Trends in biosensing platforms for *SARS-CoV-2* detection: A critical appraisal against standard detection tools

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Abstract

In this ongoing theme of coronavirus disease 2019 (COVID-19) pandemic, highly sensitive analytical testing platforms are extremely necessary to detect *SARS-CoV-2* RNA and antiviral antibodies. To limit the viral spread, prompt and precise diagnosis is crucial to facilitate treatment and ensure effective isolation. Accurate detection of antibodies (IgG and IgM) is imperative to understand the prevalence of *SARS-CoV-2* in public and to inspect the proportion of immune individuals. In this review, we demonstrate and evaluate some tests that have been used commonly to detect *SARS-CoV-2*. These include nucleic acid and serological tests for the detection of *SARS-CoV-2* RNA and specific antibodies in infected people. Moreover, the vitality of biosensing technologies emphasizing on optical and electrochemical biosensors toward the detection of *SARS-CoV-2* has also been discussed here. The early diagnosis of COVID-19 based on detection of reactive oxygen species overproduction because of virus-induced dysfunctioning of lung cells has also been highlighted.

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Introduction

Recently, coronavirus disease 2019 (COVID-19) has caused a world health disaster and socioeconomic adversity by infecting millions and killing thousands of people. This pandemic is reported to have appeared as a result of a novel mutation of coronavirus as the seventh member of corona family outbreaking in December 2019 in Wuhan, China [1]. Subsequently, it was noticed that this novel coronavirus possessed 96% genome sequence similarity with *SARSr-CoV-RaTG13* (bat SARS) and 79.5% with *SARS-CoV* [2]. The single-stranded RNA β-*coronavirus* [3] with *Nidovirales* order belongs to the *Coronaviridae* family [4] and comprises of nucleocapsid, spike (*S*), membrane (*M*), and envelope (*E*) proteins as shown in Figure 1a. The receptor binding domain (RBD) in spike-protein shares the angiotensin-converting enzyme 2 (*ACE2*) of human cells as a receptor [5]. The spike proteins are strongly attracted toward *ACE2*, which defines the virus-host array to RBD and ensures the viral fusion with host cell membrane [6].

As of today, in the absence of any recommended vaccine so far, swift and reliable diagnostic methods are of great significance for the diagnosis of symptomatic as well as asymptomatic COVID-19 cases [7]. The quick and reliable treatment decisions and quarantine strategies to slow down the spread of this infectious disease are all based on the diagnostics [8]. Some conventional testing methodologies like thoracic imaging, portable chest X-ray, flexible bronchoscopy, and computed tomography (CT) scan have been used for the preliminary diagnosis of infection. The quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) based testing [9] is regarded as the most popular test these days. Although these testing techniques are of great value but time consumption, tedious sample preparation and the need of expertise put them at the back. The infection rate and contagiousness of *SARS-CoV-2* is much higher compared with other SARS infections. Therefore, rapid and point-of-care diagnostic methods are highly desirable to overcome the limitations of conventional techniques.

For rapid and point-of-care detection, lateral flow assay (LFA), loop-mediated isothermal amplification (LAMP) method, reverse transcription-LAMP (RT-LAMP) approach, clustered regularly interspaced short

Figure 1
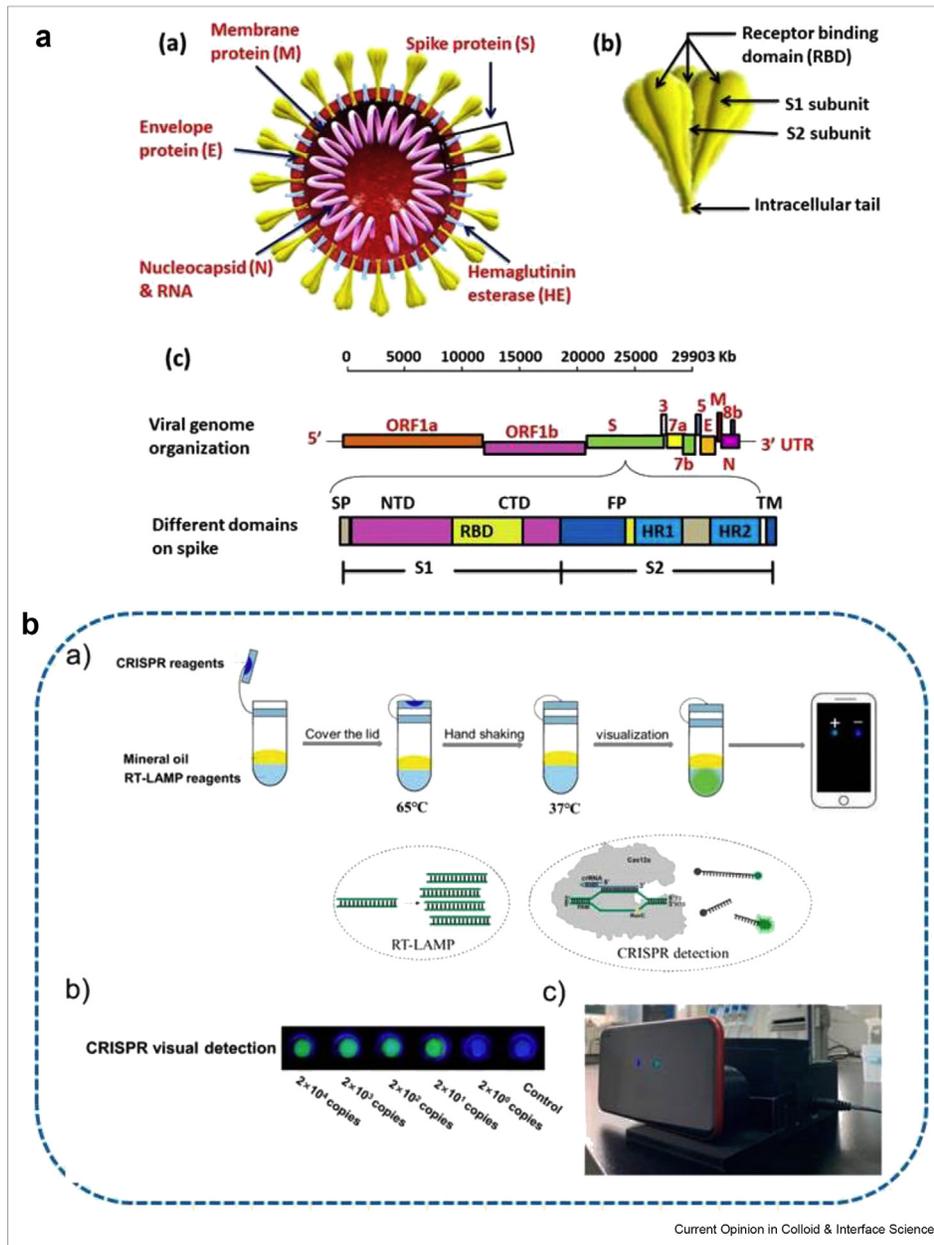
The detailed genomic representation of *SARS-CoV-2* and its mobile phone detection (**a**) Structural illustration of *SARS-CoV-2* (a) structural image of *SARS-CoV-2*, containing four different types of structural proteins (b) is the inset of key features of spike protein, representing the receptor binding domain, S1 and S2 regions, whereas (c) shows the full-length viral genome (29,903 nucleotides long) single-stranded positive RNA sequence. The leader sequence at the 5′ end and poly A tail ends at the 3′, contains structural proteins E,M,N,S along with different accessory genes (ORF 1a, 1b, 3a, 6, 7a, 7b, and 8b). The open reading frame ORF1a/b 'the replicase' is responsible for the coding of polymerase enzymes for viral RNA synthesis as well as non-structural proteins. The S1 subunit of spike protein consist of N-terminal domain (NTD), receptor binding domain (RBD) and C-terminal domain, whereas the S2 subunit comprises heptad repeat 1and 2, which plays a critical role in viral entry (**b**) The flow chart diagram describing the whole experimental process and principle for contamination-free visual detection method to detect different concentrations of RNA. Reprinted with permission from Ref. [56].

palindromic repeats (CRISPR) technology [10], and SHERLOCK [11] detection methods are generally implemented these days. Moreover, enzyme-linked immunosorbent assay (ELISA), LFAs, and microfluidic devices are the point-of-care testing methods under consideration. The technology behind testing is based on biosensing; either in the form of undeviating detection of biochemical and biotic molecules from the living sources or it is biomarker detection from bionics. The direct binding of analyte with target molecules and

biochemical reactions are two benchmark spectacles of biosensing approaches. However, easy handling, small sample size, real time analyte tracing, and flexibility to detect the target analyte of interest within seconds are the other major advantages of biosensing platforms, which can circumvent the bottlenecks of other techniques [12]. It is also worth mentioning that electrochemical biosensors are reliable analytical techniques with the potential of being modest to sample absorbance or turbidity for the detection of contagious disease.

