive tapes, equipped with two main strips on the two sides for DNA extraction (left strip), LAMP reaction (middle port) and colorimetric readout (right strip, commercially available lateral flow dipstick (LFD) strip). With tape to separate each paper layer, DNA was firstly on-chip-extracted in a lysis buffer for 10–15 min, followed by ethanol wash to remove impurities and leaving dry Glycoblue-DNA precipitates in PES membrane. After removing the extraction pad, the LAMP reaction mix was added at the port, followed by the amplification at 63 °C for 30 min. Lastly, after removing the tape barrier, 50 μL water was added at the sample port to elute the products in the LFD strip to convey visible results in 2 min (Fig. 4e). The sensor showed its sample-to-answer ability to detect the HPV in a timeframe of an hour with a limit of detection at 10^4 copies. For clinical samples, it required considerably more ethanol to remove cell debris and salts to retain the sensor performance [74]. This paperfluidic-based LAMP sensor for nucleic acid detection is affordable, portable, rapid, and sensitive. However, for real clinical samples, the purification of DNA from samples is a requirement for the amplification. Lee et al. reported a paper-based molecular diagnostic tool for direct LAMP amplification and bacterial DNA detection without complicated sample preparation. A mineral paper holder was origami-folded to serve as the Direct LAMP reactor for the DNA amplification at 65 °C for 70 min. Then a capillary tube withdrew about 2 μL mixture on the immunochromatographic strip, where antibodies against the DNA-binding labels captured the amplicons and exhibit visible lines. Using ImageJ to evaluate the RGB values, they achieved a detection limit of 10^1 cells of both *E. coli* O157:H7 and *S. aureus* in whole human blood [75]. In this

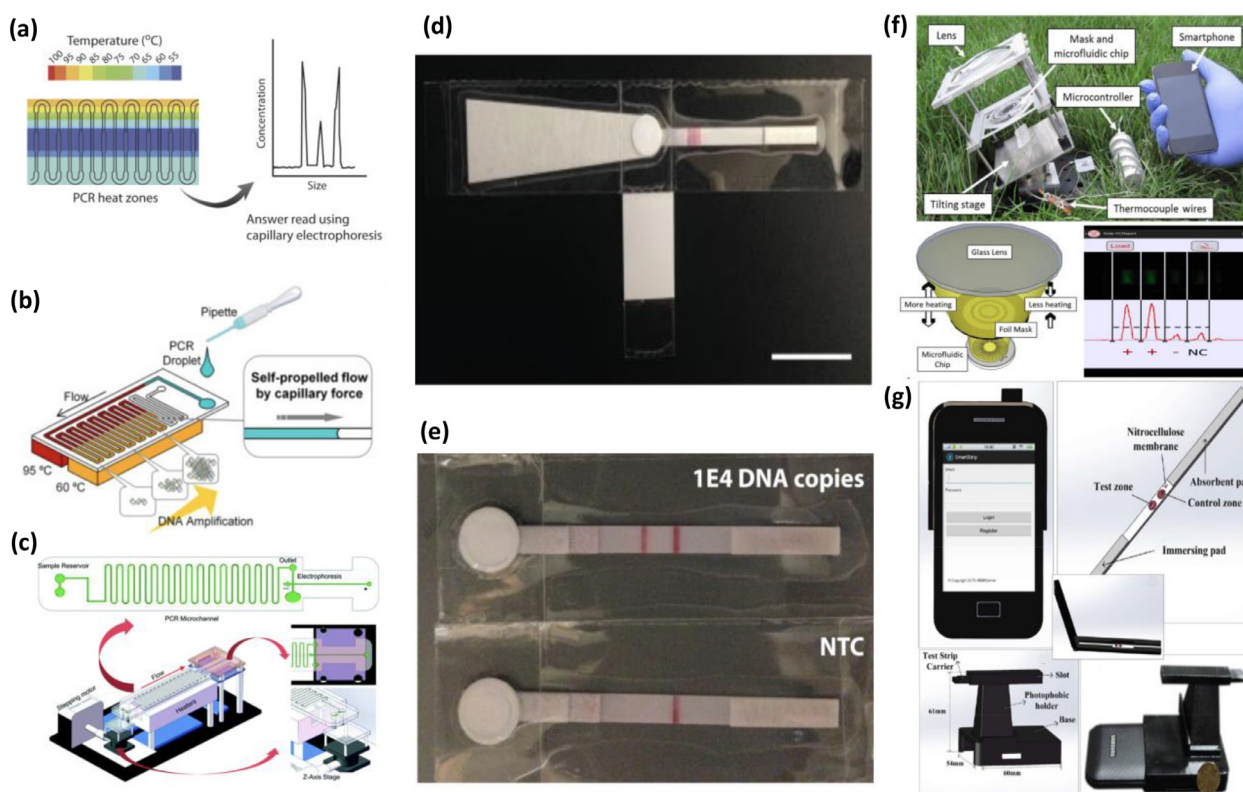


Fig. 4. Microfluidic-LoC and smartphone-assisted flow assay for nucleic acid detection. (a) A microfluidic flow assay with thin film heaters for the thermal cycles for PCR amplification, and a syringe pump (not shown) for the valveless microfluidic flow transport. Reproduced with permission from [71]. (b) A microfluidic device with self-propelled CF-PCR amplification (zoomed-in picture). Two temperature zones of 95 °C and 60 °C were provided for DNA denaturation and extension. Reproduced with permission from [72]. (c) A CF-PCR with integrated electrophoresis microfluidic all-in-one device with a microfluidic cell for nucleic acid denaturation and annealing, followed by an attached on-site electrophoresis. Reproduced with permission from [73]. (d,e) A fully integrated paperfluidic LAMP-based biosensor for the detection of DNA. The device enabled multiple functions of one-step cell lysis and DNA extraction, LAMP amplification and lateral flow detection of the amplified products. Positive results showed two red lines, while negative control showed one, as shown in (e). Reproduced with permission from [74]. (f) A solar thermal PCR system with adjustable solar energy reception ability by adjusting the lens-to-chip distance. Integrated with the HotSHOT cell lysis and smartphone-assisted detection of the fluorescence signals, PDMS chip conducted 4 tests simultaneously: two KSHV positives, one KSHV negative and one traditional PCR negative. Reproduced with permission from [76]. (g) A paper-based lateral flow immunoassay with a smartphone and application employed to capture and analyze the colorimetric signals. The target sequence would interact with the AuNP. The target-AuNP complexes were captured by the detection probes at the test zone, while the excess AuNPs were captured by the probes complementary to the detection probes. Reproduced with permission from [77].

study, the nucleic acid amplification was performed without traditional sample preparation. Instead, cell lysis, DNA extraction, purification, and amplification were achieved in the same reaction solution.

