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

Emerging biosensing technologies for improved diagnostics of COVID-19 and future pandemics

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ABSTRACT

Diagnostic tools play significant roles in the fight against COVID-19 and other pandemics. Existing tests, such as RT-qPCR, have limitations including long assay time, low throughput, inadequate sensitivity, and suboptimal portability. Emerging biosensing technologies hold the promise to develop tests that are rapid, highly sensitive, and suitable for point-of-care testing, which could significantly facilitate the testing of COVID-19. Despite that, practical applications of such biosensors in pandemics have yet to be achieved. In this review, we consolidate the newly developed diagnostic tools for COVID-19 using emerging biosensing technologies and discuss their application promise. In particular, we present nucleic acid tests and antibody tests of COVID-19 based on both conventional and emerging biosensing methods. We then provide perspectives on the existing challenges and potential solutions.

1. Introduction

Coronavirus Disease 2019 (COVID-19) is an ongoing pandemic which poses extreme challenges to public health and global economy. As of Aug. 23, 2020, there have been more than 23 million confirmed cases with 0.8 million deaths worldwide [1], and economic activities have been significantly interrupted due to mitigation measures. Combating COVID-19, as well as future pandemics, is an important task (see Table 1).

The pathogen of COVID-19 was identified as a novel coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a positive-sense single-stranded RNA virus enclosed by an envelope, as shown in Fig. 1. Its genome consists of about 29 thousand nucleotides [2], incorporating ORF1ab, S, E, M, and N genes, among other genes. The envelope of SARS-CoV-2 is mainly composed of three types of proteins, namely spike (S), envelope (E), and membrane (M) proteins [3]. A fourth protein, named nucleocapsid (N) protein, forms complexes with the genomic RNA. The virus is primarily transmitted through respiratory droplets [4]. As SARS-CoV-2 particles enter respiratory tracts, S proteins on the virus envelope bind to angiotensin converting enzyme 2 (ACE2) receptor on host epithelial cells, initiating the virus infection and replication and leading to pneumonia symptoms such as fever, cough, fatigue, and shortness of breath [5]. Though COVID-19 is less lethal compared to severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), the virus appeared much more contagious [6]. In addition, significant numbers of asymptomatic or pre-symptomatic carriers of SARS-CoV-2 have been observed, and there have been postulations that these carriers can shed virus [7], making the virus spreading very difficult to contain.

Diagnostics helps identify infected patients for timely isolation and treatment and thus plays an important role in the management of infectious diseases. In the case of COVID-19, efficient and accurate virus detection is especially important. Firstly, given its high contagiousness and rapid spread, quick and accurate identification of SARS-CoV-2 carriers is extremely important, requiring sensitive tests which can be offered in high volume. Secondly, it is increasingly believed that COVID-19 will not disappear within a year. To re-open the economy and resume normal life safely, it is important to have virus and antibody tests to keep track of infections and immunity [8].

Two types of tests have been developed for the diagnostics of COVID-

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19, namely nucleic acid test and antibody test. After initial virus infection, patients normally experience an incubation period of 4–5 days on average, when virus replicates and viral load increases [9]. Viral load usually reaches a peak in the second week after symptom onsets, before it gradually winds down, as shown in Fig. 2 [10]. Given the quick response of viral load to virus infection, test of the viral nucleic acid has been the primary means for the confirmation of infection. In contrast, antibody load shows a much slower response. It was reported that on average, IgM and IgG did not reach a detectable level until 13 days after symptom onset (Fig. 2) [11,12]. However, antibody in the serum stays at a relatively high level even after recovery. Therefore, antibody test has been prescribed for the identification of infection history and existing immunity.

The nucleic acid test of COVID-19 has been predominantly based on quantitative reverse-transcription polymerase chain reaction (RT-qPCR), which detects the presence of virus during the process of amplifying certain genes of the virus. Despite its wide use, RT-qPCR has a few limitations. Firstly, the diagnosis of early-phase infection, where the viral load is very low [13], is normally ineffective, leading to false negatives. Secondly, the testing throughput needs to be improved given the rapid increase in suspected cases before the curve is flattened. Thirdly, RT-qPCR cannot be easily adapted for point-of-care testing. In less developed areas, where life is impacted the most by the pandemic, testing laboratories are normally lacking, limiting the testing capability and timely case identification. In addition, in settings such as customs, resorting to laboratories for testing is impractical. In both scenarios, it is preferable to have the testing performed on-site without being sent to central laboratory. Ideally, such tests should require minimal human intervention for biosafety concerns and generate results in several minutes.

Biosensing is a research field that has seen rapid development in the past decade. Leveraging advances in microfabrication, nanotechnology, and novel biotechnology, researchers have developed biosensors with improved sensitivity, specificity, testing speed, and cost-effectivity [14]. As such, emerging biosensing technologies hold great promise to develop tests that could potentially address the limitations of existing tests [15,16]. Indeed, in face of COVID-19 pandemic, several diagnostic tests based on emerging biosensing technologies have been quickly developed, enabling sensitive, high-throughput, and point-of-care testing. Available diagnostic tools for COVID-19 have been summarized in a few reviews, including those focused on polymerase chain reaction [17], molecular diagnosis [18,19], FDA-approved tests [20], micro- and nanosystems [21,22], among others [23–30]. Nevertheless, reviews focusing on the biosensing perspective have been lacking [31]. Such reviews would place biosensing research in a pandemic context and provide reflections on how biosensing technologies can better assist infection tests in pandemics. Here, we consolidate the newly developed diagnostic tools for COVID-19 using emerging biosensing technologies and discuss their application promise. We first present COVID-19 tests, including both nucleic acid tests and antibody tests, based on conventional methods and discuss their limitations, before we present the recently developed tests based on emerging biosensors. We further provide comments on the advantages of emerging biosensing technologies as well as the issues that need to be solved to make them more useful in pandemics. We envision the rapid advances in biosensing technology will effectively assist the battle against COVID-19 and future pandemic outbreaks.

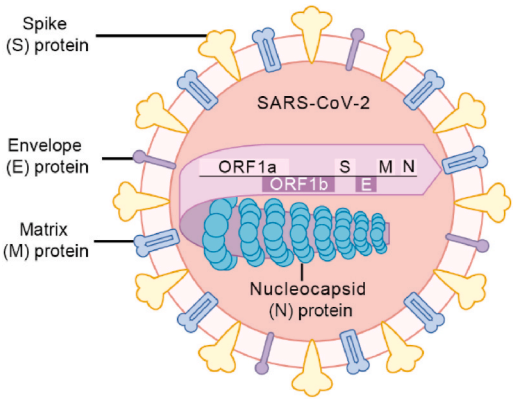

Fig. 1. Structure of SARS-CoV-2. Adapted with permission from Ref. [3].