Herein, we outline the *SARS-CoV-2* detection techniques with their pros and cons. We also present recent advances in standard testing tools and emerging biosensing platforms as portable devices for the timely detection of *SARS-CoV-2* to prevent the spread of the infection. We also aim to offer a new perspective on the biosensor-based technologies for the rapid and reliable detection of *SARS-CoV-2* to carry out the early diagnosis of COVID-19. This review concludes with a short discussion regarding current challenges and future perspectives.

Current detection approaches for *SARS-CoV-2*

The most commonly used techniques for COVID-19 diagnostics are nucleic acid test and CT scan. The procedure of identification and targeting the virus is considered more suitable when molecular approaches are used in comparison to syndromic or CT scan methods. These molecular approaches are developed after analyzing the proteomic and genomic structure of the microbes. The first genomic research on *SARS-CoV-2* was done using metagenomic RNA sequence, which is an efficient way of sequencing different genomes [13].

Designing nucleic acid tests

The RT-qPCR is a version qPCR technique, which is unequivocally developed for RNA detection. So, it is being used for *SARS-CoV-2* detection because it directly tests for the existence of virus RNA. RT-qPCR is regarded as the standard approach for COVID-19 identification. This approach is sufficiently reliable and can complete the results in few hours [14]. The commercially available kits use RT-qPCR techniques which detect more than two target regions and consequently the detection sensitivity is enhanced. This technique amplifies even a small viral genetic material in the testing samples. It works on two consecutive reactions; firstly, it converts the virus RNA to small complementary DNA sequence (cDNA) for special identification of paired sequencing on viral RNA. Secondly, cDNA is amplified through PCR using gene-specified primers and fluorescent-labeled hydrolysis probes. The DNA produced in first step is used in second step, where it is multiplied through repeated thermal cycling and finally the virus is detected using quantitative qPCR machine.

To date, numerous RT-qPCR kits have been established for the *SARS-CoV-2* detection by targeting the structural proteins and accessory genes (nucleocapsid (*N*), spike (*S*) protein (*RdRP*), envelope (*E*), or *ORF1b, ORF8* genes) as biomarkers for *SARS-CoV-2* detection. The reverse transcription of *SARS-CoV-2* RNA into cDNA takes place in RT-qPCR test case where the specific cDNA zone is further amplified to complete the process [15,16]. Zambon's research group conducted a research on alignment and investigation of several SARS-associated genome sequencing to develop the primers and probes [17]. The E, N proteins, and RNAP (RNA polymerase) genes are amid three of discovered genes at present. First two of the three genes are found to have high analytical sensitivity, whereas the third one offers feeble sensitivity for detection. The RT-qPCR tests are carried out both in single-step and double-step procedure. The single-step assay exhibits rapid response and good reproducibility, and the whole process of reverse transcription and PCR amplification takes place in one step. However, this procedure finds it difficult to optimize reverse transcription and amplification based on the simultaneous occurrence of both steps. In comparison, double-step assay shows high sensitivity but requires more time as the reactions are completed in separate tubes. Although the two-step assay is highly sensitive and flexible, one-step assay is favored for the detection of *SARS-CoV-2* because of its speed, simplicity, limited sample requirement, minimum counter time, as well as less cross contamination during the RT and real-time polymerase reaction periods [18,19]. Xiang's research group presented DNA nanoscaffold-based *SARS-CoV-2* detection to diagnose COVID-19 [20]. Recently, Lubke's research group designed RNA extraction free protocol based on RT-qPCR for the detection of *SARS-CoV-2* where the E-gene was targeted as novel primer [21]. Without dilution and heat inactivated, 5 µl respiratory samples were used. The assay was validated using 91 respiratory samples, where 81.3% samples were detected positive. It is concluded that direct RT-qPCR is a well-acknowledged alternative to conventional RNA RT-qPCR for analyzing fresh samples. Li et al. has studied 610 patients who were diagnosed with COVID-19 according to CT scan diagnosis, and reported the higher rate of false-negative RT-PCR results for *SARS-CoV-2* detection [22]. The variations in the results by RT-PCR detection might be possible due to the low viral load in the test samples at early stages of infection, prolonged RNA conversion, and transmutations in the probes target regions [23]. PCR assays are not sensitive enough to differentiate between symptomatic and asymptomatic patients [24] and susceptible to few generalizations, which limit their applications such as:

1. The small countryside hospitals are not well equipped to carry out PCR tests. Lack of expertise and infrastructure limit this facility.

2. There is a possibility of false-negative results because the appropriate sampling time for nasopharyngeal swab is still vague for the patients on ventilators [25].
3. A tedious procedure for sample preparation is required, which includes cell lysis and nucleic acid purification. Also, the extractions kits are less than the ramping up demand of tests [26].
4. During transportation, the denaturation of sample may occur, which leads to false-negative results [27].
5. In case of fully recovered patients, PCR testing can even result false-positive outcome detecting genetic material of inactive *SARS-CoV-2*, which is actually not reinfection. The genetic material never dies and can persist in the body for several days to months, so it can be detected after months of recovery [28].
6. The nucleic acid amplification is used in PCR testing, where distinct primers and probes for every target are needed that consequently limit PCR's flexibility of scaling up for other nucleic acids in an easy and prompt way.
7. If mutations of *SARS-CoV-2* (which are likely to be) are undergoing, then it may be a problem for same target. Thus, continuous monitoring of viral genetic material is mandatory to warrant the accuracy of test [29].

No doubt qPCR and RT-qPCR are evolving day by day with the number of advantages but these few considerations are needed to be improved in RT-qPCR to make quick, more sensitive, and especially cost-effective detection method.

CT scan method

In case of inadequate availability of these kits and false-negative results of RT-qPCR, a noninvasive CT scan technique has also been used in clinical diagnostics of COVID-19 [30]. The characteristics of these images are closely related to stage of infection when the symptoms are shown. At the early-stage infection, transparent, localized lesions were confined to the subpleural area of one or both lungs of patients after the appearance of symptoms [31]. When bilateral and peripheral ground-glass denseness and amalgamation of the lungs are appeared, then these are the characteristic symbols of COVID-19. With the progression of COVID-19 infection, the lungs consolidation increases in addition to crazy paving pattern. The aforementioned outcomes have revealed that CT scan method showing better false-negative rates is more sensitive in comparison with RT-qPCR but the initial level screening with CT scan is restricted. The major drawback of CT scan method in COVID-19 diagnosis is the lack of specificity because of the overlap of lungs images with other pneumonia. Moreover, the CT scan diagnoses the patients at advanced stage of infection [32].

Antibody detection

The reliable diagnosis of COVID-19 is carried out using protein tests such as antigens and antibodies [33]. The saliva test may vary with passage of time after the symptoms appear but in comparison, antibodies persist for a long time, which enable large span for indirect *SARS-CoV-2* detection [34]. The commonly used methods for antibody detection are LFAs and ELISA. In LFA, the sample is deposited onto sample pad of cassette-like device and moved through strip by capillary action. The antibodies labeled with Au NPs bind with target molecules present in sample at the first line. Owing to continuously moving sample, Au-labeled antibodies are bound by capture antibodies existing in lines. The excessive Au-labeled antibodies are captured at the control line while moving through the strip. If the target molecules are not present in sample solution, Au-labeled antibodies are surely captured at control line, which makes control line valid for test. For IgG or IgM-type antibodies or both, there should be one, two, or three stripes in display window [35]. The average test time of 10−30 min places it in the rapid diagnostic methods. It provides only qualitative analysis but has the advantages of easy handling and cost effectiveness and allows direct assessment of ongoing infection as anti-CoV antibodies are used instead of immobilized viral antigen [36]. Recently, Kong's group has developed highly sensitive, fast, easy to use, and on-site immunoassay, which can complete the assay in 15 min to detect *SARS-CoV-2* antibodies and antigens at the same time. The antibodies from human serum samples and *SARS-CoV-2* antigens from nasal swab were detected successfully from 26 COVID-19 infected and 28 healthy individuals using this integrated method [37]. Moreover, Feng's research group has constructed a simple operated point-of-care lateral flow immunoassay to simultaneously detect antibodies (IgG, IgM) developed against *SARS-CoV-2* in blood samples of affected people at various phases of infection. The achieved sensitivity and selectivity of this testing method were 86.6% and 90.6%, respectively [38]. The assay for simultaneous detection of both IgG−IgM possesses better usability and sensitivity in comparison with single IgG and IgM tests. Convalescent serum containing large quantity of neutralization IgG is extremely helpful in curing COVID-19 patients. Tan's et al. has successfully demonstrated an ELISA method to detect anti-*SARS-CoV-2* S1 IgG from human serum using just 8 μl sample [39]. The practicability of this method has been verified in detecting anti-*SARS-CoV-2* S1 IgG in 16 convalescent *SARS-CoV-2*−infected persons.

