

# Future developments in biosensors for field-ready SARS-CoV-2 virus diagnostics

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## Abstract

According to the evidence, the coronavirus disease 19 (COVID-19) is caused by a zoonotic pathogen named respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus can spread through personal contact, respiratory droplets, and also through airborne transmission. A rapid, low-cost, and effective biosensor platform is essential to diagnose patients with COVID-19 infection, predominantly the asymptomatic individuals, and prevent the spread of the SARS-CoV-2 via

transmission routes. The objective of this review is to provide a comparative view among current diagnostic methods, focusing on recently suggested biosensors for the detection of SARS-CoV2 in clinical samples. A capable SARS-CoV-2 biosensor can be designed by the holistic insights of various biosensor studies. © 2020 International Union of Biochemistry and Molecular Biology, Inc. Volume 68, Number 4, Pages 695–699, 2021

**Keywords:** biosensor, SARS-CoV-2, COVID-19, coronavirus, real-time PCR, serological assays

## 1. Introduction

Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) are two deadly coronavirus that the world experienced in 2002 and 2012, respectively.

**Abbreviations:** ACE2, angiotensin-converting enzyme 2; AuNPs, gold nanoparticles; BAL, bronchoalveolar lavage; 3CL<sup>pro</sup>, 3C-like proteases; COVID-19, Coronavirus Disease 2019; CRP, C-reactive protein; CT, computed tomography; CV, cyclic voltammetry; DPV, differential pulse voltammetry; EIS, electrochemical impedance spectroscopy; ELISA, enzyme-linked immunosorbent assay; FET, field-effect transistor; FTO, fluorine-doped tin oxide electrode; LDH, lactate dehydrogenase; LOD, limit of detection; LRT, lower respiratory tract; MERS, Middle East Respiratory Syndrome; PPT, plasmonic photothermal; RBD, receptor binding domain; RdRp, RNA-dependent RNA polymerase; S-Spike, spike glycoprotein; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); SPCE, screen-printed carbon electrode; SWV, square wave voltammetry; URT, upper respiratory tract; VTM, viral transport medium.

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Currently, severe acute respiratory coronavirus 2 (SARS-CoV-2) pandemic, which causes coronavirus disease (COVID-19), was reported in Wuhan, China [1].

Coronaviruses have a single-stranded RNA and belong to the Coronaviridae family in the Nidovirales order. The subgroups of this family based on genetic properties are alpha ( $\alpha$ ), beta ( $\beta$ ), gamma ( $\gamma$ ), and delta ( $\delta$ ) coronavirus. Since the past two decades, betacoronaviruses (SARS, MERS, and SARS-CoV-2) have been investigated by researchers due to their emerging and re-emerging characteristics. These infectious agents affect the upper respiratory tract (URT) and lower respiratory tract (LRT) and also can involve the gastrointestinal system, heart, kidney, liver, and central nervous system resulting in multiple organ failure and, generally, the flu-like symptoms of COVID-19 infection include fever, headache, joint pain, rash, and fatigue [2].

As a member of the coronavirus genus, SARS-CoV-2 showed over 80% identical to SARS-CoV (CoVZXC21 or CoVZC45) and bat SARS-CoV based on the sequencing of receptor binding spike glycoprotein (S-spike). The analysis of the nucleic acid sequence confirmed that the SARS-CoV-2 also uses ACE2 (angiotensin-converting enzyme 2) for the cell attachment, as was previously employed by the SARS-CoV. However, the current information indicate that SARS-CoV-2 is more infectious than SARS CoV [3].

The coronavirus genome encodes several structural and nonstructural proteins. S-spike glycoprotein is a viral membrane antigen and consists of two subunits of S1 and S2. The receptor binding domain (RBD) located in the S1 subunit and binds to the ACE2, on the other hand the S2 subunit provides the viral fusion and entry process into the target cell. Membrane (M) protein promotes the membrane curvature. It plays an essential role in viral assembly, envelope (E) protein is needed to release the virus, nucleocapsid (N) protein is interferon antagonistic and can support the viral replication [4].

