

Review Article

Perspectives on electrochemical biosensing of COVID-19

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Abstract

Rapid detection of human coronavirus disease 2019, termed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or COVID-19 infection, is urgently needed for containment strategy owing to its unprecedented spreading. Novel biosensors can be deployed in remote clinical settings without central facilities for infection screening. Electrochemical biosensors serve as analytical tools for rapid detection of viral structure proteins, mainly spike (S) and nucleocapsid (N) proteins, human immune responses, reactive oxygen species, viral ribonucleic acid, polymerase chain reaction by-products, and other potential biomarkers. The development of point-of-care testing devices is challenging due to the requirement of extensive validation, a time-consuming and expensive step. Together with specific biorecognition molecules, nanomaterial-based biosensors have emerged for the fast detection of early viral infections.

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Current Opinion in Electrochemistry 2021, 30:100794

This review comes from a themed issue on **Sensors and Biosensors**

Edited by **Sabine Szunerits**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 3 July 2021

<https://doi.org/10.1016/j.coelec.2021.100794>

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Keywords

SARS-CoV-2, Biosensor, Electrochemistry, Viral proteins, Antibodies, Diagnosis, Point of care.

Introduction

A single and positive-stranded ribonucleic acid (RNA) virus [1] (SARS-CoV-2) has infected people and caused deaths worldwide [2*]. To date, more than 173 M people have been infected, resulting in >3.7 M death (<https://www.worldometers.info/coronavirus/coronavirus-cases/> accessed 6.6.21). Affected subjects have experienced severe, mild, or no symptoms [3, 4]; thus, fast analysis of viral infection is of uttermost importance to isolate affected people. Although opacities in computed tomography images were observed in affected patients with pneumonia [5–7], this diagnosis is not always reliable. The viral genetic code deciphered in early January 2020 enables the development of polymerase chain reaction (PCR) for detecting its distinct RNA sequences [8]. The viral RNA consists of double-layered lipids enveloped with four major structural proteins (Figure 1).

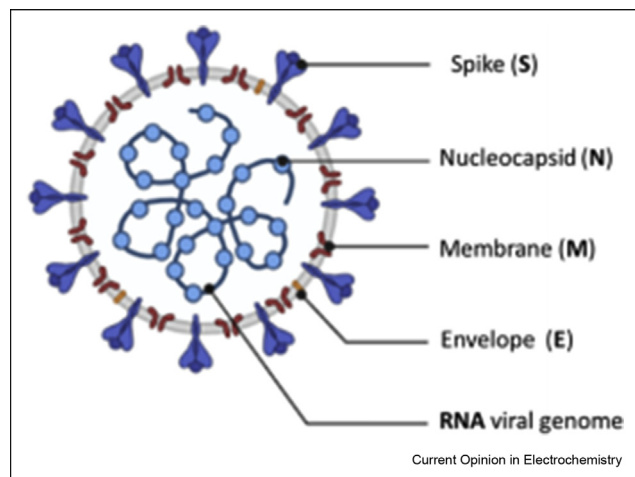
Real-time reverse transcription PCR (RT-PCR) has been developed to target various viral genes [9] (Table 1). Acute infection with SARS-CoV-2 also invokes the release of antibodies against specific viral antigens in serum within days to weeks [10].

This opinion provides an overview of emerging electrochemical detection tools for effective coronavirus diagnostic assays, encompassing the identification of viral nucleic acids, viral proteins (antigens), PCR by-products, and reactive oxygen species (ROS).

