Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
10

Electrochemical biosensors for detection of SARS-CoV-2

Yang Liu\textsuperscript{a,d}, and Blake N. Johnson\textsuperscript{a,b,c,d}

\textsuperscript{a}Department of Industrial and Systems Engineering, Virginia Tech, Blacksburg, VA, USA, \textsuperscript{b}Department of Materials Science and Engineering, Virginia Tech, Blacksburg, VA, USA, \textsuperscript{c}Department of Chemical Engineering, Virginia Tech, Blacksburg, VA, USA, \textsuperscript{d}Macromolecules Innovation Institute, Virginia Tech, Blacksburg, VA, USA

10.1 Introduction

Pathogen detection is an essential application of electrochemical biosensors \cite{1}. Through the integration of selective biorecognition elements with sensitive transducers, electrochemical biosensors have enabled the rapid, sensitive, and selective detection of viruses. While various studies have achieved impressive detection limits, in some cases a single virus or tens to hundreds of viral RNA molecules, the developed approaches for electrochemical detection of virus particles significantly vary in regard to device and measurement approach, such as the electrode, biorecognition element, electrochemical method utilized for transduction of target binding, and measurement format utilized (e.g., sample collection, preparation, and handling protocols). Thus, the reagents, materials, and measurement approach must be carefully considered to accurately assess the utility and time-to-results (TTR) for a given electrochemical biosensor-based assay for pathogen detection in a pandemic setting.

Since the beginning of the ongoing COVID-19 pandemic, several studies have examined the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using electrochemical biosensors. While
polymerase chain reaction (PCR)-based assays are the gold standard for SARS-CoV-2 detection (i.e., SARS-CoV-2 antigen and antibody testing), such assays require trained analysts, PCR analyzers, various reagents, and sample preparation and handling steps. Thus, PCR-based assays typically exhibit TTR near 2-4 hours because of a combination of sample preparation and detection time (i.e., the time to prepare the sample vs. the time dedicated to binding of the target analyte to the sensor and the associated electrochemical transduction process used for detection). The clinical and public demand for rapid assays for SARS-CoV-2 antigen and antibody testing as well as mobile real-time screening platforms has led to the investigation of various biosensors for SARS-CoV-2 detection. Among these, electrochemical biosensors have received considerable attention given their synergy with low-cost functional materials for transducer fabrication, fabrication processes, and readout systems, such as miniature impedance analyzers. While all assays for pathogen detection should exhibit high selectivity and low probabilities of false negative and positive results, the demand for safe, user-friendly, and rapid biosensor-based assays for pandemic management significantly constrains the design and measurement format associated with typical biosensors. In particular, it places significant weight on the cost, reliability, simplicity, and safety of the device and measurement approach.

The development of low-cost robust electrochemical biosensors for pandemic management will require investment in research and development. An effective biosensor for use in pandemic management should exhibit a highly stable and selective biorecognition element, safe and user-friendly measurement formats (e.g., sample preparation-free formats), and mobile data acquisition and readout platforms, such as those based on smartphones and miniature analyzers. While it may be possible for experienced analysts and researchers to establish the proof of concept for pathogen detection using electrochemical biosensors in controlled research settings, such as the various molecular targets associated with SARS-CoV-2 antigen and antibody testing, there are various challenges associated with creating robust, low-cost commercial biosensors for pandemic management.

Given their potential for mass production, commercialization, and implementation in mobile, low-cost measurement formats, here, we discuss recent developments in the application of electrochemical biosensors for detection of SARS-CoV-2 (i.e., electrochemical biosensor-based assays for SARS-CoV-2 antigen and antibody testing). In addition to highlighting various electrochemical biosensors that have enabled the detection of SARS-CoV-2, we highlight advances in biosensor design and measurement formats for use in point-of-care and field-based settings. We also highlight emerging areas in the field of electrochemical biosensors for pandemic management and future challenges and directions in applications to SARS-CoV-2 rapid antigen and antibody testing.
COVID-19 disease is caused by SARS-CoV-2 infection. SARS-CoV-2 is a positive-sense single-stranded coronavirus that exhibits structural and molecular characteristics similar to SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). Thus, the target species associated with SARS-CoV-2 antigen detection (testing) includes the active or inactivated virus, protein-containing viral fragments, or viral RNA. In addition, the detection of SARS-CoV-2 antibody serves as a critical target of interest for antibody testing applications.