The coronaviruses nonstructural proteins, such as RNA-dependent RNA polymerase (RdRp), 3C-like proteases (3CLpro, which is a main protease), and papain-like protease (PLpro) are essential for viral replication. These proteins activity result in blocking the host immune system cells expression. In fact, after the SARS-CoV-2 entrance to the host cells, the viral genome is translated into viral polyprotein and subsequently cleaved into effector proteins by viral proteinases 3CLpro and PLpro. On the other hand, PLpro can suppress the immune response via deubiquitinase of interferon factor 3 and NF- $\kappa$ B. RdRp catalyzes the replication of viral genomic from a full-length negative-strand RNA template [5].

Given the SARS-CoV-2 has been recently discovered, little immunological evidence is available. Previous reports have shown that both humoral and cellular immunity play vital roles in protective responses against SARS-CoV-2. Although antibodies against structural proteins (exclusive N and S proteins) are highly immunogenic, they have a relatively short lifespan. Compared with the humoral immunity specific reaction, the cellular immunity components such as T-helper cells, suppressor T-cells, and cytotoxic T-cells responses can largely induce long-lasting protection against SARS-CoV-2 [6].

Real-time polymerase chain reaction (real-time PCR) is known as an effective and sensitive method [7]. However, the false-negative results can occur that demand the fabrication of an accurate, rapid, and free-PCR technique for diagnosing COVID-19 infection as an alternative and first test compared to current diagnostic techniques.

In recent years, there has been an increasing interest in the biosensor, a transportable analytical device used for detecting various microorganisms and composed of biological molecules with a detector [8]. The device requires the efficient immobilization of antibodies, peptides, aptamers, or nucleic acids on the surface of a transducer responsible for the analyte recognition.

Biosensor introduced new opportunities for reliable, economical, and sensitive detection, particularly for the early detection of infectious diseases. Additionally, the materials (including graphene, gold nanoparticles, polyaniline-multiwall carbon nanotube, etc.) with the nanometer scale have been used to reach the nano structuration of biosensors. Biosensors facilitate the output signal study by cyclic voltammetry (CV), square wave voltammetry (SWV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) [9].

### Highlights

- Coronavirus disease 19 (COVID-19) is a zoonotic pathogen.
- Method detection based on biosensor systems with high sensitivity and specificity is an urgent demand for controlling the pandemic of COVID-19 infection.
- Biosensor methods have been considered innovative and promising tools for detecting other viruses.

In this review, we highlighted the limitation of current techniques. We reviewed the literature on the fabrication of biosensors for the detection of SARS-CoV-2 to encourage researchers to develop further strategies for the detection of COVID-19 disease.

## 2. Current Methods for SARS-CoV-2 Detection

Several inflammation-related parameters increase or decrease in patients with COVID-19 infection. They are used as screening tests for the prognostics of the COVID-19 infection. Particularly, C-reactive protein (CRP), lymphocyte count, interleukin-6 (IL-6), interleukin-10 (IL-10), lactate dehydrogenase (LDH), platelet count, D-dimer, and serum-ferritin [10].

Centers for Disease Control and Prevention (CDC) has recommended that four methods perform SARS-CoV-2 detection: (1) real-time PCR that is a gold-standard method because of its high selectivity and relatively high sensitivity for detection of COVID-19 infection, (2) gene sequencing, (3) serological tests, and (4) chest computed tomography (CT) [11]. Nevertheless, for asymptomatic individuals who have traveled to high-risk areas for COVID-19 infection or contacted with infected people, the preferred detection method is RT-PCR [2].

In real-time PCR assay for COVID-19 detection, the bronchoalveolar lavage (BAL) fluid, sputum, fiber bronchoscope brush biopsies, nasal swab, pharyngeal swab, or cerebrospinal fluid samples are added to a buffer solution containing probe, primers, reverse transcriptase (to gain the cDNA from RNA), DNA polymerase (to amplify the cDNA), deoxynucleotides (dNTPs), and an intercalating dye or fluorescent probes.

SARS-CoV-2 RNA has been detected in patients 1–3 days before symptom onset; therefore, the negative result of real-time PCR from the symptomatic patients should be confirmed with a new sample after at least 24 hours apart from another test of identification.

For later stages of COVID-19 infection, antibody-based techniques can be used. Usually, neutralizing antibodies against SARS-CoV-2 develop 5–15 days after infection with SARS-CoV-2 and start to decrease within 2–3 months after infection. Binding of neutralizing antibodies to the SARS-CoV-2 antigens can reduce the possibility of viral transmission [12].