In recent years, smartphone with cameras has drawn great interest in assisting the signal analysis and output for optical biosensors. The main features are the ability to photograph the optical intensities and transfer to digital data for quantifications and/or serve as a power supply/battery. The integration of the mobile device, microfluidic control component, and sensing element lays the foundations for a smartphone-assisted microfluidic biosensing system for POC applications in early diagnosis of many infectious diseases. For instance, Jiang et al. utilized the solar focusing lens to collect solar energy and created spatially distinct thermal zones for the DNA amplification (Fig. 4f). The fluorescence emitted by the SYBR green-bonded dsDNA under blue-filter LED source was recorded by a camera. Besides the employment of solar power, the system showed a low energy consumption that can be supplied by a smartphone battery for 70 h, which opens its accessibility to those in resource-deficient environments [76]. Xu et al. developed a fully integrated smartphone-based biosensor on a paper-based lateral flow immunosorbent strip for viral gene detection (Fig. 4g). A photophobic holder and the built-in application ensured self-calibration to the lighting environment. The smartphone analyzed the pictures by checking the changes in the blue color channel. A detection limit of 2.5 nM was achieved, with less than 15 min assay time [77]. Taken together, these fast, specific, and

sensitive biosensing platforms combined with portable devices for on-site sample reaction and signal output, are promising alternatives to the traditional lab-centered technologies for the diagnostic detection of viral RNA such as COVID-19.

3.5. Challenges and perspectives of nucleic acid testing

Biosensing of the SARS-CoV-2 RNA fragments usually requires similar steps of clinical sample collection, RNA extraction, reverse transcription, cDNA amplification, signal transduction and output. However, in each step, there are challenges before a biosensing device for viral RNA becomes fully ASSURED and POC compliant. Regarding sample collection, the most common method is to collect a swab from patients' nose or throat, where a notable viral load is deemed to exist [78]. However, it is human nature to feel uncomfortable when an invasive cotton stick is used. To release the discomfort, non-invasive sample can be used, such as saliva [79], exhaled breath [80], and sputum [80]. The sampling methods may affect the results, and it is essential to conform to the operation protocol to avoid false positives and negatives. As stated, compared to traditional qRT-PCR, isothermal amplifications and CRISPR-based devices are substitutes in a faster manner and, therefore, more congruous with the resource-limited settings. Nevertheless, these technologies still require a list of prescribed reagents, qualified professionals to work with biohazardous virus samples and centralized biosafety

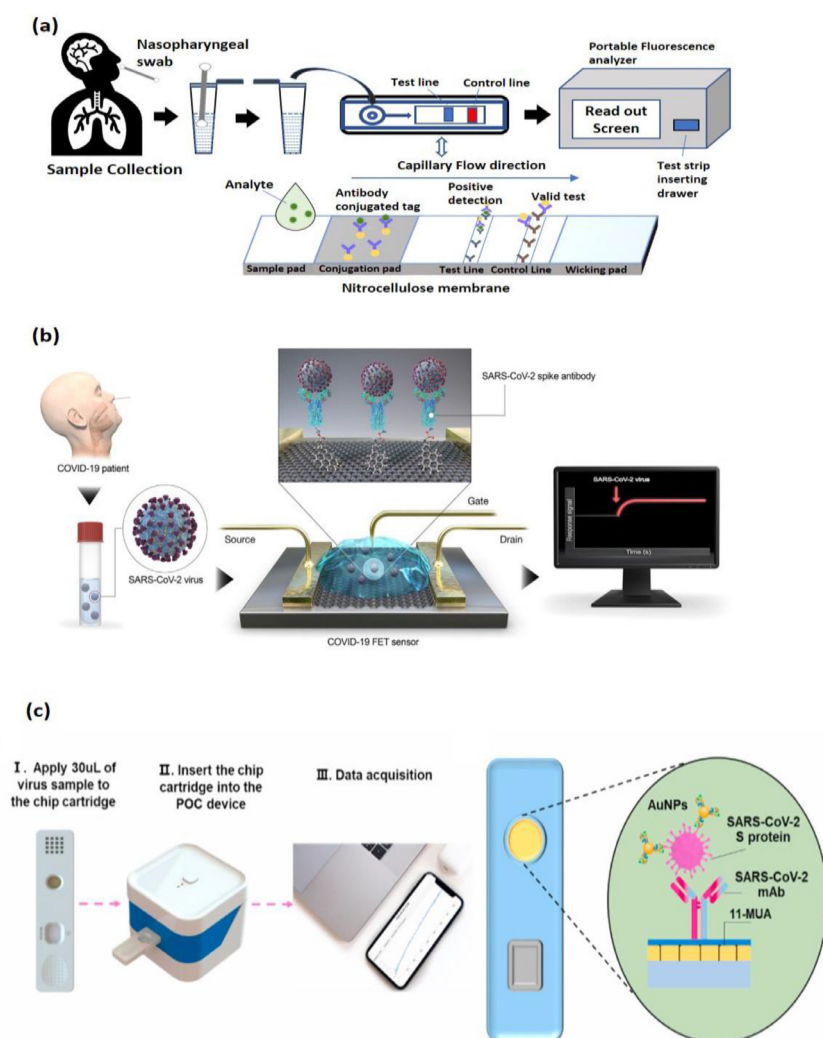


Fig. 5. Detection principle and lab-based/commercial devices for antigen-based SARS-CoV-2 diagnosis. (a) Schematic of a label-based lateral flow immunochromatographic assay with optical readout device. (b) Detection of SARS-CoV-2 using a FET sensor developed by Seo et al. The sensor detects and reports the binding event associated with the S-protein of the virus on anti-S-protein antibody coated graphene sheet. Reproduced with permission from [92]. (c) Schematic of SARS-CoV-2 pseudovirus detection with an AuNP enhanced plasmonic sensor chip and analyzed through a handheld optical readout device. Reproduced with permission from [93].

labs—a potential economic burden for resource-scarce areas. For instance, LAMP technology requires complicated primer design for every target nucleic acid; otherwise, false positives easily arise [73]. The supply shortages of buffers, enzymes, and primers lead to the short supply of test kits, causing the bottleneck for extensive screening of populations.