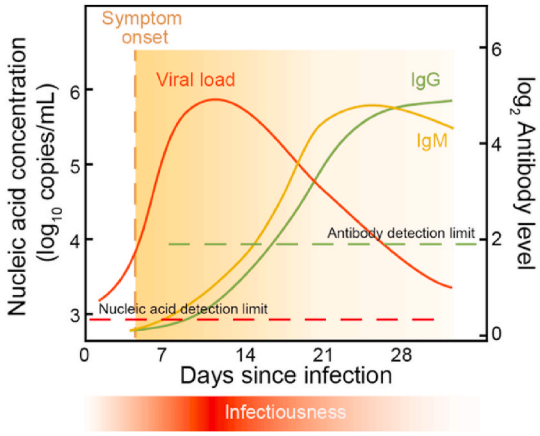

Fig. 2. Representative dynamics of viral load, antibody level, and infectiousness. Antibody level is presented as the ratio of measured values to cutoff value. Dynamic tracings were compiled based on data from Ref. [9–11], and infectiousness was compiled based on data from Ref. [109].

Table 1
Performance characteristics of representative methods.

Technique	Ref.	Detection target	Performance characteristics			
			Analytical sensitivity	Sensitivity	Specificity	Assay time
RT-qPCR	[32]	Nucleic acid	0.144 copies/μL	–	100% (310/310)	~4 h
Digital PCR	[42]	Nucleic acid	0.021 copies/μL	–	–	~5 h
RT-LAMP	[49]	Nucleic acid	1 copy/μL	–	–	~30 min
CRISPR-Cas12-based detection	[60]	Nucleic acid	10 copies/μL	–	100% (40/40)	~45 min
Barcode sequencing	[71]	Nucleic acid	–	>99.8% (estimated)	>99.8% (estimated)	~1 day
Localized surface plasmon resonance	[82]	Nucleic acid	1.32×10^5 copies/μL	–	–	~15 min
ELISA	[94]	Antibody	–	>80%	>99%	~6 h
Lateral Flow Assay	[94]	Antibody	–	>80%	>95%	~15 min
Chemiluminescence immunoassay	[96]	Antibody	–	99.9%	100% (125/125)	~30 min
Field-effect transistor	[101]	Antigen	0.242 copies/μL	–	–	~15 min
Surface plasmon resonance	[102]	Antibody	1 μg/mL	–	–	~15 min
Microfluidic ELISA	[104]	Antibody	2 ng/mL	–	–	15–20 min

1.1. Conventional nucleic acid test

Quantitative reverse-transcription polymerase chain reaction (RT-qPCR) is currently the routine method used for the detection of SARS-CoV-2. Briefly, specimens are collected from patients through nasopharyngeal swab, before the virus RNA is extracted from the medium (Fig. 3). Virus RNA is then reverse transcribed into complementary DNA, which is in turn amplified through PCR and detected using fluorescent dyes or labeled probe methods. In the PCR process, specifically designed primers are used to ensure that only chosen genes are amplified. Thus, primer design is critical to achieve high sensitivity and specificity.

A few primer and probe designs have been developed, mostly targeting ORF1ab gene, N gene, or E gene. For example, Corman et al. proposed and validated the test kit targeting RNA-dependent RNA polymerase (RdRp) gene in ORF1ab and E gene. Their test results showed a limit of detection of 3.9 copies/reaction for the E gene assay and 3.6 copies/reaction for the RdRp assay when using *in vitro* transcribed RNA identical to SARS-CoV-2 target sequences [32]. Primers and probes targeting different parts of the genes have also been tested and recommended by other research laboratories [33–35], and several RT-qPCR test kits have received Emergency Use Authorization (EUA) from the US Food and Drug Administration (FDA) and are commercially available. To gauge the quality of the test kits, Moran et al. tested the performance of Cepheid Xpert Xpress and Roche cobas SARS-CoV-2 assays [36]. The two assays generated same results on 102 out of 103 specimens, showing an agreement of 99%. However, Ct values from Cepheid assays were slightly lower, which was presumably due to the difference in primer sequences.

RT-qPCR detects nucleic acid sequences regardless of the infectivity of the virus. As such, recovered patients continued to show positive RT-qPCR results, even though replication-competent virus was not isolated [37,38]. This finding was possibly attributed to that RT-qPCR detected inactive virus remaining in the patients. In the current form of RT-qPCR test for COVID-19, specimen is normally collected by nasopharyngeal swab, which requires well-trained personnel for reliable sampling. Specimen collection with compromised quality has been postulated as a potential source of false negative test results [39]. In addition, the high-volume consumption of swabs and transportation medium have brought about supply chain issues. To address this problem, a few methods have been reported. Srivatsan et al. proposed a simplified protocol for RT-qPCR tests which performed tests on the swab directly without using transport medium or performing RNA extraction, aiming to reduce the pressure on test-related consumable supply. Results showed that dry swabs supported the virus detection at the endpoint of RT-qPCR and the sensitivity was not substantially compromised. Given the technical difficulty in obtaining nasopharyngeal swab specimen, the possibility of using alternative specimen have been explored. Researchers reported the detection of virus using saliva specimen with high sensitivity and high agreement in testing outcome with the established test protocols, suggesting that saliva could be a reliable specimen for case confirmation [40,41].

1.2. Emerging nucleic acid test

Though RT-qPCR has good sensitivity and specificity, it requires dedicated instruments in a central laboratory, resulting in turnaround time of normally more than one day. In addition, the limit of detection and the accuracy of the quantitative measurement can be inadequate for early detections. In this regard, a few new nucleic acid sensing technologies can serve as great alternatives.