TABLE 1

Comparison of reported biosensor for detection of COVID-19 infection

Ref.	Method	Sensor platform	Time	Detection limit	Protein detection
[15]	Colorimetric assay	AuNPs /ASOs	10 Min	0.18 ng/ $\mu$ L	N
[16]	Optical	Graphene sheets/ nCovid-19Ab	–	$1.6 \times 10^1$ pfu/mL/ $2.42 \times 10^2$ copies/mL	S
[17]	DPV	FTO/AuNPs/nCovid-19Ab/ SPCE	10–30 Sec	120 fM	S
[18]	SPR	AuNIs chip/DNA probe	–	0.22 pM	RdRp/ORF1ab/E
[19]	Optical	P–FAB	15 Min (Label-free bioassay)/ 5 Min (Labeled bioassay)	$10^{-18}$ M	N

By the enzyme-linked immunosorbent assay (ELISA) method, a COVID-19-infected sample is added to a well plate that is coated with an anti-human IgM-specific antibody or COVID-19 recombinant protein for detection of IgM or IgG, respectively. Then it is incubated and washed in the microwells. HRP-labeled COVID-19 tracer antibody for the detection IgM antibody or HRP-labeled COVID-19 antigen for the detection IgG antibody is added and washed away, binding specifically to the IgM and IgG of infected patients. Finally, a chromogenic substrate for quantification and stop solution are added. COVID-19-infected serums will elicit an optically detectable signal that may be correlated to the concentration of antibodies. However, the false-positive results may occur due to cross-reactivity between specific antibodies and with antibodies against other coronaviruses epitopes.

CT scan is used to confirm the false-negative results using real-time PCR from symptomatic patients or as a separate diagnostic tool for the detection of Covid-19 infection. In this method for capturing three-dimensional (3D) images, several X-ray images of the chest are taken to identify SARS-CoV2 infection, which can involve the lower parts of single or both lobes [13].

### 3. Limitation of Current Methods for the Detection of COVID-19 Infection

According to molecular techniques, laboratories detect the COVID-19 infection using real-time PCR methods within 4–6 H. However, there are limitations including designing probes-primers, sample preparation, sampling error, the requirement to equipment tools, and their need for multi-temperature sample heating for denaturation, annealing, and extension [8]. On the other hand, traveling to a laboratory for testing increases the risk of spreading COVID-19 infection. Also, it was reported that several false-positive or false-negative results, especially in the early stages of the COVID-19 infection.

The serological assay is rapid and requires minimal equipment, but its efficacy may be limited only in the detection of acute COVID-19 infection. In fact, for the detection of antibodies, it may take several days to weeks after the onset of the symptom. On the other hand, immunosuppressed people are challenged in serological assays. Moreover, a CT scan is a complementary technology with RT-PCR for detecting COVID-19 infection; nevertheless, its primary challenge is distinguishing COVID-19 infections symptoms from other flu-like symptoms or lung disorders. Besides, this assay is expensive and requires advanced skills for the analysis [14].