Electrochemical biosensors can be classified as biocatalytic or bio-complexing in nature, depending on the type of biorecognition element utilized. A comprehensive review of pathogen detection using electrochemical biosensors can be found elsewhere [1]. As shown in Table 10.1, electrochemical biosensors for SARS-CoV-2 antigen and antibody detection have been primarily based on biocomplexing reactions with immobilized antibodies or single-stranded DNA probes, which are highly selective biorecognition elements. Thus, the majority of electrochemical biosensor-based assays for SARS-CoV-2 detection can be broadly classified as antibody- or DNA-based assays.

As shown in Fig. 10.1 and Table 10.1, SARS-CoV-2 (antigens) and SARS-CoV-2 antibodies have been detected through the recognition of several target species using a variety of transducers, biorecognition elements, and measurement formats [2–27]. For example, the SARS-CoV-2 spike (S) protein, nucleocapsid (N) protein, and glycoproteins have served as the target species for several antibody-based electrochemical biosensing applications to SARS-CoV-2 antigen testing. Monoclonal and polyclonal antibodies against the aforementioned protein targets have been the most commonly used biorecognition element. Monoclonal antibodies exhibit several advantages, including high reproducibility and specificity but may be vulnerable to change of the epitope, such as via S protein mutation. Thus, tracking changes associated with the genome of SARS-CoV-2 is also a critical aspect of developing rapid and selective assays for SARS-CoV-2 detection in addition to vaccine development. For example, the spike protein, a common target of electrochemical biosensor-based assays for SARS-CoV-2 detection has mutated since the onset of the COVID-19 pandemic [9]. Alternatively, polyclonal antibodies are relatively less expensive, exhibit relatively shorter production time and higher stability, and can identify multiple epitopes of a target. However, polyclonal antibodies may exhibit relatively increased batch-to-batch variability. Thus, monoclonal and polyclonal antibodies exhibit advantages and disadvantages as biorecognition elements for use in electrochemical biosensor-based SARS-CoV-2 screening technologies for rapid antigen and antibody testing.