Lastly, the signal output is an indispensable step in conveying the results to users efficiently. Generally, qRT-PCR technologies deliver the real-time monitoring of the fluorescence signals, requiring bulky and costly thermal cyclers, fluorescence lamps, and detectors. On the other hand, RT-LAMP gives simple colorimetric, fluorescent, or turbidity readouts and are suitable alternatives for POC test to diagnose COVID-19. Besides, CRISPR-based devices combined with RT-LAMP also served as a potential for POC diagnosis for COVID-19. Recent advances in employing microfluidics in optical biosensors trigger the visible results that can be easily read by human eyes. Meanwhile, for quantitative readings, a mobile device such as smartphones is a favored choice since they are well-populated among the general public.

4. Detection of proteins

4.1. Viral proteins

Another method of COVID-19 diagnosis is through the detection of viral proteins. Among the four structural proteins (S, N, M and E), S and N proteins are of primary interest for COVID-19 diagnosis since both are

highly immunogenic and are produced abundantly during infection [68,84,85]. The detection employs antibodies against the viral proteins present in a patient's nasopharyngeal secretion. Viral protein detection can be broadly classified into labeled (such as enzyme-linked immunosorbent assay (ELISA), immunochromatography assay, chemiluminescence) and label-free (such as surface plasmon resonance (SPR), field-effect transistor (FET)) detection techniques [86]. In labeled techniques, the target binds with the capture biomolecule. Then, a secondary antibody with a label/tag attached (in most cases an enzyme/metal NPs) binds to the target forming a sandwich complex where color/fluorescence forms, which is discernible by naked eyes or optical readout devices (Fig. 5a). The labeled detection can be performed on a 96-well plate (ELISA) or lateral flow strips (immunochromatography assay). Label-free techniques, on the other hand, detect targets without labels. Label-free techniques involve determining the change in conductivity/resistance/refractive index/mass upon the binding event [87]. Compared to label-based techniques, label-free detection methods require only one (capture) antibody, are simpler and faster to perform, and are more sensitive.

Rapid antigen detection techniques have emerged as an alternative diagnostic tool to RNA for the POC diagnosis of COVID-19. These detection methods ought to be easy to perform and do not require complicated sample pretreatment. The U.S. Food and Drug Administration (FDA) has issued Emergency Use Authorization (EUA) for several rapid antigen test kits. For instance, Quidel Corporation received EUA for their product Sofia 2 SARS Antigen FIA [88] which qualitatively detects N

Table 2

Summary of the reported POC-styled diagnostic tools for COVID-19 by detecting human immune responses and viral antigen. Source: US Food & Drug Administration, unless referred otherwise.

Developer	Product	Instrument	Sample source	Target	Sensing method	Assay time	Lowest LOD reported	Comment
Serology test (Antibody detection)								
BioCheck, Inc.	BioCheck SARS-CoV-2 IgG and IgM Combo Test	MS-Fast Automated Chemiluminescent Immunoassay Analyzing System	Human serum	IgM and IgG	CLIA	30 min	Cut-off RLU for IgM: 18,500; Cut-off RLU for IgG: 26,000	Easy, qualitative readout, luminescence detection
Diazyme Laboratories, Inc	Diazyme DZ-Lite SARS-CoV-2 IgM CLIA Kit	DZ-lite 3000 Plus Chemiluminescence Analyzer	Human serum/plasma	IgM	CLIA	N/A	Qualitative	Easy, qualitative readout, luminescence detection, IgM only
BioMérieux SA	VIDAS SARS-CoV-2 IgG; VIDAS SARS-CoV-2 IgM	VIDAS® instrument	Human serum/plasma	IgM and IgG	ELFA	27 min	Qualitative	Automatic assay steps, qualitative fluorescence detection
Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.	WANTAI SARS-CoV-2 Ab ELISA	Test Cassette	Human serum/plasma	Total Antibody	ELISA	< 20 min	Qualitative	Qualitative, colorimetric detection
Siemens Healthcare Diagnostics Inc.	Atellica IM SARS-CoV-2 IgG (COV2G);ADVIA Centaur SARS-CoV-2 IgG (COV2G)	Atellica IM Analyzer	Human serum/plasma	IgG	CLIA	< 25 min	Qualitative	1-step assay protocol, chemiluminescence detection
Xiamen Biotime Biotechnology Co., Ltd.	BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test	Test Cassette	Human serum/plasma	IgM and IgG	LFIA	10 min	Qualitative	Easy, rapid, colorimetric detection
Access Bio, Inc.	CareStart COVID-19 IgM/IgG	Test Cassette	Human serum/plasma	IgM and IgG	LFIA	10 min	Qualitative	Colorimetric detection, no special equipment required
Megna Health, Inc.	Rapid COVID-19 IgM/IgG Combo Test Kit	Test Cassette	Human serum/plasma	IgM and IgG	LFIA	15 min	Qualitative	Less than 5µL of sample volume requirement, colorimetric detection, no special equipment required
Kantaro Biosciences, LLC	COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit	Microplate reader for measuring absorbance	Human serum/ plasma	IgG	ELISA	N/A	Qualitative and semiquantitative	2-step ELISA, Step-1 and Step-2 provides qualitative and semi-quantitative results respectively, not applicable for POC
Jiangsu Well Biotech Co., Ltd.	Orawell IgM/IgG Rapid Test	Test Cassette	Human serum/plasma	IgM and IgG	LFIA	10 min	Qualitative	1-step, rapid, colorimetric detection, applicable for POCT
Assure Tech. (Hangzhou Co., Ltd)	Assure COVID-19 IgG/IgM Rapid Test Device	Test Cassette	Human serum/plasma	IgM and IgG	LFIA	15 min	Qualitative	Rapid, colorimetric readout, applicable for POCT
[96]	N/A	N/A	Human serum/ plasma	IgM and IgG	LFIA	< 15 min	Qualitative	Easy colorimetric detection
[107]	N/A	N/A	Human serum/ plasma	IgM and IgG	LFIA	< 15 min	Qualitative	No special equipment requirement, sensitivity 88.66% and specificity 90.63%,
[110]	N/A	Microfluidic platform integrated with fluorescence detection analyzer	Human serum/ nasopharyngeal swab sample	IgG/IgM/Antigen	Microfluidic, multiplexed immunoassay	< 15 min	Qualitative	Applicable for POC settings, portable, multiplexed for detection of both antibody and antigen