The immunoglobulin IgG and IgM have been detected from serum sample of infected individuals using ELISA where nucleocapsid protein of *SARS-CoV-2* is used. The typical result time of ELISA is 60 min to 5 h either the test is quantitative or qualitative. ELISA being fast, flexible, robotics, high throughput with variable

sensitive range, and ability to test multiple samples is specifically suitable as point-of-care purpose [40,41]. In ELISA test, human serum sample is added to the well where the recombinant viral antigen is already coated. The bindings occur between antibodies and target antigen present in sample and then washing is done several times to make sure the complete removal of unbound substrate. Another solution comprising labeled antibodies is mixed and binding will occur if the antibodies of interest are present in the sample. After washing again to remove unbound substrate, horseradish peroxidase is used as color changing reaction to confirm the binding of target antibodies. Using a spectrometer, color change is read and concentration of antibodies is measured [42]. It has been noticed that the antibodies are developed in *SARS-CoV-2*–infected people after around 14 days of onset of symptoms. However, some studies depict the development of IgG and IgM antibodies after five days of *SARS-CoV-2* infection [43]. The level of antibodies in patients may go on increasing after five days of symptoms appear. The antibodies were also found in respiratory fluids, blood and fecal specimens.

LFAs are preferred over ELISA tests because these tests can be performed at home without the need of any expert. ELISA is the analytical technique, which can only be carried out in laboratories with the help of skilled personnel and high protocol as well as it requires different steps, long turnaround time and special instruments. Owing to these limitations, the recent studies are more focusing to enhance the sensitivity and selectivity of LFAs. Therefore, signal amplification and multiplexed detection methods are under development to achieve increased sensitivity, selectivity, and detection throughput. The development in serological tests for *SARS-CoV-2* antibody faces the issues of being cross-reactive with other family members of coronavirus. Okba observed the cross-reactivity among S proteins of SARS-CoV-2, SARS-CoV, and MERS-CoV. But there was no cross-reactivity for S1 subunit of MERS-CoV spike protein. The most conserved subunit among all coronaviruses is S2, which can increase the suitability of S1 subunit for serological tests [44].

Antigen detection
Antigen tests can be considered more reliable compared with antibodies tests because antigens are target specific and introduce antibodies [45]. Both the LFAs and ELISA methods can be used for the detection of antigens. Kim's research group developed a novel detection method where they used ACE2 receptor to detect *SARS-CoV-2* spike 1 (S1) protein [46]. The receptor is further paired with commercially available antibodies and this pair was captured and detected using lateral flow immunoassay. There was no cross-reactivity with other coronaviruses showing a

detection limit of 1.86×10^5 copies/mL in clinical samples. The fluorescent immunochromatographic LFA was designed by Diao et al. to detect nucleocapsid (N) protein of *SARS-CoV-2* [36]. The anti-N mouse antibodies and anti-rabbit IgG were used to generate test and control line, respectively. The conventional Au NPs were replaced by anti-N rabbit IgG marked with carboxylate-modified polystyrene Europium (III) chelate microparticles. The samples were consisting of urine and nasopharyngeal swab. The assay demonstrated 68% sensitivity and 100% selectivity in comparison with nucleic acid test. These are the early researches and it will take much time to be available in markets for practical use irrespective of their superb sensitivity and specificity. LFAs and ELISA are well established methods and antigen testing is the need of the hours but there is just one Sofia 2 *SARS* Antigen Test Kit available commercially so far to detect *SARS-CoV-2*. It works on the principle of immunofluorescence strip technology for the detection of N protein *of SARS-CoV-2* and *SARS-CoV* that mean the kit is unable to make differentiation between these two viruses. The kit showed clinical sensitivity of 80% and selectivity of 100% for 47 positive and 96 negative individuals [47]. Following the failures of kits, WHO discouraged the medical staff to use rapid commercial assays to detect *SARS-CoV-2*. There are two companies "QUIDEL as well as Avacta and Cytiva," which are working for the development of trustworthy antigen test kits. The targets for these two kits are N protein, and *SARS-CoV-2* viral antigens or S glycoproteins [48].

Point-of-care testing
Point-of-care testing is also much imperative to diagnose *SARS-CoV-2*–infected persons without sending them to hospitals. In pandemic, the most important is the mass testing to identify the persons with viral exposure for quarantine and treatment strategies. When the viral infection continues to spread at exponential rate, then RT-PCR and immunoassays become limited because of the turnaround time. Simple design, noncomplicated mechanism, and sample collection to result readout by anyone to everyone without any expertise are the key features of point-of-care testing, which is important to control the viral spread. LFAs are the point-of-care testing methods, which are being developed for the detection of *SARS-CoV-2*. The development in such point-of-care devices is the need-of-the-hour that anyone, anywhere with minor to moderate symptom, or even asymptomatic individuals can get the result within minutes to confirm whether they are positive or negative for *SARS-CoV-2* [49]. It is reported that currently, four point-of-care setting devices are being used under Food and Drug Administration- Emergency Use Authorization (FDA-EUA) approval that we have enlisted below with their specific characteristics [50].

- ID NOW COVID-19 test depends on the isothermal nucleic acid amplification to target the RdPR gene at specific site using 4—6 primer and a polymerase at constant temperature. It has great advantage to produce large amount of nucleic acid duplicates in a very short time with excellent sensitivity of 125 copies/mL. Moreover, the result time is approximately 13 min from upper respiratory samples [51].
- Mesa Biotech's Accula *SARS-CoV-2* test target is N gene and it is basically the combination of two techniques, RT-PCR plus lateral flow immunoassay. With the analytical sensitivity of 200 copies/mL and it approximately provides result in 30 min [50].
- Xpert Xpress *SARS-CoV-2* test offers an additional pledge in *SARS-CoV-2* detection targeting more the one gene. It targets the N and E genes and gives the turnout in 40 min. With the analytical sensitivity of 140 copies/mL and it provides selectivity of 100% [50].
- Cue COVID-19 test relies on isothermal amplification to target N gene of *SARS-CoV-2* and gives turnout approximately in 20 min. It shows 100% selectivity. Cue test offers the portable and easy to use analytical podium connected with cell phone, which is advantageous to access the health condition at fingerprint making it more promising point-of-care testing approach compared with aforementioned three methods [50].

Developing techniques for *SARS-CoV-2* detection

The imperative need of time is, to develop nucleic acid and protein-based testing for diagnosing COVID-19 to compensate the RT-qPCR drawbacks. Fascinatingly, an international company has used the combination of PCR with LFA technique as an alternate method to detect *SARS-CoV-2* within the exhaled breath condensate. Moreover, isothermal amplification of genetic material is an alternative approach to PCR for developing point-of-care devices to carry out the quick detection of nucleic acid.