Given their use as targets for PCR-based SARS-CoV-2 antigen testing assays, which remain the gold standard for COVID-19 diagnostics, the S
| Target species                  | Sample type                          | Working electrode               | Biorecognition element                  | Electrochemical method | Limit of detection          | Ref. |
|--------------------------------|--------------------------------------|---------------------------------|----------------------------------------|------------------------|-----------------------------|------|
| SARS-CoV-2 RNA                 | Cell lysate                          | Au electrode                    | CRISPR-Cas9                            | SWV                    | N/A                         | [5]  |
| SARS-CoV-2                     | Transport medium and human cells      | Perfluorocarbon SAM-modified Au electrode | Angiotensin converting enzyme 2        | EIS                    | 37.8 dC/mL                  | [23] |
| SARS-CoV-2                     | Saliva                               | Screen printed carbon electrode | SARS-CoV-2 monoclonal antibody         | DPV, CV                | 90 fM                       | [14] |
| SARS-CoV-2 N gene              | Nasal swab; saliva                   | Graphene-based Au electrode     | Antisense oligonucleotides             | N / A                  | 6.9 copies/μL               | [2]  |
| SARS-CoV-2                     | Nasal swab                           | Graphene-based Au/Cr electrode  | SARS-CoV-2 S protein antibody          | FET                    | 2.42 × 10^2 copies/mL (clinical sample) | [19] |
| N protein, immunoglobulins against SARS-CoV-2 S protein (SI) (S1-IgM and S1-IgG); C-reactive protein (CRP) | Blood; saliva | Graphene electrode               | N protein monoclonal antibody; CRP monoclonal antibody; CRP polyclonal antibody; S protein-RBD monoclonal antibody | AMP | N/A | [21] |
| SARS-CoV-2 S protein           | Saliva                               | Au electrode                    | Anti-S protein antibody                 | CA                     | N/A                         | [25] |
| SARS-CoV-2 S protein           | Saliva                               | Shrinky-Dink wrinkled Au electrodes | Aptamer                               | N / A                  | 1 ag/mL (S1 protein)       | [26] |
| SARS-CoV-2                     | Nasal swab                           | Carbon nanofiber-modified screen-printed carbon electrodes | Anti-N protein antibody                | SWV                    | 0.8 pg/mL                   | [6]  |
| SARS-CoV-2 N protein | Nasal swab | Poly-m-phenylenediamine (PmPD) modified Au-based thin-film electrodes | N protein imprinted PmPD | DPV | 15 fM | [18]  
|----------------------|------------|-------------------------------------------------|----------------------|-----|-------|-------  
| SARS-CoV-2 S protein | Nasal swab; saliva | Cu2O nanocubes modified screen printed carbon electrode | Anti-S protein monoclonal antibody | CV, EIS | 0.04 fg/mL | [16]  
| SARS-CoV-2 antibody | Serum | ZnO nanowire functionalized paper-based carbon electrode | SARS-CoV-2 S protein receptor-binding domain | EIS | N/A | [12]  
| SARS-CoV-2 S and N proteins | Saliva | Carbon black-based screen-printed electrode | Monoclonal anti-N protein antibody; polyclonal anti-N protein antibody; Monoclonal anti-S protein antibody; polyclonal anti-S protein antibody | DPV | 19 ng/mL (S protein); 8 ng/mL (N protein) | [7]  
| SARS-CoV-2 N protein | Serum | Screen-printed Au electrode | Anti-SARS-CoV-2 monoclonal N protein antibody | CA | 50 pg/mL | [11]  
| SARS-CoV-2 antibody | Serum | Graphene oxide modified graphene electrode | S protein receptor-binding domain | SWV | 1 ng/mL | [24]  
| SARS-CoV-2 antibody | Serum | Au electrode | S protein receptor-binding domain | EIS | N/A | [17]  

(continued on next page)
TABLE 10.1 Classification of electrochemical biosensors for detection of SARS-CoV-2 in terms of target species, sample type, working electrode, biorecognition element, electrochemical method, and limit of detection—cont’d

| Target species          | Sample type       | Working electrode                                      | Biorecognition element                                      | Electrochemical method | Limit of detection                                      | Ref.  |
|-------------------------|-------------------|--------------------------------------------------------|-------------------------------------------------------------|------------------------|---------------------------------------------------------|-------|
| SARS-CoV-2 S protein;   | Solution          | Graphene electrode                                     | Anti-S protein antibody                                     | SWV                    | 20 μg/mL (S protein), 5.5 × 10⁷ PFU/mL (SARS-CoV-2)     | [15]  |
| SARS-CoV-2 S S1 antibody; SARS-CoV-2 S RBD antibody | Solution          | Reduced graphene oxide-coated Au micropillar array     | SARS-CoV-2 S protein RBD-His protein; SARS-CoV-2 S protein S1-His protein | EIS                    | 2.8 × 10⁻¹⁵ M (spike protein), 16.9 × 10⁻¹⁵ M (spike protein RBD) | [3]   |
| SARS-CoV-2 RNA          | N/A               | Screen printed carbon electrode                        | ssDNA capture probe to ORF1ab                               | DPV                    | 200 copies/mL                                           | [27]  |
| SARS-CoV-2 glycoprotein | Nasal swab; saliva; blood | Glassy carbon electrode                                 | Graphene oxide with sensitive chemical compounds along with Au nanostars | DPV                    | 1.68 × 10⁻²² μg/mL                                      | [8]   |
| SARS-CoV-2 S protein    | Saliva            | MXene–graphene                                          | S protein monoclonal antibody                               | FET                    | 1 fg/mL                                                 | [13]  |
| SARS-CoV-2 S and N proteins | Nasal swab      | Single-walled carbon nanotube                           | S protein polyclonal antibody; N protein polyclonal antibody | FET                    | 0.55 fg/mL (spike antigen), 0.016 fg/mL (nucleocapsid antigen) | [20]  |
| MERS-CoV                | N/A               | AuNPs on carbon electrode                              | MERS-CoV antigen-antibody complex                           | SWV; Fe(CN)₆³⁻/⁴⁻; MERS CoV-antibody complex              | 400 fg/mL                                               | [10]  |
| SARS-CoV-2 S and N genes | Nasal swab       | Screen-printed carbon electrodes                        | S/N gene specific ssDNA probe                               | DPV; RCA               | 1 copy/μL                                               | [4]   |