PCR technology and immunoassays

The detection of RNA by reverse transcription polymerase chain reaction (RT-PCR) is time consuming and does not have the screening capacity. Point-of-care (POC) testing (POCT) alleviates the burden of the testing load on central hospitals without compromising accuracy and detection selectivity for early detection and treatment of viral infection. Loop-mediated isothermal amplification [15] and recombinase polymerase amplification can detect the lowest copy number of nucleic acids. Real-time PCR for POCT has been commercialized [16] to process a single sample or

Figure 1



Four known structural proteins of SARS-CoV-2 (created by [BioRender.com](#)). The abundant S protein (~150 kDa with 1255 amino acids, AA) mediates viral attachment to the host cell surface receptors (angiotensin-converting enzyme II [ACE2]), followed by viral infusion and entry. The motif KRSFIEDLLFNKV occurs in the spike glycoprotein that binds ACE2. The M protein (~25–30 kD with 221 AA) is the most abundant: defines the viral envelope shape and participates in viral production and maturation. The smallest E protein (~8–12 kDa, 76 AA) is involved in viral budding and assembly, directing protein–protein interceptions and inducing membrane curvature and preventing M protein aggregation. Based on its binding to the RNA genome, the N protein participates in viral assembly and budding [2]. The N protein is the most immunogenic viral structural protein that is shed just a few days after infection with a peak at around 10 days after infection. RNA, ribonucleic acid.

multiple samples, up to 80 samples [17]. The topic of POC PCR assays for COVID-19 detection was recently reviewed by Gupta et al. [18].

Serological tests based on antibodies, antigens, and some biomarkers of infected people are more rapid and inexpensive [19]. Owing to the lack of detection sensitivity with a high false-positive/negative rate, such tests cannot confirm viral infection [20]. Nevertheless, the US Food and Drug Administration has reported POC styled tools for SARS-CoV-2 by detecting its viral RNA. The detection of N1 gene by quantitative and real-time PCR requires a total assay time of 2 h, compared with 7 h for the analysis of N gene, ORF1gene, and ORF1ab gene by RT-PCR equipped with mass spectroscopy. However, the Abbott assay detects RdRp and N-genes qualitatively within 13 min [21]. Besides the presence of SARS-CoV-2, positive results are general indicators of bacterial and other viral infections. IgG and IgM occur simultaneously or sequentially, and the seroconversion for IgG and IgM happens for 14–23 days after onset of infection in most subjects. Therefore, the detection of

antibodies is unreliable during the first 14 days after onset of symptoms for all serological assays [22]. These two biomarkers cannot be used to detect acute infections, but they are useful for identifying affected subjects with asymptomatic infections and are complementary to RT-PCR for reducing false-negative testing. Commercial POC immunoassays can detect viral antigens (specific monoclonal antibodies are used to bind the SARS-CoV-2 antigens: N and S proteins), total antibody, and IgM/IgG antibodies. Detection techniques are mainly based on colloidal gold/immunofluorescence chromatography (lateral flow immunoassay), enzyme-linked immunosorbent assay, and chemiluminescence [16].

Opportunities for electrochemical sensors/biosensors

Electroanalysis of viral DNA

The electroanalysis of DNA hybridization is well investigated using a specific DNA sequence, known as a DNA probe. This short single-stranded DNA is thiolated and self-assembled on a gold surface or a gold nanoparticle (AuNP)—modified electrode. A binding event occurs when the double-stranded DNA is hybridized with its complementary DNA strand [23]. The binding event can be probed directly or by indirect measurement in the presence of an electroactive marker such as a metal complex or methylene blue [24*, 25]. A miniaturized, label-free electrochemical platform can be interfaced with a workstation or smartphone system as shown in Figure 2 [26]. One of its major drawbacks for potential POCT is the requirement of a rapid on-site extraction method for DNA. Nonspecific protein binding on gold is problematic despite unreacted gold being thoroughly blocked by small organic molecules with thiol groups.

Viral proteins (antigens) as potential targets

Among 29 possible viral proteins, both abundant N and S proteins are potential targets for antibody-based viral detection. The homology of the S and N proteins between SARS-CoV-2 and SARS-CoV-1 (SARS-associated coronavirus) is ~76% and 90%, respectively [27], and two proteins are highly immunogenic and abundantly during infections. Commercial antibodies against viral proteins, spike (S1 and S2), nucleocapsid (N), envelope (E), ORF, and non-structural proteins (NSP), are available in several countries, particularly, China. As expected, they are expensive, ranging from 5000 to 6000 \$ US/mg of protein [28]. Some methods are available to prepare antibodies or antibody-like proteins against the spike protein S1 subunit [29*].