Viral gene detection

Several organizations are striving to implement the isothermal nucleic acid amplification techniques in the detection of *SARS-CoV-2*. The isothermal amplification approaches are the best options that can be operated at a single temperature without particular laboratory apparatus providing sensitive results [52]. The helicase-dependent amplification, LAMP, and RT-LAMP approaches turned out to be established to detect *SARS-CoV-2* and are more specific, sensitive, and reaction efficient [53]. The LAMP technique is more sensitive and specific but it uses high number of primers. In comparison, RT-LAMP just uses 4 to 6 primers and DNA polymerase to target genome at 6 positions. Currently, RT-LAMP has been used to detect *SARS-CoV-2* in less than 13 min *via* reverse of transcription of virus RNA to cDNA. The assay using RT-LAMP technique was developed, which targeted Nsp3 for *SARS-CoV-2* detection and showed a detection limit of 100 copies/reaction [54]. Baek et al. designed RT-LAMP primer sets to pick out the N gene of viral RNA, which showed comparable results with RT-PCR displaying Limit of Detection (LOD) of 100 copies of RNA [55]. The test displayed no cross reactivity to other coronaviruses in the detection of *SARS-CoV-2* viral RNAs. Chen's group introduced contamination-free naked-eye detection of *SARS-CoV-2* using RT-LAMP approach as shown in Figure 1b [56]. The portable 3D printing instrument and smartphone were used and the created fluorescence was seen by naked eyes without using some devoted tools. Tanner's group used DNA-binding dye for calorimetric detection. It is reported that at the level of 480 RNA copies in cell lysates, colorimetric LAMP can recognize the viral RNA without any interference and can be the alternative of RT-PCR being fast and simple detection method. Moreover, high selectivity, sensitivity, and only requiring heating system and optical inspection make it more favorable for *SARS-CoV-2* detection. Zhu et al. developed an approach by integrating multiplex RT-LAMP with nanoparticle-based LFA to detect *SARS-CoV-2* through ORF1ab and N gene detection [57]. The *SARS-CoV-2* detection is possible in less than 13 min but the one sample/run and the optimizations of primers and reaction conditions are the major limitations associated with LAMP techniques [58]. These isothermal amplification systems may also be multiplexed at the amplification and/or readout stages. The process of multiplexing is done using organic fluorescent molecules as beads for barcoding. Furthermore, visual detection approaches are also being carried out using some dyes, which use intrinsic by-products of widespread DNA synthesis. Fascinatingly, CRISPR-Cas12 lateral flow test was used to achieve enhanced sensitivity, simplified result read, and reduced detection time for RT-LAMP method [59]. Researchers are devoting much efforts in using collateral cleavage activity of Cas nucleases (e.g., Cas12a, Cas12b, and Cas13a) to establish point-of-care viral RNA detection methods. Hou et al. achieved enhanced sensitivity with a detection limit of 7.5 copies per reaction in 40 min using RPA and CRISPR-Cas13—mediated enzymatic signal amplification [60]. A comparative study on clinical evaluation methods demonstrated that CRISPR-Cas13—based tests possess more detection capacity compared with RT-PCR methods. To make the detection process further easy, a compact dual CRISPR-Cas12a (AIOD-CRISPR) assay has been developed, where single-reaction system is used to incubate all components and high sensitivity as well as specificity has been achieved for RNA detection avoiding the need of separate preamplification steps [10]. The detection limit of 4.6 copies/reaction has been achieved for *SARS-CoV-2* N gene in 40 min. Recently,

Table 1

Recently used several analytical approaches to detect *SARS-CoV-2*.

Test type	Institute	LOD	Ref
Virus blood culture and high throughput sequence of whole genome	Wuhan Institute of Virology (WIV)	N/A	[72]
RT-PCR	Berlin Institute of Virology (BIV)	3.9 copies and 3.6 copies	[73]
High-resolution CT scan	HUST	N/A	[74]
Dual CRISPR Cas12a test	University of Connecticut Health Center	1.2 DNA copies and 4.6 RNA copies	[10]
Fast IgM and IgG combined Ab testing kit	Guangzhou Medical University	N/A	[38]
Closed tube one-stage LAMP3	University of Pennsylvania	70 copies/reaction	[75]
Closed tube two-stage RAMP4 assay	University of Pennsylvania	7 copies/reaction	[75]
RNA-based paper LFA5 PoC-based LAMP assay	National Tsinghua University	N/A	[76]
ELISA6 and GICA for combined IgG-IgM	Wuhan University	N/A	[49]
FTO-based Ab sensor	National-Institute of Animal-Biotechnology	10 fM	[77]
SPCE-based Ab biosensor	National-Institute of Animal-Biotechnology	10 fM	[77]

Wang's group designed an assay by combining RT-LAMP with Cas12a cleavage and named it as opvCRISPR to detect *SARS-CoV-2* visually [61]. This assay was validated with 50 clinical specimens of *SARS-CoV-2*–infected people. The RT-LAMP amplicon triggered collateral activity against single-stranded DNA reporters of activated Cas12a, which consequently increased the detection sensitivity and made the outcomes to be observed by naked eye. Moreover, SHERLOCK detection methods for *SARS-CoV-2* are also used based on nucleic acid testing technique. In this approach, the targeted virus RNA is reverse transcribed to cDNA where isothermal amplification takes place by reverse polymerase. Further the amplified products are transcribed back into RNA [62].

## Biosensing techniques for *SARS-CoV-2* detection

The biosensors are emerging area of analytical chemistry. The biosensing systems are able to provide quantitative analysis and measurements without demanding extra processing steps or reagents [63,64]. The biosensor-based technologies offer alternative approach to standard PCR tests, which are not only sensitive but also helpful in diagnostics and therapeutic assessments. Biosensors have the capability to detect pathogens in numerous environments without requiring tedious sample preparing steps [65,66]. Several biosensors with diverse biorecognition elements (enzyme, antibody, deoxyribonucleic acid, cell, or microorganisms) and transducers (such as mechanical and optical transducers) have been extensively applied in the detection of pathogens [67]. Labels can also be named as reporters which are molecular species including organic dyes or quantum dots which bind to the target, either in a direct way or *via* a biorecognition element to enable the detection [68,69]. The biosensors are now trending for *SARS-CoV-2* detection, specifically the CRISPR technology–based biosensing systems such as CRISPR-Chip to electrically detect genetic mutations [70] and electrochemical CRISPR-biosensing platforms for microRNA analysis [71]. These approaches are easily adapted to any nucleic acid and can be able to report the mutations of *SARS-CoV-2* in timely way. Moreover, successful differentiation in similar gene sequences with superb sensitivity has been recently reported by using combination of plasmonic photothermal (PPT) effect and localized surface plasmon resonance. Here, we have summarized the recently used several analytical approaches (see Table 1) for the detection of *SARS-CoV-2*.

Optical biosensor

To timely and efficiently diagnose COVID-19 infection, the detection of immunoglobulin A (IgA) specific to *SARS-CoV-2* is highly crucial to complement the assays used in the detection of IgM and IgG. Anfossi's research group has designed a dual functioning optical/chemiluminescence immunosensor for the detection of IgA from serum and saliva samples as presented in Figure 2a [78]. The recombinant nucleocapsid antigen was used to capture *SARS-CoV-2* antibodies present in sample in infected people. The assay was successfully used in the analysis of 25 serum and 9 saliva samples taken from infected and/or recovered persons with excellent sensitivity. This is a noninvasive immunosensor, which can be helpful to monitor early immune responses to COVID-19. Moreover, surface-enhanced Raman scattering–based lateral flow immunoassay (SERS-LFIA) has been developed by Liu et al. to detect IgM and IgG simultaneously as shown in Figure 2b [79]. To fabricate SERS, tags modified with dual-layers of Raman

Figure 2
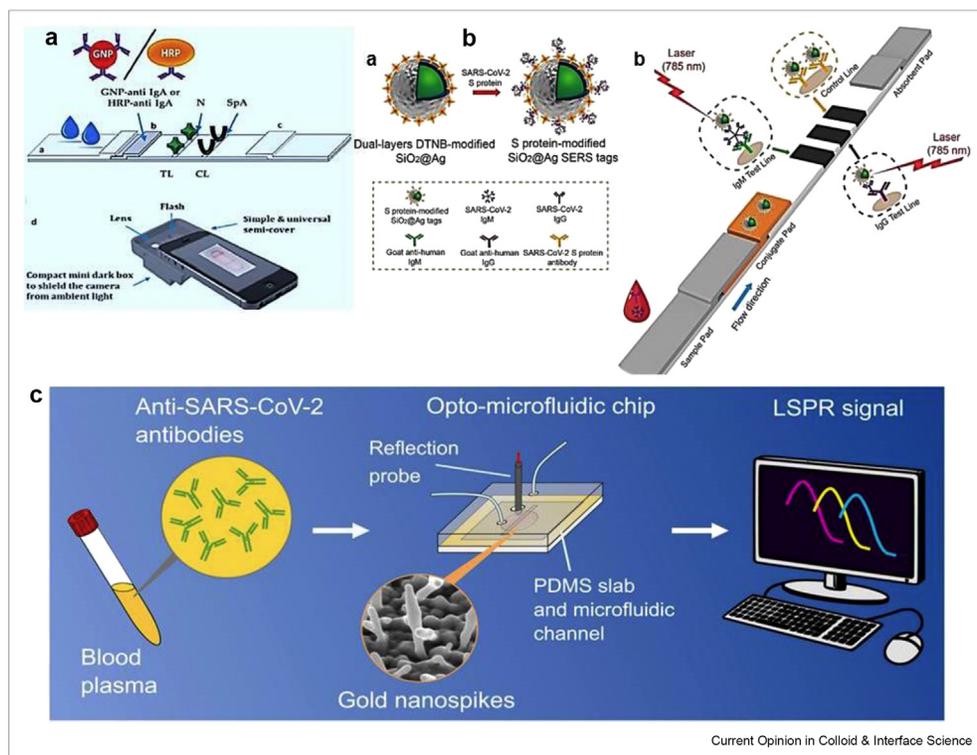
The use of optical biosensors in the detection of *SARS-CoV-2* (**a**) Schematic representation of smartphone reader used for optical immunosensor in the detection of IgA. Reprinted with permission from Ref. [78]. (**b**) Preparation and operating-principle of (a) *SARS-CoV-2* S protein coupled SiO₂@Ag SERS tags and (b) simultaneous detection of anti-*SARS-CoV-2* antibodies. Reprinted with permission from Ref. [79]. (**c**) Schematic representation of localized surface plasmon resonance−based opto-microfluidic sensor to detect *SARS-CoV-2* antibodies. Reprinted with permission from Ref. [83].