Abbreviations: EIS, electrochemical impedance spectroscopy; CV, cyclic voltammetry; SWV, square wave voltammetry; DPV, differential pulse voltammetry; CA, chronoamperometry; FET, field-effect transistor; RCA, rolling circle amplification; Au, gold; AMP, amperometry.
and N genes of SARS-CoV-2 have been utilized to develop selective single-stranded DNA (ssDNA) probes for electrochemical biosensor-based detection of SARS-CoV-2. A discussion of ssDNA probe design is beyond the scope of this chapter. Commercially available software now exists for probe design and optimization. In addition to antibodies and ssDNA, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology has recently received attention in the biosensing field as a novel biorecognition element, particularly for nucleic acid sensing applications. We direct the interested reader to various recently published reviews of CRISPR-based biosensors. As shown in Table 10.1, Dai et al. recently designed a CRISPR-based heterogeneous biochemical circuit, which enabled the detection of SARS-CoV-2 genome fragments via electrochemistry [5]. In that study, the target gene fragments were first specifically recognized and transformed by a pair of CRISPR/Cas9 D10A nucleases. The obtained strand structure was then translated into an arbitrary output and amplified into a concatemer via a primer exchange reaction mediated circuit wiring. The output of the heterogeneous biochemical circuit was examined by an electrochemical biosensing platform. The integrated platform was applied for SARS-CoV-2 genome analysis in human cell lysate.

As shown in Table 10.1, various working electrodes and electrode formats have been utilized for the electrochemical detection of SARS-CoV-2. Similar to recent trends in pathogen detection using electrochemical biosensors [1], the majority of electrochemical biosensor-based assays for SARS-CoV-2 antigen and antibody testing have examined planar and nano-structured and -functionalized (Au) electrodes. In particular,
graphene-functionalized electrodes have been used in several studies (see Table 10.1). One advantage of graphene is its ability to be integrated with flexible substrates, such as paper and polyimide films [21,24]. Another advantage is the availability of facile bioconjugation techniques for immobilization of biorecognition elements. Several methods exist for protein immobilization on graphene, which could be a SARS-CoV-2 antibody or antigen depending on the application. One method uses 1-pyrenebutyric acid N-hydroxysuccinimide ester or 1-pyrenebutyric acid as linker [15,19,21]. The pyrene group, which contains π-electrons, adsorbs to graphene allowing the carboxylic/ester group to react with available functional groups on the protein. Well-established EDC/NHS chemistry can also be utilized to immobilize proteins on graphene oxide or reduced graphene oxide [3,24]. Li et al. also showed that a method based on MXene and APTES could achieve S protein antibody immobilization on graphene [13].

As shown in Table 10.1, several graphene-based electrochemical biosensors have achieved sensitive detection of SARS-CoV-2 using various biorecognition elements and electrochemical methods. For example, Seo et al. immobilized S protein antibody on a graphene-based field effect transistor (FET), which enabled the detection of SARS-CoV-2 viral RNA in clinical samples with a limit-of-detection (LOD) of $2.42 \times 10^3$ copies/mL [19]. In another study, Ali et al. fabricated graphene oxide-functionalized aerosol jet nano-printed 3D electrodes for SARS-CoV-2 detection [3]. The sensor-enabled SARS-CoV-2 S1 protein detection at a LOD of 2.8 fM. In addition to the S protein antibody and antigen, specific antisense oligonucleotides targeting the viral N gene were also used for detection of SARS-CoV-2 viral RNA of 6.9 copies/μL using a graphene-based electrochemical biosensor chip.