(continued on next page)

Table 2 (Continued)

Developer	Product	Instrument	Sample source	Target	Sensing method	Assay time	Lowest LOD reported	Comment
[116]	N/A	N/A	Human serum	IgG	LFIA	15–20 min	Qualitative	Rapid, colorimetric detection, applicable for POC testing, no cross reactivity with severe fever with thrombocytopenia syndrome (SFTS) and avian influenza A(H7N9) patients
Antigen detection								
LumiraDx UK Ltd.	LumiraDx SARS-CoV-2 Ag Test	LumiraDx SARS-CoV-2 Ag Test	Nasal swab	Antigen (N Protein)	Fluorescence immunoassay	12 min	Qualitative	Integrated device, mobile app results. Direct detection of virus, no sample pretreatment
Becton, Dickinson and Company	BD Veritor System for Rapid Detection of SARS-CoV-2	BD Veritor™ System, BD Veritor Plus Analyze	Direct nasal swab	Antigen (N Protein)	Immunochromatographic assays	15 min	Qualitative	Integrated device, easy sample processing, POC
Quidel Corporation	Sofia SARS Antigen FIA	Sofia SARS Antigen FIA, Sofia 2 and Sofia analyzers	Nasopharyngeal (NP) and nasal (NS) swab specimens	Antigen (N Protein)	Lateral flow immuno-fluorescent sandwich assay	< 15 min	Qualitative	Integrated device, easy sample processing, POC
Celltrion USA, Inc.	Sampinute COVID-19 Antigen MIA	Sampinute™ Analyzer	Nasopharyngeal swab (NPS)	Antigen (S protein)	Magnetic force-assisted Electrochemical Sandwich Immunoassay (MESIA)	10 min	Qualitative	Not applicable for POCT, 50 µL sample volume requirement
Access Bio, Inc.	CareStart COVID-19 Antigen test	N/A	Nasopharyngeal swab (NPS)	Antigen (N Protein)	Lateral flow immuno-chromatographic assay	10 min	Qualitative	No instrument required for result interpretation, applicable for POCT
[92]	N/A	FET based biosensing device	Nasopharyngeal swab specimens	Antigen (S Protein)	Field-effect transistor sensor	< 10 min	100 fg/mL-UTM; 2.42×10^2 copies/mL-clinical sample	Label-free detection, no sample pretreatment, distinguish between SARS-CoV-2 and MERS-CoV
[117]	N/A	Image analyzer	Nasal (NS) swab specimens	Antigen (S1 Protein)	LFIA	20 min	1.86×10^5 copies/mL in the clinical specimen	ACE-2 based rapid detection of antigen (S protein), no cross-reactivity with SARS-CoV or MERS CoV S1 protein, applicable for POCT

protein from SARS-CoV-2 in nasopharyngeal and nasal swabs within 15 min using a label-based lateral flow immunofluorescent sandwich assay. A portable optical readout system detected the fluorescent signal generated due to the immunoreaction and corrects for non-specific binding in the result. A vital drawback of this test kit is its inability to differentiate SARS-CoV-2 from the SARS-CoV, a virus for which there have been no known cases since 2004 [89]. Following Sofia 2 SARS Antigen FIA, BD Veritor System for Rapid Detection of SARS-CoV-2 [90] and LumiraDx SARS-CoV-2 Ag Test [91] received FDA EUA and are claimed for POC settings. Table 2 lists some commercially available antigen test kits for POC settings with FDA EUA and lab-based devices and their salient features.

Research is on-going on the further development of POC viral particle detection with both label-free and label-based methods (Table 2). Recently, Seo et al. reported a label-free FET biosensor employing graphene functionalized with antibodies against S-protein for detection of the SARS-CoV-2 in cultured virus and nasopharyngeal swab samples (Fig. 5b) [92]. The FET biosensor selectively recognized the S protein and transduced the binding into electrical signals. The biosensor detected as low as 1 fg/mL and 100 fg/mL of S proteins, respectively in PBS and clinical transport medium. When applied for the real-time detection of virus and clinical sample, the device responded to as low as 16 PFU/mL for the virus in culture medium and 242 copies/mL in clinical samples. Besides, this device demonstrated no significant cross-reactivity with MERS-CoV antigen protein. If integrated with onsite electrical response measurement systems, this label-free, rapid and highly sensitive immunological diagnostic tool can serve as a potential POC diagnostic tool that meets the ASSURED criteria. In another study, Huang et al. reported development of a portable nanoplasmonic sensor with a smartphone-controlled handheld optical device for a single-step rapid detection of SARS-CoV-2 pseudovirus particles in POC settings (Fig. 5c). The nanoplasmonic resonance sensor device showed enhanced sensitivity and faster detection time when labeled with AuNP. The assay time was only 15 min with a linear detection range up to 10^7 viral particles (vp)/ml and a LOD of about 370 vp/ml. Moreover, it showed a high specificity to SARS-CoV-2 when compared with SARS, MERS, and VSV viruses [93]. However, this device has not been tested with real samples. Nonetheless, it is a POC device that has potential for resource-limited and home settings.

Briefly concluding, antigen-based detection techniques for COVID-19 diagnosis offer encouraging advantages for POC settings due to their rapid detection, selectivity, and low cost. However, FDA-EUA-authorized Sofia 2 SARS Antigen FIA, as well as the LumiraDx SARS-CoV-2 Ag

Test are declared unable to differentiate between SARS-CoV-2 and SARS-CoV. This necessitates additional nucleic acid tests for confirmation even when the antigenic test is negative.

4.2. Human immune response proteins

When invaded by foreign organism, the human immune system generates antibodies (immunoglobulin) against it. Thus, the SARS-CoV-2 viral infection can be diagnosed indirectly by measuring the host immune response [94]. The serological test targets antibodies against the virus in the patient serum sample, namely IgM and IgG, whose presence indicates the current and past infection status of the patient, respectively. Although IgG and IgM are not considered as direct tools for the diagnostic purposes, they are important in understanding the epidemiology in the general population and identifying groups at higher risk for infection [95]. In general, IgM and IgG antibodies are found in human serum in 2 to 3 weeks after the onset of infection. However, immediate presence of detectable IgM and IgG is not always observed in all individuals. Hence, absence of these antibodies should not rule out the possibility of infection of an individual with symptoms, rather PCR or antigen detection tests should be conducted for confirmation [95–97].