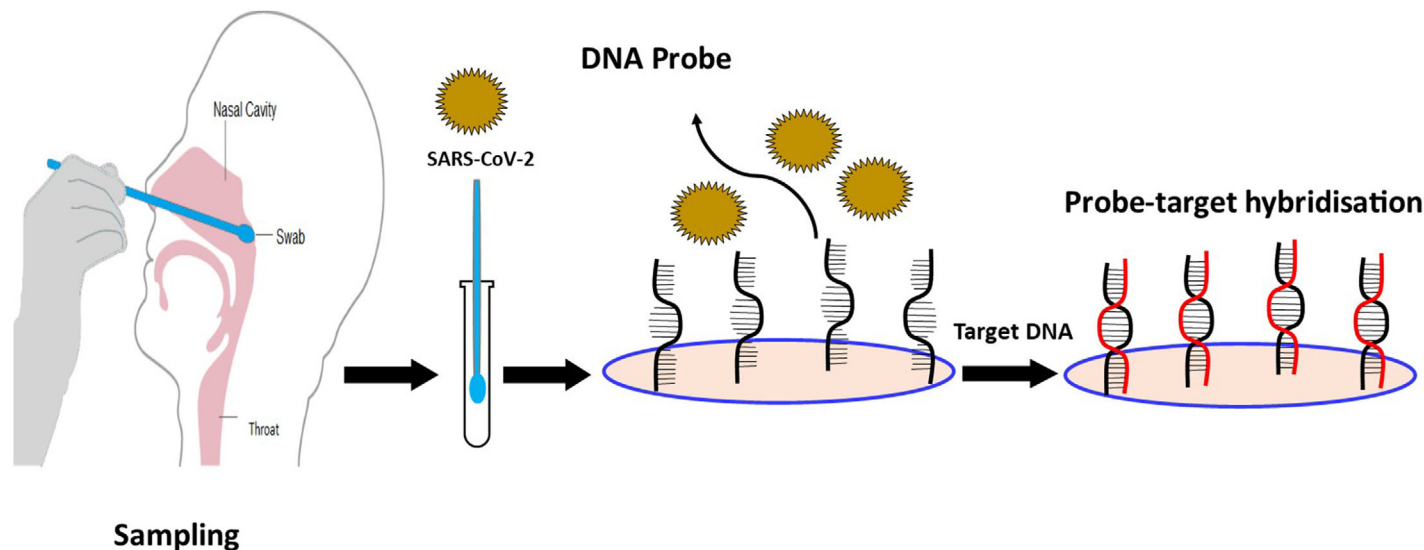
Many research groups have also focused on sensor methods to eliminate complicated stages of sample preparation and also reduce the possibility of false-positive and false-negative results and use expensive laboratory equipment.

### 4. New Developments in Biosensing Research for SARS-CoV-2 Detection

Asymptomatic patients are a major threat to public health because they are a potential source of SARS-CoV-2 transmission [1]. Therefore, new method detection based on biosensor systems with high sensitivity and specificity is an urgent demand for controlling the pandemic of COVID-19 infection.

Fabrication of biosensor platforms for the detection of SARS-CoV-2 involves three main steps: (1) identification of biology targets including viral RNA, viral proteins, human microRNA (miRNA), and human immunoglobulins, (2) selection of a bioreceptor for immobilization on a transducer including DNA or miRNA probe, aptamer, enzyme, antibody/antigen, ligand and so on, (3) and hybridization detection including electrochemical, fluorescent, colorimetric, magnetic, piezoelectric, and acoustic detection technologies.

Moitra et al. introduced a platform sensor based on combination of gold nanoparticles (AuNPs) and thiol oligonucleotides for the detection of SARS-CoV-2 N-gene with a linear range of 0.2–3 ng/ $\mu$ L and limit of detection (LOD) of 0.18 ng/ $\mu$ L within



Schematic illustration of the DNA biosensor.

FIG. 1

10 Min from the isolated RNA sample. The selectivity of the proposed biosensor has been checked against MERS-CoV RNA that it showed no change in absorbance with MERS-CoV gene [15].

In another study by Seo et al. fabricated a biosensor based on the field-effect transistor (FET) for diagnosis of SARS-CoV-2 in nasopharyngeal swab samples. FET is an electronic biosensor, and its advantages are miniaturization, portable, and mass manufacturing. In this project, the sensor was modified by graphene sheets and SARS-CoV-2 spike antibody. The performance of this sensor was checked by the other SARS-CoV-2 antigens and cultured virus, in addition to swab samples. The designed platform detected the S protein at concentrations of 1 fg/ml phosphate-buffered saline (PBS) and 100 fg/ml viral transport medium (VTM). Moreover, the LOD of this sensor in culture medium and clinical samples was  $1.6 \times 10^1$  pfu/mL and  $2.42 \times 10^2$  copies/mL, respectively [16].

In another study, Mahari et al. fabricated a biosensor by fluorine-doped tin oxide electrode (FTO) and AuNPs on the screen-printed carbon electrode (SPCE) modified with SARS-CoV-2 antibody against S protein. The LOD of this biosensor was 10 fM within 10–30 s. Surprisingly, the lifetime of these electrodes was up to 4 weeks [17].

Qiu et al. synthesized a sensitive gold nanoislands (AuNI) sensor based on the plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) for the detection of SARS-CoV-2 in clinical samples with a concentration range from 0.01 pM to 50 μM and LOD of 0.22 pM. This chip is a label-free sensor that is capable of detecting the SARS-Cov-2 RdRp, ORF1ab, and E genes [18].