Digital PCR (dPCR) utilizes tens of thousands of tiny compartments, such as droplets or microwells, and performs PCR in each compartment (Fig. 4a). Since the molecule population within each droplet follows Poisson distribution, by calculating the fraction of the fluorescing droplets, it provides a means for absolute quantification of sample concentration. Compared to traditional PCR, dPCR offers a more precise measurement. Since the outbreak of COVID-19, several groups have designed primers and implemented dPCR for the measurement of SARS-CoV-2. For example, Suo et al. reported the use of droplet digital PCR (ddPCR) for the quantitative measurement of SARS-CoV-2 and compared the results with those from RT-qPCR [42]. The primers and probes adopted in the ddPCR also targeted the ORF1ab and N gene as commonly done in RT-qPCR. Results showed that the ddPCR had a lower limit of detection of 0.109 copies/μL and 0.021 copies/μL for ORF1ab and N genes, respectively, which were more than 500 times lower than that of most RT-qPCR tests. The overall accuracy was 94.3%, and the test was able to identify 92.6% of the false negatives from RT-qPCR. Similar works using ddPCR was also reported by other groups [43–45]. In addition, instead of droplets, microwells had been implemented for dPCR assay of SARS-CoV-2, and the assay has obtained EUA from the US FDA [46].

Despite the improved quantification in measurement, a few limitations remain in dPCR. For example, the dynamic range of dPCR depends on the amount of partitions that can be generated, and with the current dPCR technologies, the dynamic range is generally lower than qPCR. In terms of operation, dPCR has lower throughput and requires specialized consumables, increasing the cost per test [47]. In addition, an inherent limitation of PCR-based methods is that PCR requires cyclic heating, which compromises the amplification efficiency and complicates the instruments, making it not ideal for point-of-care diagnosis. In contrast, isothermal amplifications are performed at constant temperature with rapid reaction and simplified instruments. The lowered demand on the instruments makes it easily adoptable for tests outside of central laboratories, which would significantly boost testing efficiency.

Loop mediated isothermal amplification (LAMP) is a DNA amplification technique performed at constant temperature. Compared to PCR, it uses more primers and thus enables better specificity in the amplification. In addition, by incorporating dyes or pH indicators in the reaction, amplification products can be visually detected by observing turbidity or simply color change, making LAMP a great technique for point-of-care virus testing (Fig. 4b). Zhang et al. reported the

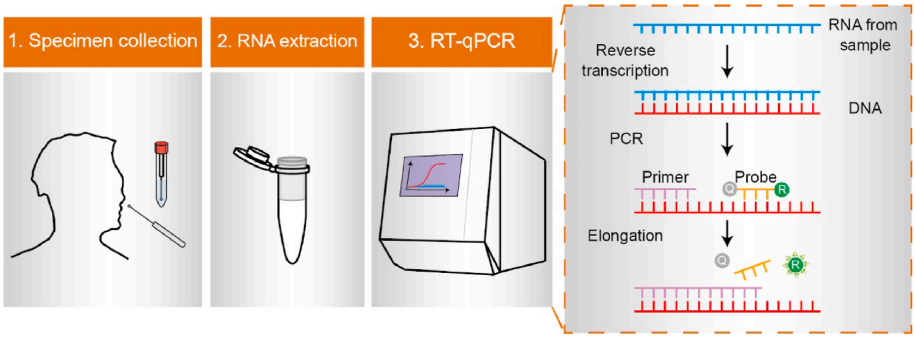

Fig. 3. The workflow of the standard nucleic acid test, RT-qPCR.

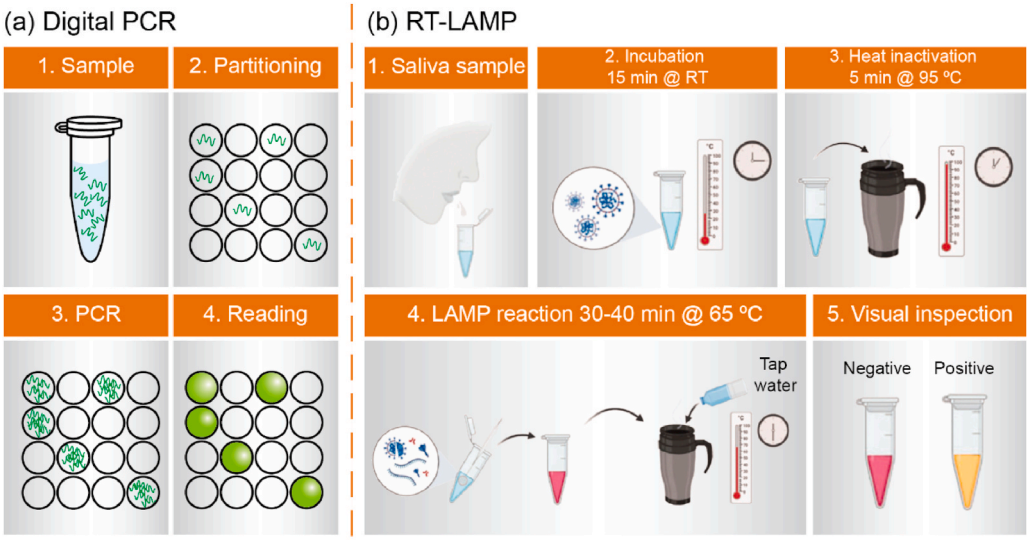

Fig. 4. Nucleic acid tests based on emerging biosensing strategies of (a) digital PCR and (b) RT-LAMP. Figure (b) adapted from Refs. [53] under the terms of the Creative Commons Attribution 4.0 License.

preliminary study of reverse transcription-LAMP (RT-LAMP) assays for SARS-CoV-2 detection with colorimetric readouts [48]. They designed five LAMP primer sets targeting ORF1a gene and N gene and tested them using synthetic RNAs. Testing results had 100% agreement with RT-qPCR, and an analytical sensitivity of 4.8 copies/μL was achieved. In addition, tests using crude cell lysates without RNA extraction also showed similar sensitivity, suggesting that RT-LAMP has the potential to serve as a point-of-care assay for field application.