dye, SiO₂ core was completely coated with Ag shell (SiO₂@Ag), which showed superb SERS signal, decent monodispersity, and excellent stability. The anti-human antibodies were immobilized which captured SiO₂@Ag-spike S protein anti-*SARS-CoV-2* IgM/IgG complexes. The as proposed SERS-LFIA biosensor showed a detection limit of 800 times higher than Au NPs based LFIA. The direct detection of S protein has been achieved using nanoplasmonic resonance sensors with a detection limit of 370 vp/mL in 15 min [80]. The assay was very economical showing good performance under clinical environment. Recently, surface plasmon resonance (SPR) sensing approach has been used to detect nucleocapsid antibodies in serum sample, which were produced in response to *SARS-CoV-2* infection [81]. Here, after activating EDC-NHS AffiCoat surface, the binding of nucleocapsid protein of *SARS-CoV-2* (rN) on the surface of the SPR chip was achieved and ethanol-amine was used for the passivation of remaining activated sites. This method is quantitative, portable and good enough to pave the way for point-of-care assays and label-free detection of antibodies. The SPR sensor had the capability to detect anti-*SARS-CoV-2* antibodies at nano-molar level after coating with peptide monolayer

and functionalizing with *SARS-CoV-2* nucleocapsid recombinant protein. The whole assay was conducted on portable SPR instrument without diluting the serum sample. This method is good enough to pave the way for point-of-care and label-free testing of antibodies. Another SPR biosensor has been developed based on pig sera-derived anti-*SARS-CoV-2* antibodies [82]. Pig-sera were used to purify *SARS-CoV-2* antibodies against nucleocapsidprotein (NP). The assay presented a detection limit of 1.02 pM for the detection of *SARS-CoV-2*. More recently, Shen's group detected antibodies against *SARS-CoV-2* spike protein using Au nanospikes in opto-microfluidic sensing platform as shown in Figure 2c [83]. The opto-microfluidic sensor was developed based on the principle of localized surface plasmon resonance (LSPR), and Au nanospikes were electrodeposited for the detection of antibodies against *SARS-CoV-2* spike protein 1 µL of human plasma. The label-free microfluidic sensor showed a detection limit of ∼0.5 pM and completed the assay in ∼30 min. The wavelength peak shift of Au nanospikes is measured, which is then correlated with the amount of target antibodies. This platform is very cheap, easy-to-use, fast, and offers point-of-care diagnostics. Furthermore, circle-

to-circle amplification and optomagnetic analysis have been used to quantify *SARS-CoV-2* RdRp coding sequence [84]. The proposed optomagnetic approach was successfully used to detect *SARS-CoV-2* cDNA with a detection limit of 0.4 fM. In comparison to previous literature on circle-to-circle—based sensors, this method showed subfemtomolar level detection, reduced the time consumption and labor work. These biosensors possess the advantages to execute fast naked-eye diagnosis of viral infection with real-time analysis and without requiring many reagents. Consequently, preferable method of choice is the used of optical biosensors. They can detect analyte with very low quantity, high sensitivity and specificity as well as detect the variants of viral strains.

Dual-functional LSPR and PPT biosensor
The biosensors are considered most trustworthy diagnosis approaches as an alternate way out to clinical

analysis, real-time and nonstop monitoring to promote epidemic prevention and control [85,86]. It is well-documented that huge optical cross sections are shown by plasmonic nanoparticles and abundant heat energy is produced as a result of nonradiative relaxing process of absorbed light [87]. More recently, Wang's research group fabricated a dual-functionalized plasmonic biosensor using PPT effect in combination with localized surface plasmon resonance (LSPR) sensing transducer for sensitive and promising diagnosis of *SARS-CoV-2* in clinical specimens [88]. The schematic drawing of hybridization, real-time hybridization of RdRp-COVID and its cDNA sequence, and RdRp-COVID sequence detection at various concentrations have been shown in Figure 3a. The nucleic acid hybridization process has been used in the detection of selected sequences of virus with super sensitivity where two-dimensional Au nanoislands combined with cDNA receptors perform as biosensing platform. To achieve

Figure 3
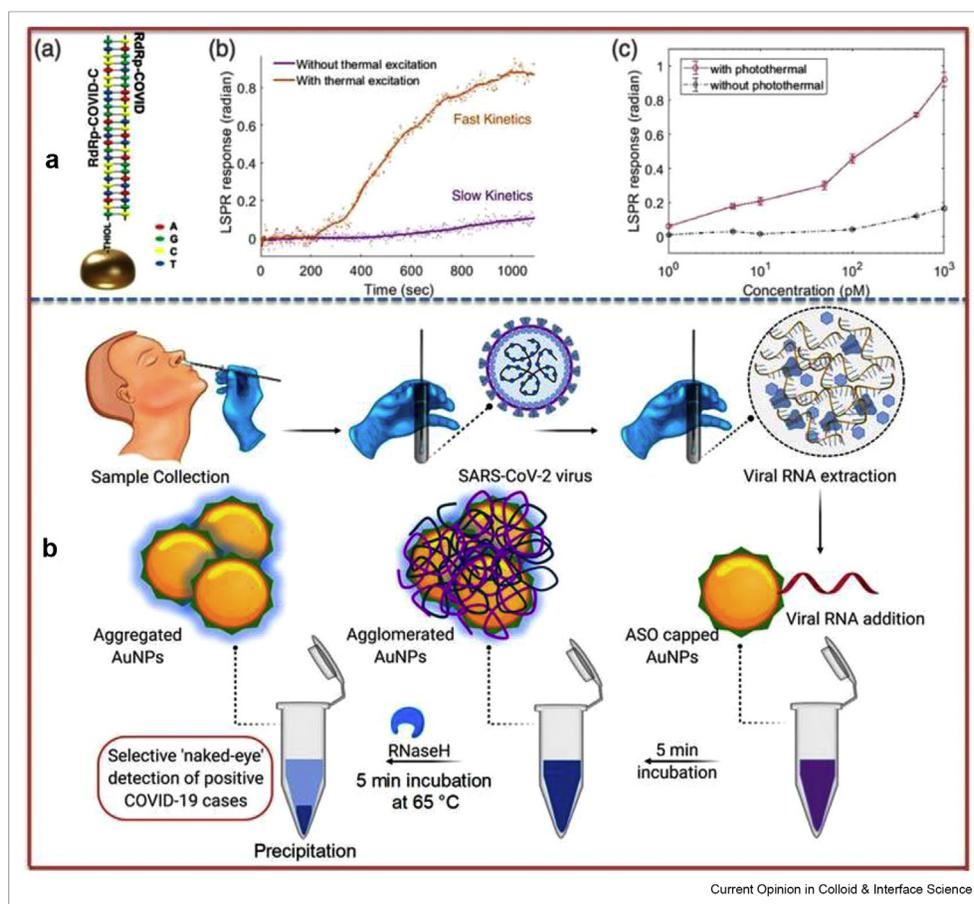
Dual-functional plasmonic biosensing and naked-eye detection of *SARS-CoV-2* (**a**) LSPR biosensing. (a) Schematic drawing of the hybridization and inhibited hybridization, (b) real-time hybridization of RdRp-COVID and its cDNA sequence, (c) RdRp-COVID sequence detection at various concentrations. Reprinted with permission from Ref. [88]. (**b**) Scheme illustrating the naked-eye detection of *SARS-CoV-2* using ASO-capped AuNPs. Reprinted with permission from Ref. [91].

improved performance, the same Au nanoislands chip is used to generate plasmonic heat when illuminated. Furthermore, the localized PPT heat has the capability to increase the *in-situ* hybridization temperature, which in turn enables the perfect differentiation of two same gene sequences. The proposed LSPR biosensor has successfully demonstrated superb sensitivity in the selective determination of *SARS-CoV-2* sequences showing low detection limit of 0.22 pM and has allowed accurate determination of particular target in mixed genes. This research gains insight into thermoplasmonic enhancement and envisions the practical application of LSPR biosensor in nucleic acid testing and viral infection diagnosis. Moreover, the integration of these biosensors with microfluidic systems may increase overall sensitivity of device helping in miniaturization process [89].