In a parallel work, Murugan et al. proposed both approaches (two plasmonic labeled and label-free immunoassays) based on the U-bent optical fiber sensor system (P-FAB) for

the detection of SARS-CoV-2 N gene in the saliva sample. In the label-free bioassay, a U-bent fiber-optic probe as a platform covers with gold nanoparticles, followed by covalent conjugation of anti-N protein monoclonal antibodies via a thiol-PEG-NHS based coupling chemistry. In this way, the results can be obtained within 15 Min. The labeled bioassay manner is based on the sandwich immunoassay. The U-bent fiber-optic probe is immobilized with anti-N protein monoclonal antibodies, and gold nanoparticles are subsequently treated with bovine serum albumin (BSA) solution to minimize the nonspecific interactions. Then, the modified platform is exposed to saliva samples for 5 Min. The signal response can be obtained within 10–15 Min. Therefore, the P-FAB system has excellent potential in the detection of COVID-19 infection [19]. The analytical performance of current biosensors for detection of COVID-19 infection were listed in Table 1.

## 5. Conclusion

This review paper provides an overview of the conventional methods and biosensor-based techniques that have been recently used to detect the SARS-CoV-2. Currently, the real-time PCR is used as the main and powerful assay for the detection of COVID-19 infection. We found out that conventional methods cannot meet the rapid detection demands and challenges in the viral analysis.

In conjunction with the real-time PCR, CT scan significantly increases the sensitivity, facilitating clinical counseling, and improving treatment outcomes. Although the real-time PCR is an accurate method compared with the current assays, it has some limitations. As mentioned above, due to the drawbacks of current diagnostic methods in the early stage infection, the employment of some advanced methods, such as microfluidics, biosensors, and lab-on-a-chip systems, will be recommended as suitable methods for the diagnosis of SARS-CoV-2.

TABLE 2

Our suggested sequences of target genes for detection of SARS-CoV-2 DNA

Gene	Probe sequence (5'-3')	target sequence (5'-3')
ORF1ab	CCGTCTGCGGTATGTGGAAAGGTTATGG	CCATAACCTTCCACATACCGCAGACGG
RdRp	CAAGTGGGGTAAGGCTAGACTTT ACTTAGGATAATCCCAACCCAT	AAAGTCTAGCCTTACCCCACTTG ATGGGTTGGGATTATCCTAAGT
N	TTGCTGCTGCTTGACAGATT	AATCTGTCAAGCAGCAGCAA
	ACACCAAAGAUCACAUUGG	CCAATGTGATCTTTTGGTGT
	CCCGCAAUCCUGCUAACAAU	ATTGTTAGCAGGATTGCGGG
	CAGAACAAACCCAAGGAAU	ATTCCTTGGGTTTGTCTG
	ACUGAUUACAAACAUUGGCC	ATTCCTTGGGTTTGTCTG
E	CTAGTTACTAGCCATCCTTACTGC	GCAGTAAGGATGGCTAGTGTAAGTAG
S	CCTACTAAATTAATGATCTCTGCTTACT CAAGCTATAACGCAGCCTGTA	AGTAAAGCAGAGATCATTAAATTTAGTAGG TACAGGCTGCGTTATAGCTTG

In recent years, biosensor methods have been considered innovative and promising tools for detecting other viruses, which, in contrast to the conventional methods, are less complicated to use and free of prolonged experimentation processes. The biosensor systems are rapid and specific for infection detection, and a physician can quickly decide whether the treatment is needed or not.

Generally, a DNA-based biosensor can detect the pathogens and record the information of them in clinical diagnostics. DNA biosensor is mainly composed of a bioreceptor and a transducer. A bioreceptor is a DNA probe designed from a conserved sequence to recognize the pathogens DNA by a transducer using converting the biological signal into the desired signal.

DNA biosensor as an alternative method for current techniques can provide rapid response and also is the high sensitivity and low cost. A schematic diagram of the DNA-based biosensor is summarized in Fig. 1.

It is recommended that further research should be undertaken on the developing DNA biosensor in the following subjects. First, choice of a more conserved and specific gene of SARS-CoV-2 (we provide SARS-CoV-2-specific probes and target based on highly conserved regions of the S, E, and N proteins and nonstructural (RdRp and ORF1ab) proteins in Table 2). Second, the biosensors' study of performance with the large sample size. Third, a comparison of the sensitivity and specificity of the biosensor with current methods.

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