The conventional assays for human antibodies detection include enzyme linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), lateral flow immunoassay etc. ELISA is a microtiter plate-based assay and demonstrates a colorimetric change via an enzymatic reaction that correlates to the presence and/or concentration of the antibody [98,99]. In CLIA, luminophore markers (direct method) or enzymatic markers with corresponding substrate (indirect method) are used to produce luminescence (emission of visible or near visible light) signal [100]. Compared to ELISA, CLIA tests are deemed preferable in POC settings because of higher sensitivity, selectivity and speed, wider dynamic range, and less labor and reagents consumption [99,101]. Lab-based antibody detection techniques use bulky and expensive laboratory instrumentation for signal readout, adequate infrastructures, and complicated sample processing, making them unsuitable for field-deployable detection. In comparison, cassette-based portable systems employing lateral flow immunoassay (LFIA) test strips rely on color changes due to the antibody-antigen binding reaction that are perceivable by naked eyes or portable optical signal readers (Fig. 6a). The immunochromatography-based test requires simple sample pretreatment since all necessary reagents are integrated, awaiting a small sample volume and buffer to be dropped on the designated inlet and wicks through the porous nitrocellulose membrane. The antibodies in the serum sample bind with the

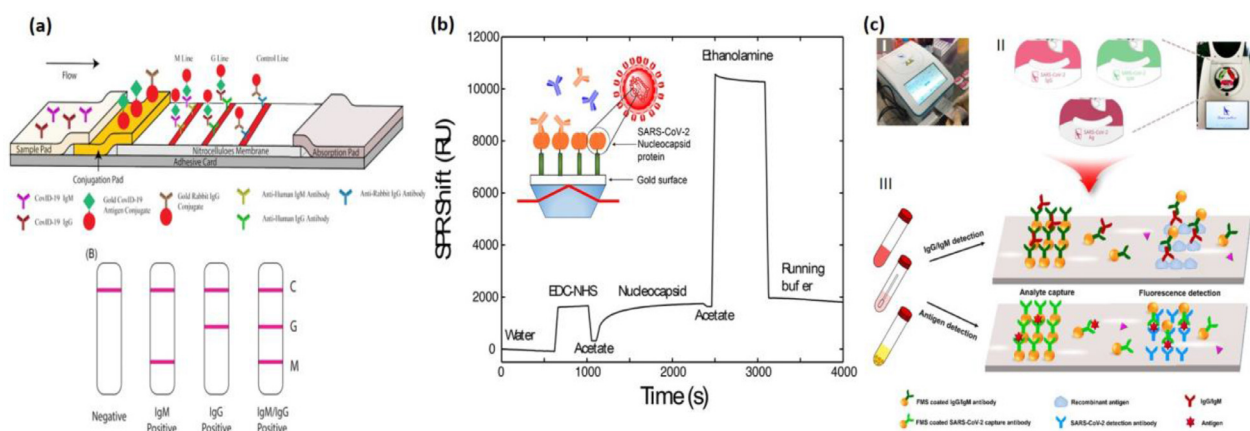


Fig. 6. Detection principle and lab-based/ commercial devices for detecting human immune responses. (a) Representative image of antibody testing in serum sample in LFIA cassette. Reproduced with permission from [107]. (b) Sensorgram of SPR gold surface functionalization. N protein was bound to the sensor surface through EDC/NHS chemistry and unbound sites were blocked with ethanolamine. Reproduced with permission from [108]. (c) Microfluidic device for antibody/antigen detection. I: Portable fluorescence detection kit. II: Immunoassay microchip to be used for detection. III: Schematic diagram of IgG/IgM/Antigen detection through microfluidic fluorescence assay. Reproduced with permission from [110].

recombinant viral antigen tagged with a nanomaterial indicator such as AuNPs, dye loaded latex particles or quantum dots. In the test region, the immobilized anti-human antibody-antigen-capture antibody complexes accumulate, forming a narrow color band detectable by naked eyes [102]. The advantages of LFIA strips over lab-based assay systems are that they are miniaturized, low-cost, simple to operate, need a small amount of sample volume, and can operate with minimum human intervention—all of which makes them fulfill criteria for POC use. However, compared to ELISA and CLIA, these tests are less sensitive and less specific [103]. Although less reliable, the quick detection, onsite applicability and low cost are particularly useful for large-scale seroprevalence study [104].

Numerous biotech companies are developing serological test kits, as summarized in Table 2. In the United States, Cellex™ [105] was the first company to receive FDA EUA for the qSARS-CoV-2 IgG/IgM Rapid serology test product [106]. This LFIA device provides qualitative detection of IgM and IgG antibodies in serum, plasma, or venipuncture whole blood samples within 15–20 min. Subsequently, more test kits based on 1) LFIA, including Assure Tech® COVID-19 IgG/IgM Rapid test Device by Hangzhou Co., Ltd, Anti-SARS-CoV-2 Rapid test by Autobio Diagnostics Co., Biohit® SARS-CoV-2 IgM/IgG Antibody Test Kit by Biohit Healthcare Co., Ltd, and COVID-19 IgG/IgM Rapid Test Cassette by Healgen Scientific LLC; 2) CLIA, including Babson Diagnostics aC19G1 from Babson Diagnostics, Inc., and Beckman Coulter Access SARS-CoV-2 IgG from Beckman Coulter, Inc.; and 3) ELISA, such as Platelia® SARS-CoV-2 IgG assay by Bio-Rad laboratories, Anti-SARS-CoV-2 ELISA (IgG) by EUROIMMUN US Inc., COVID-19 ELISA IgG antibody test by Mount Sinai Laboratory etc., have been introduced in the market for IgG/IgM detection. Table 2 lists some FDA EUA test kits and recent research works on serological tests for COVID-19 diagnosis.