In another study, Rabe et al. developed similar RT-LAMP assays and optimized virus inactivation and purification protocols [49]. The

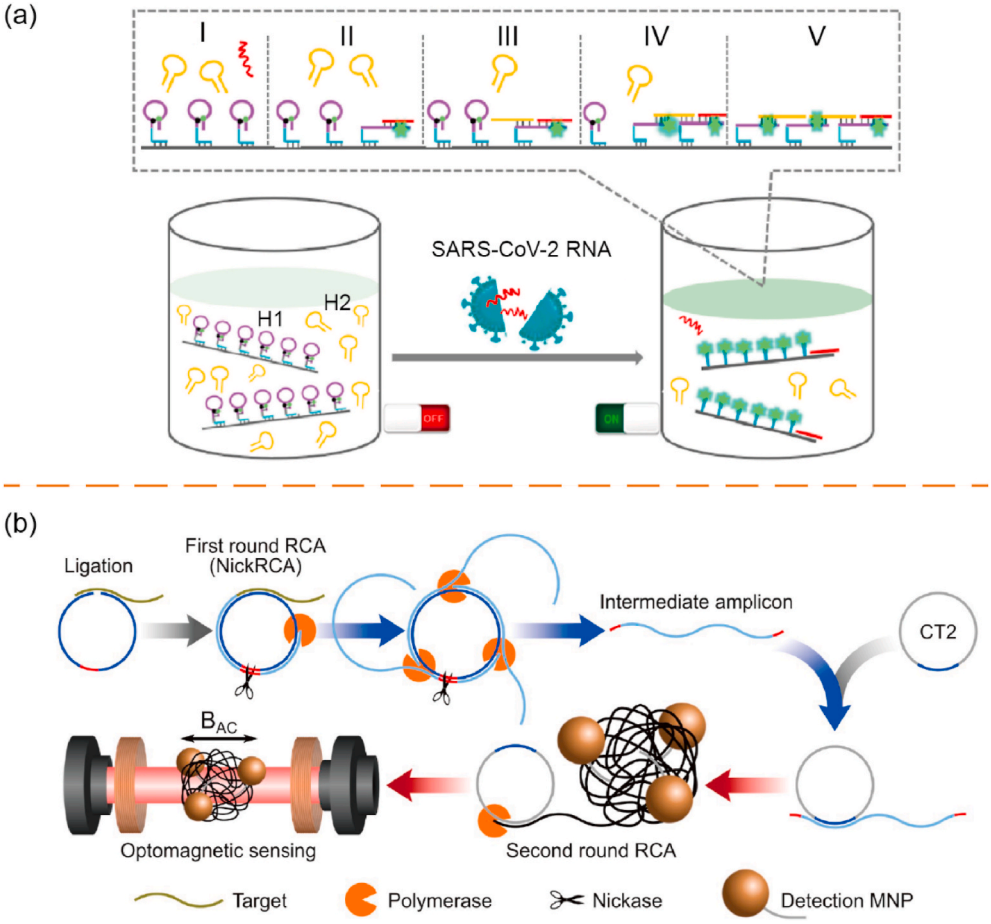

Fig. 5. Nucleic acid test based on emerging biosensing strategies of DNA (a) nanoscaffold hybrid chain reaction and (b) circle to circle amplification. Figure (a) adapted with permission from Ref. [57]. Figure (b) adapted with permission from Ref. [58].

optimized assay was able to be completed in 30 min, and the analytical sensitivity was 1 copy/μL following the purification protocol. Impressively, the sample inactivation and purification process did not require commercial reagent kits, and the overall cost was only 0.07 US dollars per sample, making it affordable for massive testing. Similar works have also been reported by other research groups [50–55].

Though LAMP is a convenient technique for nucleic acid amplification, a few limitations shall be addressed to achieve widespread adoption. First, LAMP uses a set of four or six primers, making primer designs subject to more constraint and difficult to implement in certain settings [56]. Second, multiplex detection using LAMP is less developed than using PCR, since larger number of primers imposes greater chance of primer-primer interference.

Other nucleic acid amplification tests have also been explored and implemented for the detection of SARS-CoV-2. Jiao et al. reported the development of SARS-CoV-2 detection based on DNA nanoscaffold hybrid chain reaction (DNHCR) [57]. This method adopted two hairpin probes, namely H1, which is self-quenching, and H2, as shown in Fig. 5a. In the presence of SARS-CoV-2 RNA, H1 hybridize with the target RNA and unfold, restoring the fluorescence and allowing H2 to hybridize with H1. The unfolded H2 then hybridizes with adjacent H1, thus amplifying the fluorescence signal. DNHCR detected target within 10 min without the need of heating, suggesting its potential as an alternative to the standard nucleic acid test. Nevertheless, the acquisition of fluorescent signals requires bulky equipment, making it suboptimal for point-of-care testing. Further efforts may be necessary to miniaturize the signal acquisition setup. In another work, Tian et al. designed and implemented a detection method based on circle-to-circle amplification, as shown in Fig. 5b [58]. The first round of rolling circle amplification generated intermediate amplicons, which annealed to a second circular template. The resultant amplicon coils assembled to specially designed magnetic nanoparticles, which were then detected through an opto-magnetic sensor. This proposed method achieved a detection limit of 0.4 fM (~240 copies/μL) when using synthetic complementary DNA of SARS-CoV-2 and a total assay time of roughly 100 min. Since clinical samples are much more complicated than synthetic DNA sequences, further efforts shall be devoted to assay validations using clinical samples.

CRISPR-Cas system is an emerging technology which enables nucleic acid sequence recognition and subsequent cleavage. The system consists of a guide RNA (gRNA) and a CRISPR associated (Cas) protein. Upon the binding of the gRNA to the target site, Cas protein is activated and cuts nucleic acids. Among the various Cas proteins, Cas12 and Cas13 showed collateral cleavage activities on RNA or DNA, making them great tools for biosensing of virus nucleic acids [59]. Broughton et al. developed a CRISPR-Cas12-based assay for the detection of SARS-CoV-2, named SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR), and validated its efficacy [60]. Primers and gRNA were designed to target the E gene and N gene of SARS-CoV-2, and single-stranded DNA (ssDNA) probes tagged with FAM-biotin reporter were incorporated in the detection reagent, as shown in Fig. 6. When the target sequences were recognized by the gRNA, Cas12 effector cleaved the probes, generating visual readout on test band on the lateral flow assay. The DETECTR-based lateral flow assay enabled virus detection in fewer than 40 min, with limit of detection of 10 copies/μL, sensitivity of 95%, and specificity of 100%. A similar assay based on Cas12 b was also reported [61].

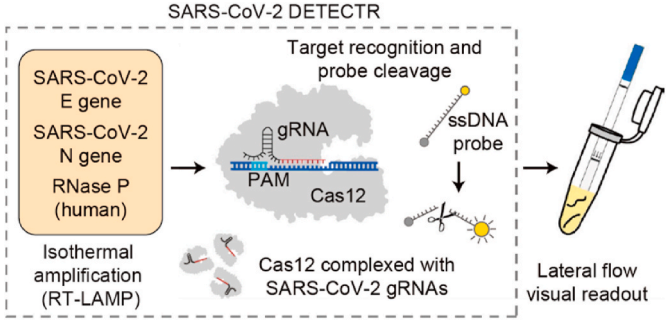

Fig. 6. Nucleic acid test based on CRIPSR/Cas systems, namely SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR). Adapted with permission from Ref. [60].