Microfluidic colorimetric biosensor
In last decades, microfluidic devices have been used for the contagious ailments like *HIV, SARS-CoV,* and others, which target the biomarkers in less than 5 min consuming only 6 μL of blood plasma sample [90]. For COVID-19 and other infectious viral diseases, microfluidic devices are raising the panorama of swift, low cost, and point-of-care diagnostic to avoid further transmission of virus. Moreover, microfluidic devices fulfill the "ASSURED" (affordable, selective, sensitive, user-friendly, rapid and robust, equipment-free, and deliverable) criteria for biosensing and point-of-care testing having the benefits of high precision, transportability, quick response, high reproducibility, less reagent usage, easily applied, and high throughput parallel processing. These biosensors offer multiplexing, miniaturization, and capability of integration with various techniques. Conversely, sample preparation and analysis, sample's matrix effect reduction, and full automation in microfluidic devices are still challenging.

Colorimetric biosensors present the exciting properties including straightforwardness, rapid response, accurateness, and cost-effectiveness, and produce signals visible to naked-eye for detection of viral infectious diseases. The thiol-modified antisense oligonucleotides (ASOs) capped Au NPs (Au-ASOs) have been used as colorimetric biosensing platform to detect *SARS-CoV-2* by Pan's research group [91]. The schematic illustration of Au-ASOs—based biosensor for naked-eye detection of *SARS-CoV-2* has been shown in Figure 3b. The Au-ASOs—based assay enabled the specific detection of N-gene from nasal swab to detect *SARS-CoV-2* infection in 10 min. The detection mechanism involved the agglomeration of Au-ASOs architecture with target *SARS-CoV-2* RNA, which led to red-shift. The cleavage of RNA strands from RNA—DNA hybrid was done by adding RNaseH and finally the precipitations in solution owing additional agglomeration of Au NPs were visible

to naked-eye. The biosensor showed a detection limit of 0.18 ng/μL for *SARS-CoV-2* RNA with no cross-reactivity with *MERS-CoV* viral RNA. Moreover, Kumar et al. developed another similar colorimetric-based biosensing system for the detection of RdRp gene of *SARS-CoV-2* form nasopharyngeal sample in less than 30 min [92]. The biosensor demonstrated low detection limit of 0.5 ng for *SARS-CoV-2* RNA and no cross-reactivity toward cervical DNA sample, which was acquired from human papillomavirus-infected women. This biosensing platform may facilitate the mass screening during pandemic management in a cost-effective manner. The simultaneous detection of immunoglobulin IgM and IgG for *SARS-CoV-2* has been carried out using Au NPs—based immunoassay [38]. The assay detected antibodies from human blood sample in 15 min with a clinical detection sensitivity and selectivity of 88.66% and 90.63%, respectively.

Electrochemical biosensing systems
Moreover, electrochemical biosensors have attracted immense attention of the researchers because of their capabilities to offer sophisticated ways to interface at molecular level, DNA recognition and signal transduction elements and are economical requiring low volume and power for DNA detection [93,94]. The electrochemical biosensing podium to detect different viruses including *SARS-CoV-2* has been described in Figure 4a [95]. A hybridization-based genosensor was developed on a 100 nm sputtered gold film to detect 30-mer sequence, which is distinctive to SARS [96]. The alkaline phosphatase (AP) was attached with AP-conjugated streptavidin *via* eminent streptavidin—biotin binding on electrochemical nanoarchitectured screen printed carbon electrode and was used as a genosensor for electrochemical detection of SARS-associated coronavirus [97]. Here, the Au NPs act as immobilization surface through thiol—gold interaction and transduction surface as well. This enzymatic electrochemical biosensor demonstrated a linear range between 2.5 and 50 pmol/L with low LOD of 2.5 pmol/L in the detection of targeted DNA sequence. In this work, the usability of Au NPs immobilized surfaces has considerably improved the electrochemical sensing performance. Fung's research group developed a biosensor based on piezoelectric quartz crystal (PQC)-aptamer coupled with paramagnetic nanoparticle for specific detection of SCV helicase protein created by *SARS-CoV* replication. The fabricated aptamer-PQC biosensor showed low detection limit of 3.5 ng/mL having reproducibility of 6.8% (%RSD, n = 3) and good recoveries of 102% and 119% [98].

Recently, Pan's research group fabricated Au NPs capped with specific antisense oligonucleotides (ssDNA) based electrochemical biosensor chip, which enabled digital detection of *SARS-CoV-2* nucleocapsid

Figure 4
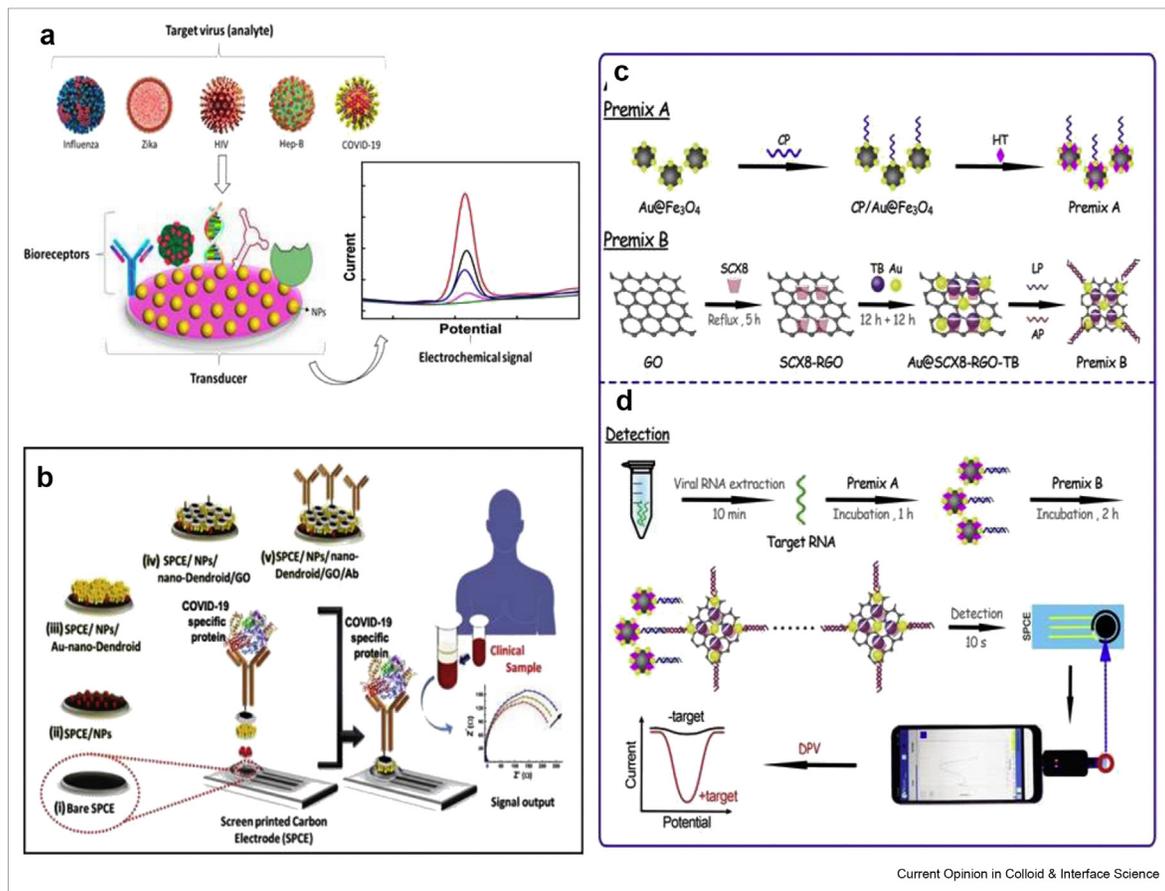
The vitality of electrochemical sensing techniques to carry out *SARS-CoV-2* detection (**a**) Electrochemical biosensing podiums to detect different viruses including *SARS-CoV-2*. Reprinted with permission from Ref. [95]. (**b**) Scheme representing the concept of SPCE/NPs/nano-Dendroids/GO/Ab probe fabrication to diagnose COVID-19. Reprinted with permission from Ref. [100], Schematic illustration of (**c**) Preparation of premix (**d**) Use of smartphone in *SARS-CoV-2* detection using the electrochemical biosensing platform. Reprinted with permission from Ref. [104].

phosphoprotein (N-gene) [99]. The paper-based electrochemical system was used to immobilize sensing probes to construct nucleic acid testing device. The as-fabricated biosensor outperformed, exhibiting a sensitivity of 231 (copies $\mu L^{-1})^{-1}$ and detection limit of 6.9 copies/μL when applied to test samples obtained from vero cells infected with *SARS-CoV-2* as well as patients' specimens. The biosensor showed 100% accuracy, sensitivity, and selectivity when the results of 22 COVID-19 positive patients and 26 healthy individuals acquired by the proposed sensor with standard RT-PCR diagnostic kit. The sensor was also feasible during genomic mutation of *SARS-CoV-2* because design of ssDNA probes which concurrently targeted two regions of *SARS-CoV-2*.