The S protein assists in binding the virus to the cellular human receptor; thus, it is responsible for inducing the

Table 1

Current target analytes and detection methods.

Detection procedures	Target analytes and other key points	Ref.
RT-PCR	S-gene, N-gene, E-gene, M-gene, RdRp (RNA-dependent RNA polymerase), and ORF1ab (open reading frame). Detection sensitivity and selectivity of such target genes vary as per the manufacturer studies. Such laboratory-based analyses are time consuming and required the extraction of RNA from patient specimens and specialty reagents/enzymes to deactivate RNase and convert RNA to the complementary DNA, followed by an amplification step. Proteinase K is frequently used to lyse the virus and it must be destroyed by subjecting the lysis buffer for 5–10 min at 95°C. All available RNA detection formats can be adapted to detect SARS-CoV-2. The quality and relevant abundance of RNA in the collected samples are significantly dependent on the type and site of collection. Several commercial POC devices with automated instrument operation are available. Both integrate nucleic acid extraction and RT-PCR amplification into a cassette.	[10–14]
Immunoassays	Infection with SARS-CoV-2 initiates the release of antibodies against specific viral N and S antigens: specific anti-S protein antibodies against the spike's1 protein subunit and receptor-binding domains (RBD). These antibodies are released in serum within days to weeks after acute infection. Thus, serologic testing cannot be used to diagnose acute SARS-CoV-2 infection. However, serologic tests can identify persons with resolving or past SARS-CoV-2 infection.	[10]
Biosensing/diagnostic assays	The detection of two viral proteins (S and N) opens the possibility for probing parts of the intact virus without any sample step.	

RT-PCR, reverse transcription PCR; PCR, polymerase chain reaction; RNA, ribonucleic acid; POC devices, point-of-care devices.

infection [30]. Graphene with a high surface area offers fast electron transfer kinetics and excellent detection sensitivity. A biosensor based on a graphene field-effect transistor can detect the virus in clinical samples [31]. The S protein antibody is covalently conjugated to a graphene sheet (Figure 3(a)). A multiplexed telemedicine platform-based laser engraved graphene electrode detects several specific biomarkers in saliva and blood [32]. A cell-based biosensor can detect the viral S protein using an array of eight Au screen-printed electrodes [33]. The potentiometric signal is generated upon the addition of the S protein into the membrane-engineered mammalian kidney cells down to 1 fg/mL within 3 min [33]. Amperometry using monoclonal antibodies and AuNPs can detect the viral antigen spiked in saliva samples with linearity of 1 fM to 1 μ M [34].

The binding of the S-receptor-binding domain (RBD) to the host ACE2 receptor is crucial for the viral invasion into human cells [35]. The interaction of S-RBD and ACE2 is the basis for neutralization antibody tests, which provide critical information on whether the subjects have developed neutralization antibodies to counteract SARS-CoV-2 after convalescence or vaccination. The electrochemical sensor consisting of cobalt-functionalized TiO₂ nanotubes can detect viral S-RBD in clinical specimens within 30 s with a limit of detection (LOD) of 0.7 nM [36] (Figure 3(c)).