Based on the fact that rapid serological testing can be a potential supplementary diagnostic approach to time- and resource-consuming RT-PCR process, growing research has been spotlighting the development of sensitive, specific, and fast serological detection of SARS-CoV-2 infection. Li et al. reported a rapid, simple, equipment-free, and point-of-care LFIA which detects the virus-induced antibodies simultaneously in human serum within 15 min. SARS-CoV-2 recombinant antigen-conjugated AuNP and rabbit IgG-conjugated AuNPs were prepared on the conjugation pad. Upon the lateral flow of sample, antigen-AuNPs captured the human IgG/IgM and were bonded with the pre-deposited anti-IgG/anti-IgM at the test lines to form pink colors. The rabbit IgG-AuNPs, on the other hand, was captured by the anti-rabbit IgG antibody at the control line (Fig. 6a). The LFIA colorimetric strip was tested on 525 cases, and the sensitivity and specificity were found to be 88.66% and 90.63%, respectively, indicating good reliability of the test kit [107]. Djailab et al. reported the detection of the human antibody against the SARS-CoV-2 virus N protein in serum by a surface plasmon resonance (SPR)-based biosensor (Fig. 6b). The gold surface of the SPR biosensor was coated with a peptide monolayer and functionalized with recombinant N protein as the biorecognition element. This SPR sensor can detect the antibody with an LOD in the nanomolar range (100 ng/mL) within 15 min [108]. This label-free and rapid detection tool can serve in potential point-of-care settings. Abedin and coworkers demonstrated a novel surface plasmon resonance-based optical sensor that uses the nonlinear optical response of gold films to detect biomolecular binding events in a rapid, high-throughput, and label-free manner. The authors showed real-time quantitative analysis of individual nucleic acid hybridization events with sub-diffraction limit resolution in their proof-of-concept study. Because the signal came from the nonlinear optical response of the gold film itself, this sensor can potentially be used for label-free detection of SARS-CoV-2 in real-time, through either nucleic acid-based detection or antibody-based detection by simply changing the analyte of interest placed on the gold film [109]. By modifying commercially available portable SPR devices with a pulsed laser and integrating with the sensor, this technique can be implemented in POC settings.

Besides, microfluidics-based immunoassay devices have been considered a featured component in detecting infectious diseases due to their automated manipulation of reagents, miniaturized footprint, and multiplexed detection capability. In a recent study, Lin and coworkers demonstrated a portable sample-in-answer-out microfluidic immunoassay device suitable for rapid (< 15 min), sensitive and multiple target detections. Their POC analyzer included a fluorescence-based portable instrument (3.8 kg), with centrifuge, diagnostic microchip, and multiple immunoassays for the detection of IgM, IgG, and antigen of SARS-CoV-2 (Fig. 6c). The detection antibody of each marker (IgG/IgM/antigen) and antigen against SARS-CoV-2 was patterned on the microchip. The fluorescent microspheres (FMS) were functionalized with the capture antibody against IgG/IgM/antigen and deposited on the capture region of the microchip to ensure the sensitivity and specificity. The assay protocol included addition of sample (10 μ L serum/nasopharyngeal fluid) and buffer solution in the sample loading chamber of each microchip, followed by incubation, centrifugation of microchips to remove residual liquids and finally fluorescence intensity measurement [110]. The device could simultaneously detect IgG, IgM, and viral particles through three separate, disposable and replaceable chips. This combined approach is suitable for simultaneous detection of infected and convalescent individuals. Determination of the three targets at the same time but in different chambers reduces the chance of contamination and serves for multiple detections. Overall, the high sensitivity and selectivity of the microfluidic setup were attractive for POC applications. In conclusion, serological tests can help identify antibodies that are generated as immune response to coronavirus infection but are not able to detect the virus directly. Therefore, antibody tests should not be used for direct diagnosis of COVID-19 patient. However, considering their fast assay time, portability, and easy operation, LFIA based serological test kits that analyze the levels of IgG and IgM can help identify the infected persons for epidemiology studies.

4.3. Challenges for protein-based diagnosis

Nucleic acid-based detection techniques for coronavirus are currently considered as the gold standard for COVID-19 diagnosis. However, the requirement for resource intensive facilities and longer processing time do not meet the tremendous demand for rapid testing and screening of the masses. In comparison, the reported technologies for viral protein detection and serology tests require lower cost, less labor, less assay time, and reagents, therefore more affordable and accessible in resource-limited settings. However, although protein-based detection techniques meeting the ASSURED criteria are suitable for POC use, they have inherent weaknesses. Reduced sensitivity and increased cross-reactivity are the two main disadvantages of protein-based detection of SARS-CoV-2 disease [29]. In particular, cross-reactivity among antibodies for SARS-CoV-2 and other coronaviruses, especially between SARS-CoV and SARS-CoV-2, is a major concern to sabotage the specificity and sensitivity of immunoassays. Lack of specificity leads to a large number of false positive and false negative results [111]. In a study by Lv et al., antibody responses of serum samples from 15 patients infected with SARS-CoV-2 and 7 infected with SARS-CoV found that the cross-reactivity in antibody binding to S protein was frequent [112]. Moreover, variable viral load and inconsistency in sampling for antigen-based tests might lead to false negative results. Despite these drawbacks, antigen-based diagnosis offers several advantages over nucleic acid-based detection, such as rapid detection time, ease of readout and requirement of limited technical expertise as well as resources. On the other hand, antibody-based (serological) detection is susceptible to incubation period of the SARS-CoV-2 virus in human body. Backer and coworkers tracked and analyzed travel history data and infection symptoms of 88 positive cases and found a mean incubation time of 6.4 days, with a range of 2 to 11 days [113]. Furthermore, the host's immune response to SARS-CoV-2 infection was usually detected from week one or later [114]. This required time for developing a detectable antibody or viral

particle load makes it difficult for the antibody-based detection techniques to identify, separate, and treat the patients promptly. As a result, asymptomatic patients can cause the spread of the infection to their surrounding environment before they are accurately diagnosed with other confirmatory tests [115].

5. Other bio/markers-based diagnosis

Besides viral genes, viral proteins, and human antibodies against viral proteins, there are a variety of supplementary markers of clinical importance for COVID-19 diagnosis. These include, cytokines, reactive oxygen species (ROS), C-reactive protein, serum amyloid A, lactate dehydrogenase, D-dimer, etc. [25] Detection of these markers provides supplementary evidence with clinical significance to confirmatory tests. In the following sections we review some of the bio/markers that have been considered for COVID-19 diagnosis and summarize them in Table 3.

5.1. Detection of Smell dysfunctions

Reports suggest that some sensory dysfunctions are useful as a marker for diagnosis of COVID-19. For example, a study showed that 98% (59/60) patients exhibited smell dysfunction to some extent, which can be divided to 58% (35/60) patients were anosmic or severely microsmic, 27% (16/27) with moderate microsmia, 13% (8/60) with mild microsmia and 2% (1/60) normosmia. Therefore, a quantitative smell test that identifies the smell dysfunction can potentially diagnose COVID-19 patients in the early quarantine [118].