The scheme representing the concept of SPCE/NPs/nano-Dendroids/GO/Ab probe fabrication to diagnose COVID-19 has been shown in Figure 4b [100]. The label-free electrochemical impedimetric sensor was

prepared by Rashed et al. to rapidly detect *SARS-CoV-2* antibodies [101]. The sensor showed 100% accurate performance when results displayed by this sensor for clinical samples of COVID-19 negative and COVID-19 positive persons were compared with ELISA method. Moreover, Kim's research group has designed field-effect transistor (FET)-based biosensor in the detection of *SARS-CoV-2* from clinical specimens [102]. Graphene nanosheets modified FET was coated with specific antibody against *SARS-CoV-2*. The as-constructed FET biosensor has shown excellent sensing capabilities in the detection of *SARS-CoV-2* in culture medium and clinical samples with detection limits of 1.6×10^{1} pfu/mL and 2.42×10^{2} copies/Ml, respectively. The detection of S and N proteins from saliva samples has also been carried out using an electrochemical immunosensor based on magnetic beads as support of immunological chain and antibody with AP as immunological label [103]. The sensor depicted low detection limits of 19 ng/mL and 8 ng/mL for S and N

proteins in buffer solution and untreated saliva, respectively. In this assay, saliva sample is used, which yields high sensitivity compared with nasopharyngeal sample owing to self-sampling of saliva. Magnetic bead—based sensor offers the possibility of loading enhanced amount to capture antibody because of high surface area. More recently, Li's research group has introduced an electrochemical sensing platform using smartphone for the detection of *SARS-CoV-2* from infected patients as shown in Figure 4c and d [104]. Using supersandwich-type recognition approach, electrochemical smartphone was able to detect RNA of *SARS-CoV-2* without nucleic acid amplification and reverse transcription. The detection capability of this biosensor (85.5% and 46.2%) was higher than RT-PCR (56.5% and 7.7%) when practically applied in to detect RNA of *SARS-CoV-2* from 88 RNA extracts of 25 confirmed patients and 8 recovered individuals, respectively. The measured LOD was 200 copies/mL and only two copies (10 μL)/assay of *SARS-CoV-2* were needed. The different biosensing platforms for virus detection have been presented in Table 2. These biosensors not only demonstrate the very low detection limit, enhanced sensitivity and selectivity in detecting infectious agents without sample pretreatment but also produce rapid and cost-effective results in comparison with RT-PCR detection methods. Rapid testing, ease of handling, and miniaturization are also offered by electrochemical biosensors. The integration of theses biosensors with microfluidic platforms, and multiplexing can easily be achieved, which can further increase the sensitivity of device.

Viral-induced reactive oxygen species detection based on EC sensor

The biomarker-based detection of COVID-19 without using viral RNA, antigen, antibodies, and whole virus particles for detection, may be an interesting strategy in pandemic. It is well-documented that the existence of viral RNA is acknowledged to initiate the activation of NLRP3 inflammasome through RNA-modulating proteins and to trigger the generation of reactive oxygen species (ROS) [108]. Moreover, Zika virus has also been found to induce oxidative stress, which ultimately lowers antioxidant enzyme activities. During *in-vitro* and *in-vivo* studies, it has been confirmed that the infection caused by Zika virus significantly enhances ROS production and lipid peroxidation products, whereas decreases superoxide dismutase and catalase activities [109]. Previously, in case of *SARS-CoV*, Li's group investigated that the ROS level was noticeably augmented in *SARS-CoV* 3C-like protease expressing cells. The authors confirmed the role of *SARS-CoV* in virus-induced cell apoptosis in Vero-E6 cells [110]. It is very crucial to carry out early diagnosis of COVID-19 pneumonia to avoid care consumption and mortalities at mass level. Considering the early diagnosis, Miripour et al. presented a diagnostic method based on early traces of overproduced mitochondrial ROS in lungs due to viral-infected lung epithelium [111]. The recent investigation on COVID-19 diagnostic mechanism has been carried out monitoring overproduction of early traces of mitochondrial ROS as lung cells' dysfunctions triggered by *SARS-CoV-2*. The scheme of side effects of virus in lungs producing mitochondrial ROS which promote viral replication has been shown in Figure 5a. The electrochemical sensor for the detection of ROS consisting of three steel needle electrodes with working electrode modified by multiwalled carbon nanotube (MWCNT), reference electrode and counter electrode in a triangular form with 3 mm distance from each other, comparison among DPV responses of confirmed and healthy persons and their corresponding CT scan images

Table 2

Different biosensing platforms for virus detection.

Biosensor	Pathogen	Detection target	Limit of detection	Linear range	Ref.
EC	*SARS-CoV-2*	S or N protein	19 ng/mL and 8 ng/mL	–	[103]
EC	*SARS-CoV-2*	Antibodies	–	–	[101]
EC	*SARS-CoV-2*	N-gene	6.9 copies/μL	585.4 to 5.854 × 10^7 copies/μL	[99]
Cell-based	*SARS-CoV-2*	Antigen	1 fgmL^{1-}	10 fg and 1 μg mL^{1-}	[105]
Optofluidic	*SARS-CoV-2*	Antibody	0.5 pM	1-	[83]
Nanoplasmonic	*SARS-CoV-2*	Virus particles	370 vp/mL	0 to 10^7 vp/mL	[80]
SPR	*SARS-CoV-2*	Antibody	1.02 pM	2−1000 pM	[82]
LSPR	*SARS-CoV-2*	RNA	1 pM	1 nM to 1 μM	[106]
Lateral flow optical/ chemiluminescence	*SARS-CoV-2*	Serum IgA	–	–	[78]
SERS-LFIA	*SARS-CoV-2*	Antibody	1.28 × 10^7-fold dilution	–	[79]
Lateral flow	*SARS-CoV-2*	RNA	12 copies/reaction	–	[57]
Liquid crystal	*SARS-CoV-2*	RNA	–	–	[107]
Optomagnetic	*SARS-CoV-2*	RdRp	0.4 fM	0.1−10 fM	[84]