### 10.2 Future directions

#### 10.2.1 Rapid and sample preparation-free assays

While Table 10.1 shows that various studies have examined the detection of SARS-CoV-2 antigens and antibodies, the target species often required sample preparation, such as extraction and amplification prior to, and sometimes during, detection. Sample preparation steps were most commonly reported in nucleic acid-based SARS-CoV-2 biosensing applications. While such approaches may provide sensitive and robust assays when performed in controlled laboratory settings by experienced analysts, they present a number of challenges for field and public use. For example, sample handling should be minimized to prevent cross-contamination. Further, the reagents associated with amplification reactions exhibit stability concerns and may impose challenging handling and storage requirements on end users. In contrast, as shown
in Table 10.2, several recently developed antibody-based electrochemical biosensor assays for SARS-CoV-2 antigen and antibody detection exhibit sample preparation-free formats. Ultimately, it is desirable to establish low-cost electrochemical biosensors for SARS-CoV-2 antigen and antibody testing that exhibit sample preparation-free measurement formats. It is desirable to avoid sample preparation steps within assays as sample preparation can increase biosafety hazards, TTR, the potential for false results, and assay cost. A list of ‘rapid’ electrochemical biosensor-based assays for SARS-CoV-2 antigen and antibody testing is provided in Table 10.2. Although the assays in Table 10.2 were classified as ‘rapid’ given the reported detection time was less than two hours (i.e., the time for target binding to be transduced to a point that the concentration can be identified or quantified), their actual TTR may be significantly increased based on sample preparation requirements, which must be well understood for each assay. In the assessment of electrochemical biosensors for SARS-CoV-2 antigen and antibody testing, we recommend that one should also consider the sample matrix (e.g., type of body fluid), the sample collection method, and the required sample volume, all of which also impact the environmental safety hazards associated with the assay. We recommend that these aspects of biosensor-based assays be described in future studies related to pathogen detection.

10.2.2 Mobile- and smartphone-based measurement platforms

In addition to creating safe, reliable, and user-friendly electrochemical biosensor-based assays for SARS-CoV-2 antigen and antibody detection, there remains a demand for mobile screening platforms. Zhao et al. recently established a smartphone-based electrochemical sensor for SARS-CoV-2 antigen testing [27]. In that study, a SARS-CoV-2 S protein RBD His protein-functionalized reduced-graphene-oxide-coated Au micropillar array electrode was interfaced with a smartphone. The smartphone-based platform enabled detection of SARS-CoV-2 antibody via electrochemical impedance spectroscopy [27].

10.2.3 Mass production of biosensors – Considerations in biosensor design and packaging

While mobile electrochemical biosensing platforms are now emerging for SARS-CoV-2 antigen and antibody testing [27], additional research is required to understand the stability of biorecognition elements used in field-based mobile biosensing applications. Creating highly stable biosensors for field, home, or clinical use is a multi-faceted challenge that will require innovative solutions in electrode and biorecognition layer design, biorecognition element engineering, packaging, and perhaps even
| Target species                                                                 | Sample collection method and type | Working electrode                                      | Biorecognition element                                      | Electrochemical method | Detection time | Limit of detection  |
|--------------------------------------------------------------------------------|----------------------------------|-------------------------------------------------------|-------------------------------------------------------------|------------------------|----------------|---------------------|
| S-RBD protein                                                                  | Protein solution                  | Cobalt-functionalized TiO2 nanotubes                  | Cobalt-functionalized TiO2 nanotubes                        | AMP                    | ~30 s           | 0.7 nM              |
| SARS-CoV-2 virus                                                               | Saliva spiked with Covid-19       | Screen printed carbon electrode                       | SARS-CoV-2 monoclonal antibody                              | DPV, CV                | 10 - 30 s       | 90 fM               |
| SARS-CoV-2 N-Gene                                                              | Nasal swab; saliva                | Graphene-based Au electrode                           | Antisense oligonucleotides                                 | N/A                    | < 5 m           | 6.9 copies/μL       |
| SARS-CoV-2                                                                     | Nasal swab                        | Graphene-based Au/Cr electrode                        | SARS-CoV-2 S protein antibody                               | FET                    | < 10 m          | 2.42 × 10² copies/mL (clinical sample) |
| N protein, IGs against SARS-CoV-2 S protein (S1) (S1-IgM and S1-IgG); C-reactive protein (CRP) | Blood; saliva                     | Graphene electrode                                    | N protein monoclonal antibody; CRP monoclonal antibody; CRP polyclonal antibody; anti-S protein RBD monoclonal antibody | AMP                    | 1 min           | N/A                 |
| SARS-CoV-2 S protein                                                           | Saliva                            | Au coating                                            | Anti-S protein antibody                                     | CA                     | 5 min           | N/A                 |
| SARS-CoV-2 S protein                                                           | Nasal swab, saliva                | Cu₂O nanocubes modified screen printed carbon electrode| Anti-S protein monoclonal antibody                           | CV, EIS                | 20 min          | 0.04 fg/mL         |