Instead of cleaving ssDNA, Cas13 cleaves single-stranded RNA and has also been adapted for virus detection. Myhrvold et al. developed a Cas13-based virus detection platform, named specific high-sensitivity enzymatic reporter unlocking (SHERLOCK), and incorporated the biosensing technology into lateral flow assays [62]. Using SHERLOCK, Dengue and Zika virus were successfully tested in a rapid, quantitative, and portable fashion. Since the outbreak of COVID-19, the same groups quickly developed an assay protocol to detect SARS-CoV-2 along with other respiratory viral pathogens simultaneously, which could potentially enable fast surveillance of circulating viruses [63]. Using synthetic SARS-CoV-2 RNA as the test sample, an analytical sensitivity of 10 copies/μL was demonstrated. Similar Cas13-based SARS-CoV-2 assays were also achieved by different groups [64,65].

A shortcoming of CRISPR-based biosensors is that they require protospacer-adjacent motif (PAM) sequences adjacent to the target sequence, which could pose a challenge when performing single nucleotide polymorphism (SNP) discrimination or detection of short sequences [59]. In addition, current CRISPR-based biosensors require amplification and detection in different tubes, complicating the operation and increasing the chance of contamination. Joung et al. developed an optimal combination of LAMP primers and gRNAs to allow for amplification and CRISPR-mediated detection in a single-step reaction, in an assay named SHERLOCK-Testing in One Pot for COVID-19 (STOPCovid) [66,67]. The assay only required micropipettes and 60 °C water bath, which greatly increased its accessibility. The limit of detection was shown as 100 copies, and the assay could be accomplished within 70 min on a lateral flow assay, making it a powerful tool for point-of-care testing.

It is noteworthy that recently CRISPR-Cas system has also been adopted to develop a massively multiplexed virus detection platform [68]. Ackerman et al. designed multiple primers to generate amplicons from samples and multiple crRNAs for the detection of different viruses. The amplified samples and Cas13 detection mix were color coded based on the primer or crRNA and encapsulated into droplets, which were then pooled and paired on a microwell chip. Contents in the droplets could be identified based on the coded fluorescence, and virus presences were detected based on a different fluorescence emitted by the probes. Using this platform, the authors demonstrated the simultaneous detection of 169 viruses. In addition, the detection of a coronavirus panel including SARS-CoV-2 was designed and implemented, which demonstrated the capability of this platform for quick adaption to ongoing pandemic.

DNA sequencing provides a means to examine the nucleic acid sequence of the pathogens in detail, thus enabling both case confirmation and mutation surveillance, which is extremely important for public health decision making [69]. Indeed, an application note on the workflow for an NGS-based SARS-CoV-2 detection was posted by Illumina [70]. However, such assays are expensive, time-consuming, and difficult to scale up. To address this challenge, Schmid-Burgk et al. proposed a barcoded nucleic acid amplification protocol, named **LAMP-seq**, for massively parallel sample sequencing [71]. After sample collections, RT-LAMP was performed for nucleic acid amplification and indexing, with each sample using a unique set of primers with unique barcode, before samples are pooled, further amplified, and sequenced. The unique barcode primers ensured that each sample could be identified in the pooled sequencing data. This method could be scaled to analyze millions of samples per day on an NGS facility, with each sample potentially costing only about 2 US dollars. A potential limitation is the skewing of sample representation due to the amplification process at the

pooling stage, though initial assessment showed promising results. In addition, further work is necessary to investigate the sensitivity and stability of RT-LAMP reaction using unpurified swab samples.

Nanopore sequencing is a third-generation sequencing technology which provides long-read, real-time readout. Combining targeted amplification and nanopore sequencing, Wang et al. developed **nanopore targeted sequencing** for the detection of SARS-CoV-2 as well as other respiratory viruses [72]. As shown in Fig. 7, sample RNA was first extracted before being reverse transcribed and going through target amplification. The amplification products were then barcoded and ligated with adapters for sequencing on the nanopore sequencer. The detection method could be completed within 6–10 h with a limit of detection of ten standard plasmid copies per reaction.

Coinfection with microorganisms other than SARS-CoV-2 has likely contributed to the morbidity and mortality of COVID-19. To detect SARS-CoV-2 while simultaneously assessing the background microbiome, nanopore sequencing was used to perform metagenomic analysis [73]. Results identified the coinfection with other microorganisms such as Fusobacterium periodonticum and human betaherpesvirus 5 among patients. Though in terms of diagnosis, the sensitivity is not as good as RT-qPCR, this method can potentially be used to guide coinfection treatment and monitor viral evolution. A nanopore sequencing based protocol for the molecular diagnosis of COVID-19 was later published by Oxford Nanopore Technologies Inc. and have been widely adopted in the United Kingdom [74,75].

In addition, a few other sequencing-based SARS-CoV-2 detection and surveillance methods have been reported. Chandler-Brown et al. developed a protocol based on Sanger sequencing [76]. Briefly, specimen was directly added to PCR master mix without the step of RNA extraction, before being amplified and sequenced. Using frame-shifted spike-in as the specimen, it was shown that a limit of detection comparable to RT-qPCR was achieved. Given that automated Sanger sequencing instruments are common in clinical laboratories and that each instrument can handle up to 3840 samples per day, this method could be a powerful supplementary test. In another work, St Hilaire et al. developed a whole genome sequencing method, named Pathogen-Oriented Low-cost Assembly & Re-sequencing, for rapid, low-cost, and highly sensitive SARS-CoV-2 diagnosis [77]. The assay showed a limit of detection of 86 genome equivalents per milliliter, with a cost of ~$30/patient and turn-around time of 24 h. In a different work, Credle et al. developed capture RNA-mediated oligonucleotide Annealing Selection and Ligation with next generation DNA sequencing (cRASL-seq) for targeted, multiplexed virus detection [78]. In cRASL-seq, a biotinylated capture probe and two ligation probes were adopted. Upon hybridization with target RNA, ligation probes are ligated, before the targets were captured and enriched through biotin. Ligation probes were then sequenced, enabling the identification of target virus RNA. Combined with sample barcoding, the assay showed high scalability and extremely low per-sample cost. In another study, Guo et al. developed a targeted sequencing method, named V-seq, using densely tiled reverse transcription primers across SARS-CoV-2 genome [79]. Specially designed hexamers at the 3' end were incorporated to minimize mis-matching with non-viral RNA and increase specificity. This protocol could be completed within 5 h with a cost of only $6 per sample.