Other potential target analytes: PCR products and ROS

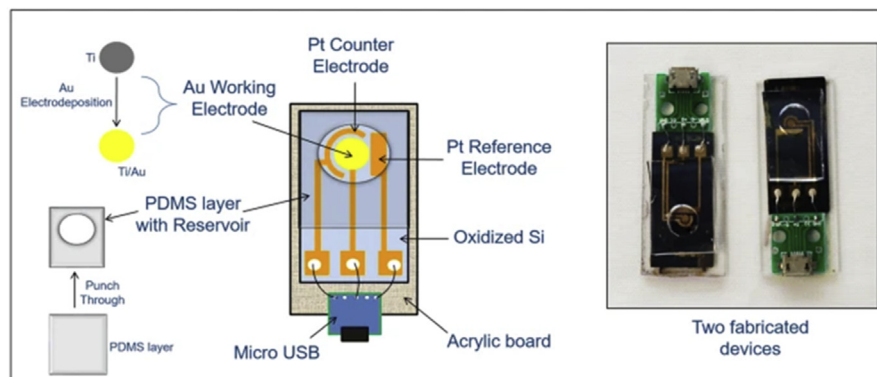
Reactive PCR products

GenMark Diagnostics launched a rapid test, known as ePlex® to detect amplified products of PCR. Qualitative viral detection in nasopharyngeal swab specimens is performed by a single-use cartridge and proprietary surface electrowetting. The US Food and Drug Administration has approved this test for Emergency Use Authorization [37]. Based on the hybridization of competitive DNA, electrochemical detection by a capture probe immobilized on a gold-plated electrode. The viral RNA extracted from the specimen by magnetic solid-phase extraction is converted to cDNA followed by PCR amplification. A ferrocene-labeled signal probe is then mixed with the target DNA. Upon the hybridization of the target DNA, the ferrocene-labeled signal probe generates an electrochemical response. The test has a LOD of 1×10^5 copies/mL for the SARS-CoV-2 DNA [38], which is comparable with the approved RT-PCR for Emergency Use Authorization [39].

Reactive oxygen species

Upon the inflammation of the lungs with SARS-CoV, the respiratory system underlays molecular mechanisms: pyrin domain-containing 3 (NLRP3), a nod-like receptor family, is activated by the virus [40] and then releases ROS to damage mitochondria [41,