(continued on next page)
| Test System                                           | Matrix      | Electrode/Carbon Source                                      | Analyte/Target                                      | Technique  | Incubation Time | Detection Limit/N/A          | Reference |
|------------------------------------------------------|-------------|----------------------------------------------------------------|-----------------------------------------------------|------------|-----------------|-------------------------------|-----------|
| SARS-CoV-2 antibody                                  | Serum       | ZnO nanowire functionalized carbon electrode                  | SARS-CoV-2 S protein receptor-binding domain         | EIS        | 30 min          | N/A                           | [12]      |
| SARS-CoV-2 N protein                                 | Serum       | Screen-printed Au electrode                                   | Anti-SARS-CoV-2 monoclonal N protein antibody        | CA         | < 1 h            | 50 pg/mL                      | [11]      |
| S protein; SARS CoV-2                               | Solution    | Graphene electrode                                             | Anti-S protein antibody                              | SWV        | 45 min           | 20 μg/mL (S protein); 5.5 × 10⁵ PFU/mL (SARS-CoV-2) | [15]      |
| SARS-CoV-2 S1 antibody; SARS-CoV-2 Spike RBD antibody| Solution    | Reduced graphene oxide coated Au micropillar array            | SARS-CoV-2 S protein RBD-His protein; SARS-CoV-2 S protein S1-His protein | EIS        | Seconds         | 2.8 × 10⁻¹⁵ M (spike protein); 16.9 × 10⁻¹⁵ M (spike protein RBD) | [3]       |
| SARS-CoV-2 S protein                                 | Saliva      | MXene−graphene − graphene − graphene array                    | S protein monoclonal antibody                        | FET        | ~ 50 ms          | 1 fg/mL                       | [13]      |
| SARS-CoV-2 S protein; N protein                      | Nasal swab  | Single-walled carbon nanotube                                  | S protein polyclonal antibody, N protein polyclonal antibody | FET        | 2 min            | 0.55 fg/mL (spike antigen); 0.016 fg/mL (nucleocapsid antigen) | [20]      |
| SARS-CoV-2 S gene; SARS-CoV-2 N gene                 | Nasal swab  | Screen-printed carbon electrodes                               | S/N gene specific ssDNA probe                       | DPV        | < 2 h            | 1 copy/μL                     | [4]       |

Abbreviations: EIS, electrochemical impedance spectroscopy; CV, cyclic voltammetry; Au, gold; AMP, amperometry.
machine learning and artificial intelligence. For example, while ssDNA has enabled selective detection of SARS-CoV-2 via viral RNA, it may be advantageous to consider probes based on alternative oligonucleotide chemistry, such as probes that employ locked nucleic acids or peptide nucleic acids. In addition to considering alternative biorecognition elements, it may be useful to consider materials-based biorecognition technology, such as molecularly-imprinted polymers, as opposed to molecular biorecognition elements (e.g., antibodies, ssDNA, and enzymes). Raziq et al. recently used a nucleocapsid-imprinted poly-m-phenylenediamine (PmPD) biorecognition layer on Au thin-film electrodes for detection of SARS-CoV-2 N protein with a detection limit of 15 fM [18].