Localized surface plasmon resonance (LSPR) sensing uses metal nanoparticles, commonly gold, to localize surface plasmon and enhance the peak signal in the absorption spectrum, making it a sensitive biomolecule detection technology. Efforts have been devoted to develop LSPR-based biosensors for the detection of a wide range of substances [80,81]. Recently, LSPR has been adapted to develop an assay for the detection of SARS-CoV-2 nucleic acid [82]. DNA complementary to RdRp sequence was linked to the surface of gold nano-island, and its hybridization with RdRp sequence led to a shift in the detected spectrum, which was subsequently used to calculate the virus concentration. In addition to LSPR, plasmonic photothermal effect was utilized to implement localized heating, which reduced the hybridization between mismatched oligonucleotides and increased detection accuracy. Using synthetic RdRp sequence, it was shown that a detection limit of 0.22 pM (~1.32×10^5 copies/μL) was achieved with good specificity. Since LSPR required dedicated optics and instruments for signal acquisition, further efforts may be necessary to miniaturize the test before it can be applied in the field. In another work, thiol-modified oligonucleotides were used to functionalize gold nanoparticles and allow for agglomeration upon hybridization with target RNA sequence of SARS-CoV-2 [83]. This method resulted in a colorimetric assay, which eliminated the need of sophisticated instruments, though the sensitivity was inevitably compromised compared to spectrum-based measurement. In addition to measurements using synthetic oligonucleotides, measurements using clinical samples may also be necessary to further validate the assay.

1.3. Conventional antibody test

Antibody test provides important serological information and can serve as a supplement to nucleic acid test. First, it assists contact tracing after a suspected infection by showing whether antibody is present in the serum [84]. Second, it helps reveal the population of asymptomatic cases to facilitate epidemiological study and public health policy making, considering that pre-symptomatic or asymptomatic transmission could happen [85]. Third, antibody profiles of participants in treatment or vaccine trials provides important information on immune status for the result analysis [86]. Therefore, developing reliable antibody test is of great importance.

S protein, particularly the receptor binding domain (RBD), is thought to be highly antigenic and has been predominantly used as the antigen for antibody capturing. In addition, N protein has also been used in some tests. Based on these antigens, a few antibody tests have been developed, including both conventional assays and assays based on novel biosensing technologies. Since antibody tests are generally based on specific binding between antigen and antibody, antigen detection can also be achieved using similar detection scheme.

Enzyme-linked immunosorbent assay (ELISA) is a commonly used antibody test. The detection is based on specific binding of antibody and subsequent enzyme reaction, which generates a colorimetric readout.

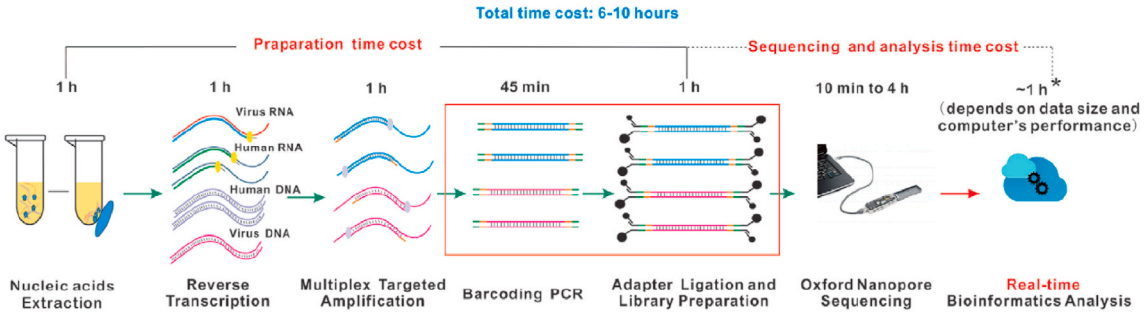

Fig. 7. Nucleic acid tests based on nanopore targeted sequencing. Adapted with permission from Ref. [72].

Briefly, a surface, such as a cell plate, is coated with antigen to capture target antibody in the sample, as shown in Fig. 8a. Afterwards, an enzyme-linked antibody binds to the target antibody, which enables the subsequent chromatic reaction. Since the outbreak of COVID-19, several ELISA kits have been developed. For example, Amanat et al. reported the preliminary results on the development of an ELISA assay for the detection of COVID-19 antibody [87,88]. The authors developed two different assays using recombinant full S protein and RBD domain, respectively, as the antigens. As shown by the results, full S protein showed better reactivity with COVID19 sera, and good sensitivity and specificity were observed in both assays, though not strictly characterized.

Lateral flow assay (LFA) is a paper-based point-of-care immunoassay. Paper substrates generate capillary action and drive sample to flow into conjugate pad and then testing region. In the conjugate pad, antibodies in the sample specifically bind to the colloidal gold-conjugated antigen, which are subsequently immobilized in the testing region, showing a color band due to the presence of colloidal gold. A myriad of LFAs have been developed by the industry sector as well as research laboratories. For example, Li et al. reported the development of an LFA which detected IgG and IgM of COVID-19 simultaneously, using RBD of S protein as the antigen, as shown in Fig. 8b [89]. The assay could be completed in 15 min using a blood drop from fingerprick, and it showed a sensitivity of 88.66% and a specificity of 90.63%, demonstrating the efficacy of LFA for rapid screening of SARS-CoV-2 infections. Two other works used lanthanide-doped polystyrene nanoparticles in lieu of colloidal gold for band visualization and achieved similar performance [90,91]. In addition, gold nanoparticles (AuNP) have also been adopted as an alternative to colloidal gold for antibody conjugation and test line visualization, given their long-term stability and little biotoxicity. Huang et al. developed AuNP-LF assay and achieved an assay time of only 15 min with serum consumption of 10–20 μL [92]. Besides detecting antibody, LFA can also be used to detect virus antigens. Since seroconversion causes significant delay in the presence of serum antibody after infection, the detection of antigen could potentially hold better sensitivity. Grant et al. developed a half-strip LFA to detect SARS-CoV-2 nucleocapsid antigen and achieved a limit of detection of 0.65 ng/mL (~13 pM) [93]. Though the assay was a simple dipstick without lateral flow instead of a full LFA, it proved the feasibility of the implementation of LFA on SARS-CoV-2 antigen detection.