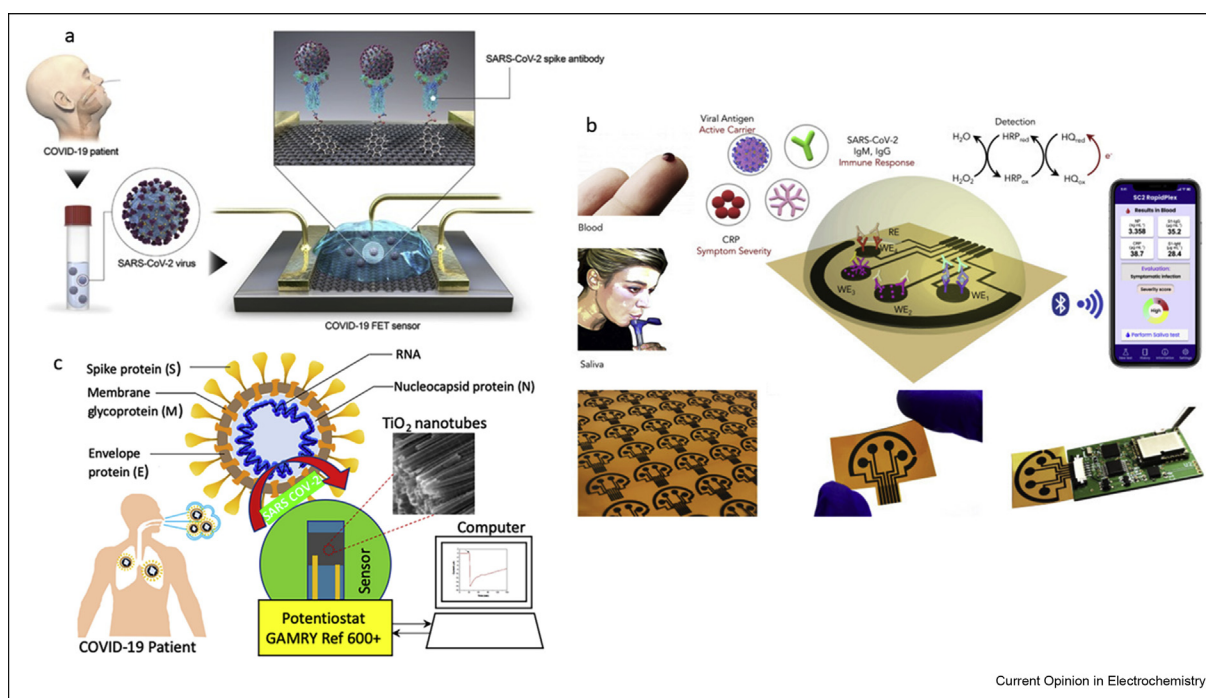
Figure 2



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Schematic representation of the miniaturized electrochemical platform showing different components, with an actual image of two fabricated devices [26]. Reproduced with permission from the study by Tripathy and Singh. [26].

Figure 3



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(a) Schematic diagram of a graphene-based field effect transistor (FET) sensor to detect viral proteins. The specific binding between the S protein and GFET results in dose dependently changing the surface potential [31]. The GFET biosensor can detect SARS-CoV-2 in phosphate-buffered saline, universal transport medium (UTM), the culture medium, and in-patient samples at 1 fg/mol, 100 fg/mol, 16 pfu/mL, and 242 copies/mL, respectively [31] (b) A wireless graphene-based telemedicine platform equipped with a mobile user interface. This platform detects several specific biomarkers including N-protein, IgG and IgM antibodies, and inflammatory biomarker C-reactive protein. Different recognition receptors are covalently conjugated to graphene, followed by blocking unreacted sites by bovine serum albumin. Reproduced with permission from the study by Torrente-Rodríguez et al [32] (c) A schematic diagram of a Co-functionalized TiO₂ nanotube (Co-TNT)-based sensing platform [36]. Reproduced with permission from the study by Vadamani et al. [36]. GFET, graphene field-effect transistor.

42]. Sputum is one of the most secreted samples with a high viral load in the lung epithelium [43]. A real-time sensor based on the fabrication of multiwalled

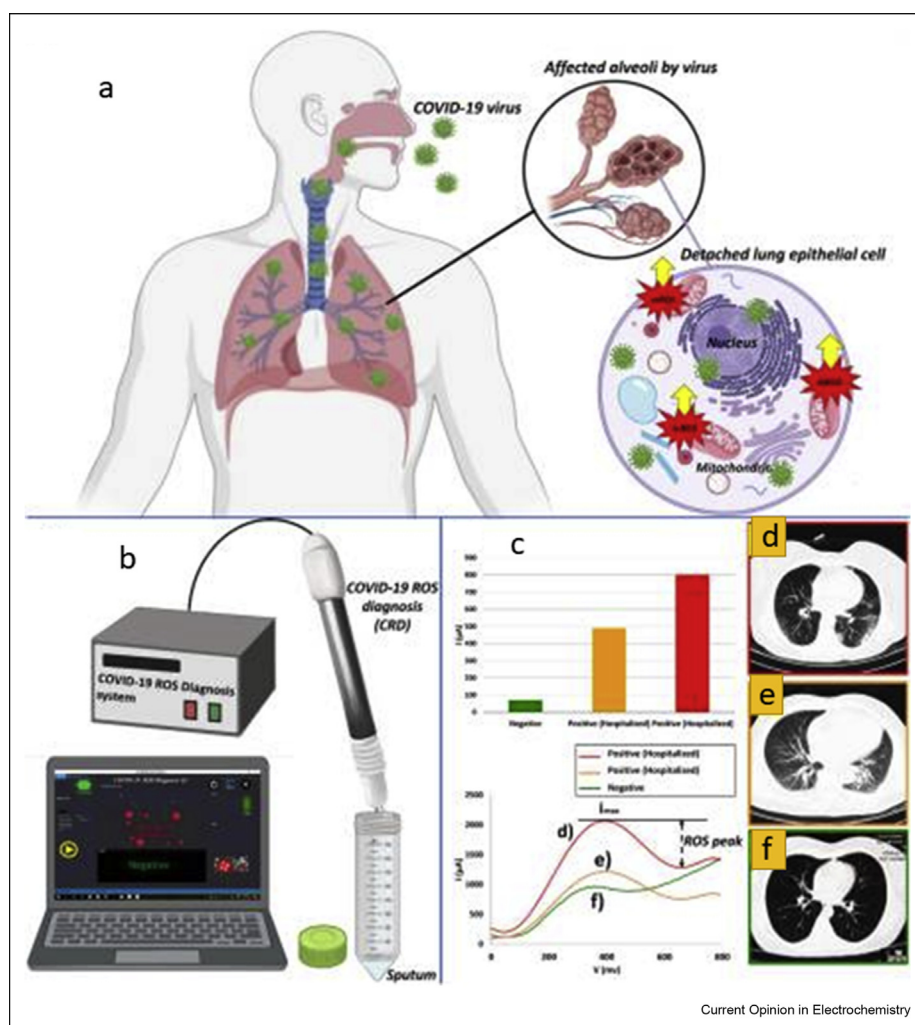
carbon nanotubes on the steel needle tip was designed to detect ROS in patients' samples [44] (Figure 4).