5.2. Detection of Cytokines

Among the reported biomarkers suggested for monitoring SARS-CoV-2 infection, cytokines play an important role [25–28]. Cytokines are a broad group of small proteins and glycoproteins secreted by the immune system and control the interaction among the immune cells and stimulate the target cells to produce more cytokines [119]. Cytokines have

been recognized as potential biomarkers towards the detection and monitoring of the COVID-19. Studies have shown that interleukin-6 (IL-6) levels increase significantly during the manifestation of COVID-19 [120]. Based on activity, cytokines are divided into two types: pro-inflammatory and anti-inflammatory cytokines. During the infection of SARS-CoV-2, the host patient's immune system can become hyperactive, resulting in a release of an excessive amount of pro-inflammatory cytokines, which is often referred to as “cytokine storm” [121]. Consequently, a large number of immune cells are produced, and their signaling molecules cause excessive inflammatory reactions in the lungs, which can lead into acute respiratory distress syndrome, low oxygen level in blood, and multi-organ failure, ultimately causing death in severe cases [122]. Researchers have reported significantly increased concentration of pro-inflammatory cytokines IL-1, IL-6, and TNF- α in the serum of coronavirus hosts [27,123]. IL-6, a pro-inflammatory cytokine, is one of the critical mediators in the acute inflammatory response. Reports indicate that the mean levels of IL-6 of patients with complicated manifestations of COVID-19 are 2.9-fold higher than those without [124]. The normal concentration of IL-6 is < 7 pg/mL in the human peripheral blood [125], while Wang et al. have reported a higher level of IL-6 (20 pg/mL) in COVID-19 patients' samples [126]. Thus, monitoring the cytokine levels at point-of-care settings will not only facilitate treating the patients before they need intensive care treatment but also aid immunomodulatory therapies [127]. Recently, commercial products have been released for the detection of IL-6. Roche Diagnostics received FDA EUA for their commercial product Elecsys IL-6 [128]. Elecsys IL-6 is an electrochemiluminescence immunoassay device for quantitative detection of IL-6 in human serum or plasma with a dynamic concentration range of 1.5 to 5000 pg/mL. The test requires less than 30 μ L sample and has an assay time of 18 minutes. It is a promising tool for early detection of SARS-CoV-2-triggered hyper inflammation, thereby aiding health workers to monitor the disease's prognosis. However, this device is not for POC settings. To meet the ASSURED criteria, colorimetric paper-based immunosensor has been reported [129]. Using plasmonic gold nanoprobe, colorimetric detection of IL-6 in unprocessed whole blood in sepsis is achieved with a LOD of 12.5 pg/mL within 17 min

Table 3

Summary of the reported POC-styled diagnostic tools for COVID-19 by detecting other bio/markers. Source: US Food & Drug Administration, unless referred otherwise.

Developer	Product	Instrument	Sample source	Target	Sensing method	Assay time	Lowest LOD reported	Comment
Other biomarker detection								
Roche Diagnostics	Elecsys IL-6	Cobas e immunoassay analyzers	Human serum and plasma	Interleukin-6 (IL-6)	ECLIA	18 min	1.5 pg/mL	Identify severe inflammatory response (Cytokine storm) in serum/plasma
Beckman Coulter, Inc.	Access IL-6	Luminometer	Human serum and plasma	Interleukin-6 (IL-6)	Paramagnetic particle, chemiluminescent immunoassay	~35 min	≤ 2 pg/mL	Identify severe inflammatory response in serum/plasma, determine need of incubation with mechanical ventilation in confirmed cases.
[129]	N/A	N/A	Whole blood	Interleukin-6 (IL-6)	Plasmonic immunosensor	< 17 min	0.1 pg/mL	Paper-based plasmonic biosensor, paired with smartphone app for colorimetric detection
[131]	N/A	Portable fluorescence strip reader	Human serum	Interleukin-6 (IL-6)	Quantitative LFIA	15 min	0.37 pg/mL	Portable, applicable for POC, patient of severe sepsis cannot be diagnosed as their IL-6 level is beyond linear detection range

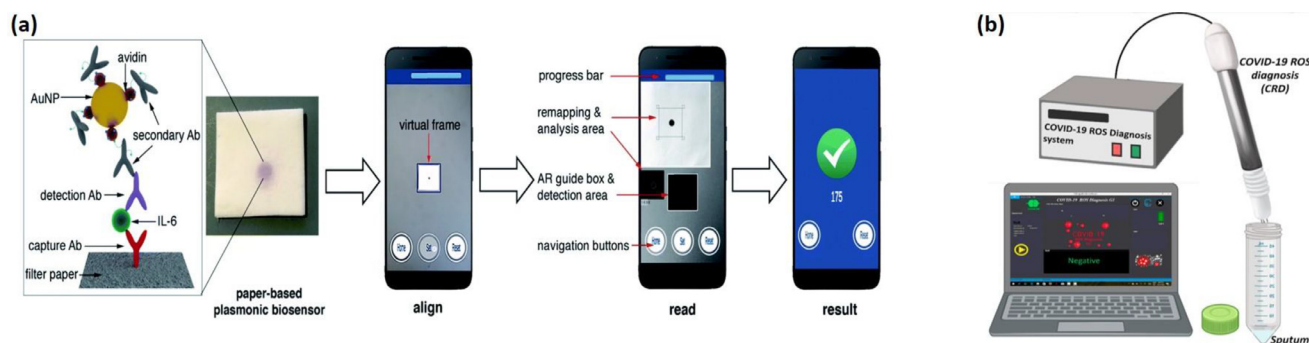


Fig. 7. Detection methods for other bio/markers. (a) Schematic illustration of smartphone based plasmonic biosensor. Antibody-functionalized AuNP produces colored spots on paper substrates. The pixel intensity is detected and quantified with an augmented reality aided app through a smartphone and displays the quantitative detection of interleukin-6 (IL-6). Reproduced with permission from [129]. (b) Real-time electrochemical diagnosis system for reactive oxygen species (ROS) in sputum. Reproduced with permission from [132].

(Fig. 7a). The colorimetric signal is easily processable with an augmented reality system supported smartphone application. Moreover, Russel et al. reported a needle-shaped microelectrode device for real-time electrochemical detection of IL-6 at a physiologically relevant concentration level (pg/mL) from human serum samples. Their reported setup can also perform real-time monitoring of the IL-6 level in blood when integrated into the bloodstream with a cannula [130]. In another study, Huang et al. reported a europium nanoparticle-based double-antibody sandwich immunofluorescent LFIA kit for on-site detection of IL-6. The quantitative detection range was 2–500 pg/ml with a sensitivity of 0.37 pg/ml, and the total assay time was < 15 min [131]. As discussed, although cytokine and other bio/markers of clinical significance measurements are only indicative—it is not a confirmatory test for diagnosing the disease. Researchers and commercial manufacturers are still working on developing real-time and point-of-care tools for measuring the cytokine levels in COVID-19 patient serum.