Figure 5
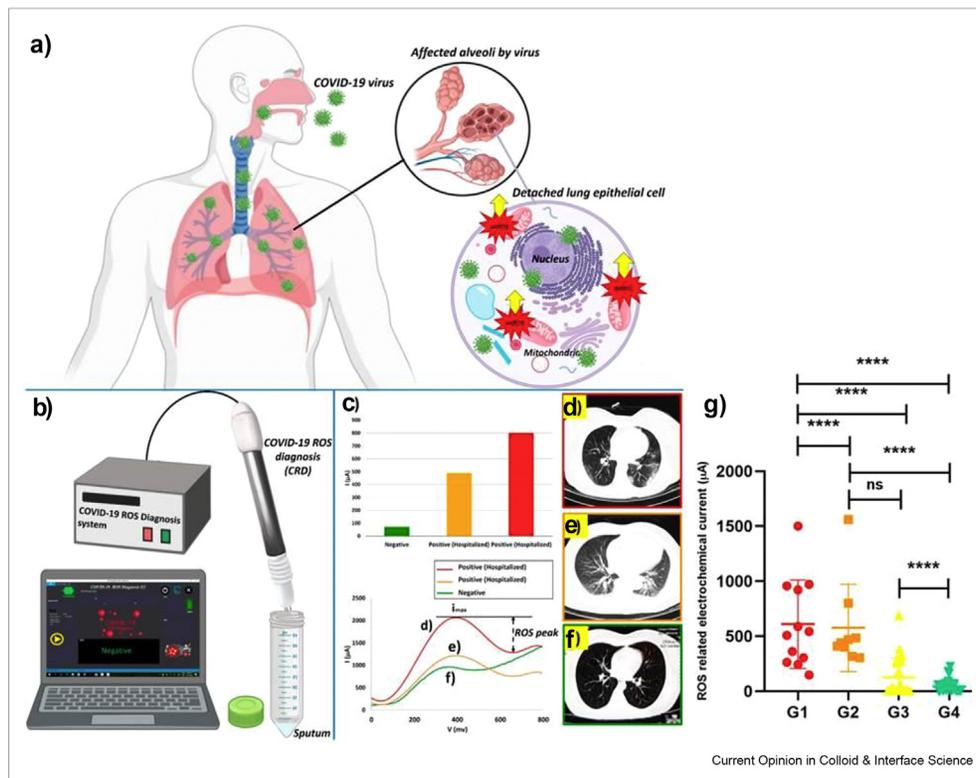
The ROS-based detection of COVID-19 using an electrochemical biosensor consisting of three needle electrodes modified with functionalized multiwalled carbon nanotubes (**a**) Scheme representing the side effects of virus in lungs producing mitochondrial ROS which promote viral replication. (**b**) Electrochemical detection of ROS. (**c**) Comparison among different fresh sputum samples from patients and their corresponding CV. (**d**–**f**) CT-scan images of infected patients and healthy individual. (**g**) G1; hospitalized in ICU (n = 25), G2; hospitalized without need to ICU care (n = 36), G3; PCR-positive nonhospitalized (n = 45), G4; PCR-negative healthy controls (n = 36). Reprinted with permission from Ref. [111].

have been displayed in Figure 5b–f. The practical application of proposed electrochemical sensor in differentiating patient samples by measuring the produced current under sweeping potential range of −0.8 to 0.8 V and with scan rate of 100 mV/s has also been presented in Figure 5g. So far, this is the first report on construction of electrochemical sensing platform, which can specifically detect concentration of ROS in sputum specimens to screen the individuals with or without COVID-19 infection. The sensor can work with a volume of less than 500 μl. The MWCNT-decorated electrode showed a sensitivity of 97% when applied in ROS detection in sputum samples of more than 140 healthy and confirmed cases and compared the results with clinical diagnostics. The sensor displayed the diagnosis results in less than 30 s and it can be a supreme assistant in rapid screening of patients. However, the authentication on specificity of clinical analysis would be needed further. Though, this type of portable, light weight, and sensitive biosensing platform is extremely needed to execute rapid screening of patients for further

medical checkup to decrease the workload on front line medical workers during pandemic.

Comparison among various detection approaches
The detailed comparison among various detection approaches has been discussed with their distinguish characteristics as given in Table 3. There are different detection methods such as immunofluorescence approaches, cell culture–based methods, molecular assays, and electrochemical biosensing–based approaches.

The issues need to be addressed
No doubt, there are lots of successful advancements in detection and diagnostic assays which are effectively contributing to control the *SARS-CoV-2* spread worldwide but some unmet problems still need to be solved for better control and prevention of COVID-19. Especially in the case of asymptomatic, presymptomatic, and the patients with less viral load, the point-of-care devices that work without RNA extraction, met the

Table 3

Comparing the pros and cons of conventional and electrochemical biosensing techniques.

Techniques	Significant points	Drawbacks	Ref.
Immunofluorescence (IF) approaches	Highly specific and sensitive.	Sensitivity is less as compared to cell culture−based methods, as well as, being highly specific cannot be used for all types of viruses, exhibit poor sensitivity against some viral particles. Moreover, need expertise.	[112]
Cell culture−based methods	Sensitivity is higher as compared to most antigen testing methods. Specific viral particles can be isolated even from the mixed culture medium. Facilitate the Antiviral, serotype as well as epidemiological studies.	Long incubation period and need of expertise are major disadvantages related to cell culturing methods.	[113]
Molecular assays	Sensitivity and specificity are good enough, turnout time is less in real-time analysis, even appropriately can detect the viral particles that cannot be cultured by cell culture methods.	These assays need highly specific primer and probes, need skills to be intramural use, as well as are expensive and most of them can only be done in research laboratories. In case of mixed infections, chances of false results are higher. Moreover, FDA-approved kits are not available for all types of viruses.	[114]
Electrochemical biosensors	Portable biosensors facilitate the in-house healthcare services without the need of highly trained personals. Being highly cost effective, with frequent response, less result turnout time, small sampling size, as well as admirable detection limits biosensors are key technologies in health care systems.	Sensitivity toward the samples matrix effect and poor stability are the bottleneck of biosensing technology.	[115,116]

accuracy problem for the sensitivity and detection of *SARS-CoV-2*. The long detection time also does not fit into the fast and on-site conductive screening of COVID-19 suspected individuals [117]. In case of COVID-19, it has been observed clinically that most of the infected persons have low viral load as well as less expression of nucleocapsid protein in infected cells, which ultimately produce a less nucleocapsid concentration in body fluids comparing to other CoV viruses. This thing by putting the question marks on the accuracy of the antigen and viral particle detection, suggested the development of ultrasensitive and POC detection technologies for the fast and *in-situ* sensing of antigen in suspected cases for the early diagnostics purposes [118].

Furthermore, low seroconversion is the main reason of trace antibody level (anti SP-antibody) in most of the COVID-19 patients, specifically the level of anti-RBD and anti-NP-antibodies is less at the start of infection, which results in the false results during the examination. During 5−6 days of incubation period, due to the trace level of targeted *SARS-CoV-2* antibodies, there are more chances that infected patients could be neglected by the commercially available kits. Ultimately, these pre-symptomatic and asymptomatic persons will be the source of virus spread to their close contacts [119]. To solve these unmet problems, in addition to smarter strategies, there is an urgent need to develop the ultrasensitive and cost-effective detection technologies for the accurate detection of antigen, antibodies, and integration of RNA release through preconcentration, amplification, and detection in portable microfluidic-based chips.

Conclusion and future outlook

The ultrasensitive and specific diagnostic methods are necessary for the early detection of *SARS-CoV-2* to expedite the treatment and isolation of infected individuals. In addition, diagnostic techniques are highly mandatory to empower medical staff with direct resources and to better combat with COVID-19. These days, CT scan, RT-PCR, and lateral flow immunoassays have been developed for the diagnosis of COVID-19. The nucleic acid testing methods and RT-PCR kits

being sensitive and specific offer the best way to detect *SARS-CoV-2*, but the reduction in false negative results is needed to increase the applicability of RT-PCR test. To overcome the bottleneck of standard diagnostic tools, emerging diagnostic approaches with low cost, wide availability, rapid response, and superb reliability are very essential to identify and handle the viral spread. Here of, we have critically discussed the state-of-the-art biosensing strategies for *SARS-CoV-2* detection. Biomarker-based diagnosis potential of biosensors will be the captivating approach in combination with clinical observations and risk factors to treat the patients according to the severity of their disease. Recently, biosensors have successfully detected *SARS-CoV-2* from biological samples; therefore, the use of biosensing technologies is the best way to diagnose the disease at its earliest stage.

With the innovation of nanotechnology, the sensing performance of biosensor devices should be further improved in terms of low detection limits, specificity and reproducibility, making them more trustworthy for *in-vitro* and *in-vivo* diagnostics. To improve the accuracy, combined detection of various biomarkers with multiplex biosensors can be the alternative sensitive approach. To guarantee the fast measurements at the point-of-care, sensing devices should be inexpensive and easy to handle. More work is required to connect sensing devices through databases with medical staff to realize a decentralized healthcare, which would be an important tool for the diagnosis of emerging infectious disease and to determine the herd-immunity regions. Furthermore, colorimetric strips and smartphone-based biosensors that can target antibody or antigen possess great potential as home-used point-of-care testing. Moreover, rapid translation of laboratory research on biosensors into commercially practicable prototype by industry is the main challenge to bridge the gap between laboratory work and industrial needs. The wastewater-based epidemiology should be further explored to develop proficient analytical devices for accurate and fast detection of trace level of *SARS-CoV-2* to determine the virus carrier regions. The society needs the massive defense to contain the enormous *SARS-CoV-2* like viral attacks and we believe that the best way to deal with such situations is, the development of the highly sensitive, swift, easily accessible point-of-care testing kits, which can be used even by the common man.

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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* of special interest
** of outstanding interest

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