Trends and future endeavors

Asymptomatic cases of SARS-CoV-2 have made the diagnosis more complex by physical examination or chest X-rays. Portable PCR and immunoassays are commercially available for different viral genes, human immune proteins, and pertinent biomarkers to alleviate the burden of testing. AuNPs together with a recombinant viral antigen and rabbit-IgG can be used in lateral flow immunoassays of IgM and IgG in human blood within 15 min [45]. Nevertheless, simple electrochemical approaches are powerful tools for POCT, doctors' offices in remote areas, or countries with limited resources. S protein and N protein are considered the prime targets owing to their abundance and high immunogenicity, and the biorecognition molecules are

based on exorbitantly expensive polyclonal/monoclonal antibodies for these two proteins. The envelope (E) and membrane (M) proteins are required for virus assembly [46]; however, they are nonspecific biomarkers for SARS-CoV-2. The biorecognition molecules are polyclonal or monoclonal antibodies, which are commercially available from different sources. The ACE2 has rarely been used, due to its commercial high cost, and must be kept at -20 to -70 °C to conserve its stability [47]. Single-stranded RNA or DNA oligonucleotides can also serve as specific recognition biomolecules. A search of small molecules with selective binding to S and N proteins is critical for developing POCT. For POCT, a 'true' one-step is desirable as the user simply swirls the sample to the detection device. To date, applications of

Figure 4



(a) Common clinical symptoms of lung host cells as the virus induces mitochondrial ROS overproduction for its replication (b) A detection scheme based on three needle electrodes, which are coated by functionalized CNTs (c) The released ROS detected as a cathodic peak from the fresh sputum of two hospitalized patients (d, e) and compared with a confirmed normal case (f): $I = 800$ μA (red column) and $I = 490$ μA (orange) with severe lung affected by SARS-CoV-2, compared to ~ 71 μA (green column, normal case). The ROS peak current is correlated with the amount of released ROS in the sputum. The results are corroborated by lung CT scanning (d, e, and f). The sensor offers a rapid response time of 30 s, showing a great potential to be used as a POC tool for detecting viral-induced infection. Reproduced with permission from the study by Miripour *et al.* [44]. ROS, reactive oxygen species; CNTs, carbon nanotubes; POC tool, point-of-care tool.

nanomaterials are limited [48], and electrochemical approaches have competed with colorimetry and surface plasmon resonance. Both S and N proteins must be analyzed simultaneously to circumvent potential cross-reaction with other endemic coronaviruses or viral mutations. Other biomarkers such as C-reactive protein, interleukin-6, D-dimer, and so on also assist the prognosis of SARS-CoV-2. The commonest sample is a swab from the patient's nose or throat with an expected notable viral load. Noninvasive samples, for example, saliva, sputum, breath, and stools have been explored to establish sampling protocols that provide minimum false positives and negatives. Several fully quantitative immunoassays for IgG have been launched, and the recent one is Beckman Coulter. There is a need for detecting both N and S proteins of SARS-CoV-2. As an increasing amount of the population is getting vaccinated with mRNA vaccine, they will develop antibodies against the S protein. Therefore, the Centers for Disease Control and Prevention recommends the use of N protein for detecting anti-SARS-CoV-2 antibodies [49].