5.3. Detection of reactive oxygen species (ROS)

ROS is a potential indirect marker for COVID-19 diagnosis. ROS are highly reactive radicals or non-radical derivatives of oxygen (hydroxyl radical ($\text{OH}^\cdot$), superoxide anion ($\text{O}_2^{\cdot-}$), singlet oxygen ($^1\text{O}_2$), oxygen peroxide (H_2O_2), and ozone (O_3)) [133]. Several studies have shown the correlation between ROS production and the depletion of antioxidants in the human immune system due to viral infections [134,135]. Based on this correlation, Miripour and coworkers reported a simple, rapid, and point-of-care, electrochemical diagnostic device where sputum samples of patients were used to measure the electrical signal due to increased ROS concentration (Fig. 7b). The working electrode was functionalized with multi-walled carbon nanotubes, which reacted with the ROS. The released electric charges from the reaction were transferred through the counter electrode. The setup with an integrated readout board and disposable sensor produced results in just 30 seconds and had both sensitivity and accuracy of 97% [132]. This device may serve as a potential diagnostic tool for on-spot testing; however, the accuracy needs to be increased further for more accurate detection.

As discussed above, the identification of sensory dysfunctions, cytokine storm and ROS in sputum samples are indirect and indicative detection for identifying potential COVID-19 patients, rather than being confirmatory tests. However, in the time of high demand for reagents, tools, and necessary accessories required for confirmatory tests, these indirect, point-of-care diagnostic tools can serve as potential alternatives to reduce the load on other SARS-CoV-2 testing methods. Thus, monitoring these markers can help the patients monitor themselves in the initial stage rather than going to the hospitals where there may be a higher level of contagion.

6. Conclusions, perspectives and outlooks

The detection of the viral gene (i.e., viral RNA) is considered the most reliable evidence of the presence of the SARS-CoV-2 virus, the source of which can be not only the host human body but also surfaces of common objects. Even though many governmental authorities and institutions have built up the large capability of COVID-19 diagnosis to suffice the need for thoroughgoing screening to prepare for the reopening of the economy and society, the COVID-19 pandemic has profoundly changed human life and tests of infectious diseases such as COVID-19 will be a prevalent and necessary societal activity in the future. To develop affordable, reliable, fast and portable nucleic acid tests, increased funding and research should be encouraged. One promising trend is miniaturizing and integrating the test on a chip (LoC) where pre-set reagents, preprogrammed microfluidics, and portable measurement instruments are equipped to simplify and accelerate the sensing result reporting process. As mentioned earlier, a large number of commercial tests are now available to detect various bio/markers for COVID-19, while more advances are being made in research labs for higher sensitivity and specificity, lower detection limits, and lower production cost. Compared to the conventional RT-PCR method, novel biosensing methods and functions are employed and integrated with the LoC-styled systems, including isothermal nucleic acid amplification, gene editing, microfluidics, and mobile devices.

Among all COVID-19 biomarker detection methods, protein-based tests are relatively easy to be integrated on a miniaturized sensing device due to the nature of the sensing process that is less demanding for reagents, skilled operators, controls of fluids and temperatures. Numerous commercial antibody test kits have been developed and pushed to the markets. In the future, people may obtain such products easily to quickly check the antibody levels towards a specific disease at home. Nevertheless, the most common causes of the low sensitivity and specificity of the immunoreaction based diagnostic tools can be attributed to these reasons—non-specific antibody/antigen binding, biochemical interferences and cross-reactivity, inappropriate/insufficient surface blocking of the sensor in serological immunoassay, and instability of the reagents [136]. Care must be taken, and attention must be paid to these issues while designing a rapid and point-of-care immunosensor meeting the ASSURED criteria. Moreover, protein-based tests offer rapid and low-cost methodologies while lacking good sensitivity and specificity compared to PCR-based diagnosis. However, PCR requires complicated lab-based facilities and much longer detection time. Therefore, a combination of the biosensing with clinical routines offers much higher accuracy for detecting the infected.

Additionally, it is worth noting that the existence of antibodies against COVID-19 does not equal the lifetime immunity to the disease as reports indicate some suffer worse or lose lives on a reinfection of the SARS-CoV-2 [137,138]. Thus, it is dangerous to conclude the

equivalence of antibodies and immunity. Meanwhile, it is worth stating that the antibody test is supplementary evidence, and the results from large scale antibody tests do not ensure herd immunity. As of now, it is merely a year since the COVID-19 was reported, and there is not enough scientific proof verifying the entire role of the immune responses with many mechanisms still unclear. Researchers worldwide are working diligently to develop the diagnostic tests, vaccines, drugs, and treatments. However, it often takes months or years before a vaccine, or a drug is corroborated as effective in introducing human immunity and curing without severe side effects.

Lastly, an advanced diagnostic test for quick, sensitive, specific identification of COVID-19 cases, irrespective of the target analytes (viral RNA, viral protein, human IgG IgM, or other bio/markers), is only one element to break the chain of transmission of COVID-19. The keys to breaking the transmission include controlling the contagion (diagnostic test and isolation), protecting the susceptible (vaccine and isolation of the infected) and breaking the transmission path (disinfection, face coverings, and handwashing). In the particular case of COVID-19, the spread of the virus is mainly through contaminated objects, close contacts with the salivary or respiratory secretion droplets, and close contact with infected people via mouth and nose secretions. Therefore, wearing proper PPE, self-hygiene, and social distancing can effectively break the transmission chain by avoiding contact with these virus-containing particles. Tools such as masks and hand sanitizers are low-cost and low-tech, yet highly effective in preventing the COVID-19 transmission. The quarantine of patients or suspected cases was proven in many areas to control the infection source and therefore reduce the number of victims and thus flatten the curve. Taken together, a test is not a final solution; rather it is a “stepping-stone” to the collaborative job of the whole human to fight the COVID-19. It requires great efforts from all communities on earth, ranging from diagnostic tests, medical care workers, supply manufacturers, ordinary people’s staying-at-home to scientific and therapeutic advances, to eradicate the threat of COVID-19 to human life.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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