Given the ubiquitous use of ELISA and LFA in *in vitro* diagnostics industry, ELISA and LFA have been quickly developed and commercialized by many companies. Whitman et al. tested the performance of 10 commercial LFAs, one commercial ELISA, and one in-house ELISA [94]. It was shown that IgM detection had more variations compared to IgG detection, and combined testes had highest sensitivity. The authors also showed that the test performances varied depending on the infection stage, and the specificity spanned from 84.3% to 100% in negative controls (pre-COVID-19 specimens). Therefore, detailed serological characterization covering the full spectrum of infections would be required to guide result interpretation.

Chemiluminescent immunoassay (CLIA) is commonly used in centralized laboratories for high throughput immunoassays. The testing principle is similar as ELISA, but instead of using enzyme reaction, CLIA uses chemiluminescence to generate light for signal detection. Since the outbreak, several commercial CLIA test kits have been quickly developed. However, due to the short validation time, it was observed that many assays have inadequate or unverified performance [95,96]. Therefore, a few groups took the initiative and evaluated the commercial CLIA test kits. For example, Padoan et al. assessed the performance of the SARS-CoV-2 IgM and IgG CLIA kits produced by Snibe (Snibe Co., Ltd., Shenzhen, China) [97]. Results showed that imprecision and repeatability were acceptable and the detection of IgG at day 12 had a sensitivity of 100%. In another study, Bryan et al. evaluated the performance of Abbott SARS-CoV-2 IgG test kits on Abbott Architect (Abbott Laboratories, Illinois, US), and results showed a specificity and sensitivity of 99.9% and 100%, respectively [96]. Performance evaluation on other IgG and IgM test kits were also reported [98,99]. These testing results demonstrated the validity of these CLIA test kits on the market and showed that these CLIA tests could facilitate the serological study of COVID-19.

1.4. *Emerging antibody test*

In addition to these conventional methods, a few antibody assays based on emerging biosensing technologies have been reported as well. These assays are fast and highly sensitive and hold great potential for rapid diagnosis.

Graphene-based field-effect transistors (GFET) have been shown as a great biosensing unit [100]. Based on this technology, Seo et al. developed a biosensor for the detection of SARS-CoV-2. In this GFET device, Graphene sheets were coated with a specific antibody against S protein [101]. As shown in Fig. 9a, upon sample loading, SARS-CoV-2 bound to the antibody through S protein, altering the electrical properties of the graphene sheet and thus the electric current. The amplitude of the current change was monitored by a source-measure unit to deduce the S protein concentration. It was shown that a detection limit of 242 copies/mL was achieved, which was significantly lower than that of ELISA. Nevertheless, similar as other emerging biosensing strategies, miniaturization of the instrumentation and further validation on test stability is needed for practical applications.

Surface plasmon resonance (SPR) sensing detects large biomolecules based on the shift in the reflectance spectrum upon specific binding on the surface, in a similar principle as LSPR. Djaileb et al. reported the preliminary results of detecting SARS-CoV-2 using SPR [102]. In that work, gold surface was linked with recombinant N protein for the capturing of COVID-19 antibody. The SPR biosensors showed good response to antibodies in the serum, with a limit of detection of about 1 μg/mL. The assay could be performed in 15 min using a portable SPR instrument, making it a possible method for rapid point-of-care testing. A potential limitation is the cost of the assay. The assay required SPR surface for antibody immobilization and SPR instrument for signal

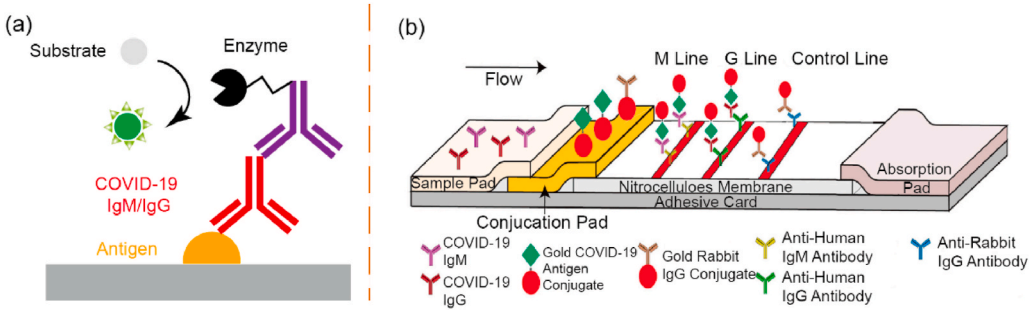

Fig. 8. Conventional antibody tests of COVID-19, including (a) ELISA and (b) lateral flow assay. Figure (b) adapted from Ref. [89] under the terms of the Creative Commons CC BY license.

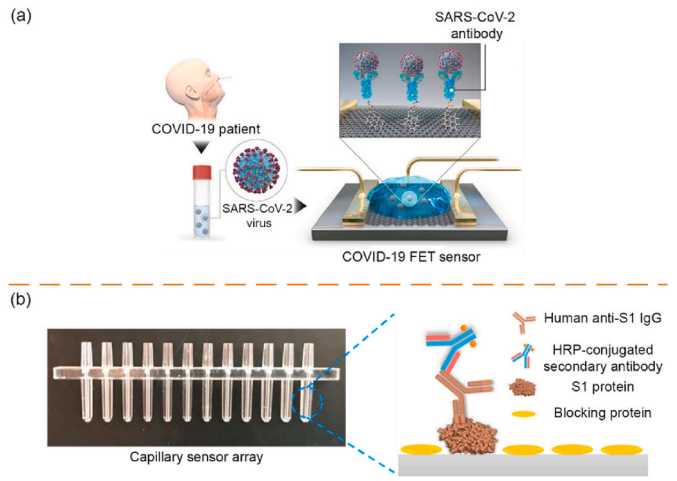

Fig. 9. Antibody tests based on emerging biosensing strategies of (a) graphene-based field-effect transistor and (b) microfluidic ELISA. Figure (a) adapted with permission from Ref. [101]. Figure (b) adapted with permission from Ref. [104].

detection, which added up to higher cost compared to conventional assays such as ELISA and LFA. In addition, further validation experiments may be necessary to improve the test reliability.