Owing to the anticipated nonelectroactivity of antibodies, viral antigens, and nucleic acids, impedance spectroscopy is a detection method of choice over other electrochemical methods. Biosensors for POCT are also needed to detect viral infection in urine, blood, saliva, and stools. POCT must be validated in appropriate populations and settings, a time-consuming and expensive step. Electroanalysis can be combined with complementary methods, mainly microquartz balance and surface plasmon resonance toward the development of more reliable biosensors [50]. Of importance for POCT are the paper-based sensors for nucleic acids [51] and SARS-CoV-2 antibodies [52].

Another important parameter is the dissociation constant (k_D), which can be used to differentiate the binding of SARS-CoV-2 S to human ACE2 ($k_D = 14.7$ nM) over SARS-CoV S ($k_D = 325.8$ nM) [53]. Transplant patients generate T-cell responses but not anti-SARS-CoV-2 antibodies after infection [54]. Therefore, it is of uttermost importance to engineering a multiplex biosensor that can detect both T cells and antibodies [55]. Of further interest is the development of various clustered short palindrome repeats with regular interval—clustered short palindrome repeats with regular interval—associated protein detection platforms for detecting COVID-19 [56–59]. Immunoassays have been miniaturized and adapted for POCT to target antibodies in blood, serum, and plasma [45*, 60, 61] or viral antigens in the nasopharynx [21,62–67]. Rapid PCR methods [68–72] and isothermal amplification [73, 74] for POCT are also commercially available. Apart from clinical diagnostics, the demand for surveillance systems and devices that can detect this virus in the air (aerosols) and water is likely to increase. The vast literature information of electrode

materials, nanomaterials, and sensing methods is available for viral detection.

Conclusions

Current electrochemical biosensors have emerged to target two structural proteins: spike (S) and nucleocapsid (N) with antibodies as the biorecognition molecules, whereas E and M proteins are nonspecific biomarkers for early viral infection. Biorecognition molecules are polyclonal/monoclonal antibodies, aptamers, single-stranded RNA, or DNA oligonucleotides. Considering nucleic acids, biomarkers, antibodies, proteins, and so on are not highly electroactive or nonelectroactive, electrochemical impedance spectroscopy (EIS) is an obvious choice in the development of electrochemical POC sensors. This approach is considered the simplest detection procedure without any bioconjugation of labeling steps. Of course, cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV), and so on can be used if the biorecognition molecule (antibody, aptamer, small organic molecule, and so on) is conjugated with a small electroactive compound, for example, a ferrocene derivative. Biosensors must be able to detect viral infection in different clinical samples and are subject to rigorous validation. The long-term efficacy of vaccines remains unknown considering the levels of antibodies are lower in vaccinated subjects (Pfizer/BioNTech vaccine) with increasing age and the emergence of new coronavirus variants [75]. Some drugs, particularly immunosuppressive medications, also lessen the benefit of vaccination [76]. It is still unknown if the antigen tests work for all SARS-CoV-2 spike variants [77]. Therefore, continuous improvement in diagnostic assays is critical to ensure reliable detection of SARS-CoV-2 infection from different variants [78]. The coronavirus infection produces a large repertoire of cytokines [79], which could be exploited toward the development of POC devices. POCT eliminates the storage and delivery of collected samples to central laboratories as collected samples must be stored at 2–8 °C to preserve sample stability. POC sensing also reduces the risk of sample cross-contamination. As sample transportation might take a few hours or longer, POCT ultimately reduces the turnaround time appreciably, that is, the time duration between sample registrations and reporting of results.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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 article.

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