Microfluidics is a technology that manipulates fluids in microscale with high surface-to-volume ratio, which enables miniaturized assays for fast and sensitive detection with reduced sample consumption. Tan et al. developed a **microfluidic ELISA** system using capillary tubes as the immobilizing surface (Fig. 9b). The small diameters of the capillaries facilitated rapid reaction and reduced sample consumption. Consequently, the ELISA assay could be completed within 30 min using only a few microliters of samples [103]. Using the same platform, assays for the detection of SARS-CoV-2 specific IgG and S protein was developed [104]. The adapted assays coated S1 protein on the capillary wall to capture IgG; alternatively, SARS-CoV-2 antibody was coated to capture S protein in the serum sample. The developed assays took only 15–20 min and consumed only 10 μL serum for each test, and the lower limit of detection of SRAS-CoV-2 S1 protein was 0.4 ng/mL. The authors demonstrated the assays' application in antibody affinity screening, showing that antibody D003 has a relatively higher affinity and could potentially be used for future assay design. Nevertheless, a few limitations remain in the reported microfluidic ELISA. Commercial ELISA tests require consumables with good manufacturability to keep the cost low. Further efforts may be needed to explore the manufacturability of the surface-coated capillary tubes. In addition, the level of automation shall be further improved to achieve throughput that is comparable to the automated ELISA systems in hospitals.

Magnetic nanoparticles and corresponding surface functionalization techniques provide a means to perform detection with magnetic readout. Pietschmann et al. developed a magnetic immunoassay for SARS-CoV-2 antibodies using magnetic nanoparticles, along with an immunofiltration column and magnetic readout devices [105]. Immunofiltration columns were coated with spike protein peptide, which enriched the antibodies in blood samples, before biotinylated labelling antibodies were flushed in and bound to the captured antibodies. Magnetic nanoparticles, functionalized by streptavidin, were then added and conjugated to the labeling antibodies. The magnetic strength was then measured and used to infer the antibody concentration within the blood sample. The assay showed a limit of detection of ~3 ng/mL, with a test time of 42 min, offering a convenient point-of-care antibody test. Nevertheless, this work aimed to demonstrate the concept and involved several steps of manual operations, which inevitably compromised the measurement consistency. Further validation and optimization would be necessary.

Agglutination tests, as commonly used in blood typing, are high-throughput, fast, and low cost. Alves et al. utilized this test and developed an antibody test for COVID-19 [106]. An antiglycophorin antibodies-viral peptide conjugate was first synthesized and used to functionalize reagent red blood cells (RRBC). These RRBCs were then mixed with patient serum for testing. In the presence of SARS-CoV-2, the RRBCs agglutinated and enabled visual inspection, as shown in Fig. 10a. The assay was tested on 14 clinical samples and compared with ELISA, suggesting the assay's feasibility. Detailed investigation into the false-positive and false-negative rates would be needed to further characterize the assay performance.

Aptamers are oligonucleotides that can specifically bind to target molecules. Compared to antibodies, aptamers have advantages such as smaller size, better stability, easy synthesis, and better quality control. The first step towards the development of aptamer-based assay for the detection of SARS-CoV-2 is the identification of aptamer sequence. To this end, Song et al. used an ACE2 competition based aptamer selection strategy and identified a 51-base hairpin-structured aptamer and a 67-base aptamer, targeting the RBD of SARS-CoV-2 (Fig. 10b) [107]. High-binding affinity was demonstrated, with K_d values of 5.8 nM and 19.9 nM, respectively. These developed aptamers can potentially be used to develop new COVID-19 diagnostic tools.

2. Concluding remarks

Pandemic is a major threat that challenges global health and economic stability, and it is generally agreed by the public health experts that pandemics will keep occurring [108]. When an outbreak does occur, it is extremely important to have effective diagnostic tools that can be deployed in a matter of weeks or even days. Conventional testing methods, such as RT-qPCR, ELISA, and colloidal gold-based lateral flow assays, have shown limitations such as suboptimal sensitivity, throughput, and portability. Biosensing technologies based on nanomaterials and microfluidics have made great progress in the past few years, bringing about emerging biosensors that are fast, portable, and highly sensitive with minimal sample consumptions. Nevertheless, very few novel biosensors have practically contributed to the testing so far in the COVID-19 pandemic. Two issues could have contributed to this situation.

The first issue lies in the facilities required for emerging biosensing technologies. Most new biosensing technologies require dedicated instrumentation facilities, such as laser, spectrometers, or source-measure units, the installation and calibration of which are costly both time and finance wise. In pandemics, when time is critically important, tests that can be performed on the existing facilities are highly favorable. For example, if a new test can be adapted and performed on high throughput automated *in vitro* diagnostics systems, such as Abbott Architect and Roche cobas, it would be more actionable to incorporate this test in the clinical laboratory. As for point-of-care testing, lateral flow assay is commonly used, and the main "facility" is the dipstick, which is commercially available. As such, a new test which can be implemented on lateral flow assay would have a better chance to be adopted.

Another issue is related to the test reproducibility and reliability. Despite that many emerging biosensors have shown superior sensitivity and lower limit of detection, due to their immature nature, most of them suffer from high testing variance and suboptimal reliability, which prevent them from being admitted to clinical settings. Before they can be widely accepted, tests might have to go through iterations of troubleshooting in order to meet the validation and verification requirements. The long troubleshooting process stops them from being prioritized in pandemics. Therefore, biosensing research should consider the compatibility with existing testing infrastructures, as well as repeatability and reliability, so that these biosensors can be readily applicable in pandemics.

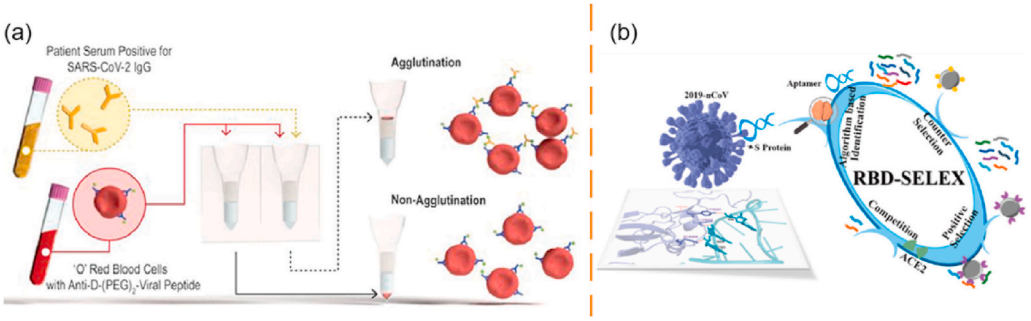

Fig. 10. Antibody tests based on emerging biosensing strategies of (a) agglutination test and (b) aptamer. Figure (a) adapted with permission from Ref. [106]. Figure (b) adapted with permission from Ref. [107].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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