10.3 Conclusions

Here, we summarize recent progress in SARS-CoV-2 antigen and antibody detection using electrochemical biosensors. A comprehensive analysis of studies reported since the beginning of the COVID-19 pandemic was provided in terms of transducer design, biorecognition element, electrochemical method, and measurement format. Critical aspects of biosensor and assay design and performance characteristics for pandemic management applications are highlighted including rapid, sample preparation-free, and mobile measurement formats.

References

[1] E. Cesewski, B.N. Johnson, Electrochemical biosensors for pathogen detection, Biosens. Bioelectron. 159 (2020) 112214. http://doi.org/10.1016/j.bios.2020.112214.
[2] M. Alafeef, K. Dighe, P. Moitra, D. Pan, Rapid, ultrasensitive, and quantitative detection of SARS-CoV-2 using antisense oligonucleotides directed electrochemical biosensor chip, ACS Nano 14 (12) (2020) 17028–17045. http://doi.org/10.1021/acsnano.0c06392.
[3] M.A. Ali, C. Hu, S. Jahan, B. Yuan, M.S. Saleh, E. Ju, et al., Sensing of COVID-19 antibodies in seconds via aerosol jet nanoprinted reduced-graphene-oxide-coated 3D electrodes, Adv. Mater. 33 (7) (2021). http://doi.org/10.1002/adma.202006647.
[4] T. Chaibun, J. Puenpa, T. Ngamdee, N. Boonapatcharoen, P. Athamanolap, A.P. O’Mullane, et al., Rapid electrochemical detection of coronavirus SARS-CoV-2, Nat. Commun. 12 (1) (2021). http://doi.org/10.1038/s41467-021-21121-7.
[5] Y. Dai, W. Xu, R.A. Somoza, J.F. Welter, A.I. Caplan, C.C. Liu, An Integrated multifunction heterogeneous biochemical circuit for high-resolution electrochemistry-based genetic analysis, Angewandte Chemie - International Edition 59 (46) (2020) 20545–20551. http://doi.org/10.1002/anie.202010648.
[6] S. Eissa, M. Zourob, Development of a low-cost cotton-tipped electrochemical immunosensor for the detection of SARS-CoV-2, Anal. Chem. 93 (3) (2021) 1826–1833. http://doi.org/10.1021/acs.analchem.0c04719.
[7] L. Fabiani, M. Saroglia, G. Galatà, et al., Magnetic beads combined with carbon black-based screen-printed electrodes for COVID-19: A reliable and miniaturized electrochemical immunosensor for SARS-CoV-2 detection in saliva, Biosens. Bioelectron. 171 (2021). http://doi.org/10.1016/j.bios.2020.112686.
[8] S.A. Hashemi, N.G. Golab Behbahan, S. Bahrani, et al., Ultra-sensitive viral glycoprotein detection NanoSystem toward accurate tracing SARS-CoV-2 in biological/non-biological media, Biosens. Bioelectron. 171 (2021) 112731. http://doi.org/10.1016/j.bios.2020.112731.

[9] Korber, B., Fischer, W. M., & Gnanakaran (2020). et al. Tracking changes in SARS-CoV;182:812–827.

[10] L.A. Layqah, S. Eissa, An electrochemical immunosensor for the corona virus associated with the Middle East respiratory syndrome using an array of gold nanoparticle-modified carbon electrodes, Microchim. Acta 186 (4) (2019) 224. http://doi.org/10.1007/s00604-019-3345-5.

[11] J. Li, P.B. Lillehøj, Microfluidic magneto immunosensor for rapid, high sensitivity measurements of SARS-CoV-2 nucleocapsid protein in serum, ACS Sensors 6 (2021) 1270–1278. http://doi.org/10.1021/acssensors.0c02561.

[12] X. Li, Z. Qin, H. Fu, R. Peng, Z. Li, J.M. Rini, et al., Enhancing the performance of paper-based electrochemical impedance spectroscopy nanobiosensors: An experimental approach, Biosens. Bioelectron. 177 (2021) 112672. http://doi.org/10.1016/j.bios.2020.112672.

[13] Y. Li, Z. Peng, N.J. Holl, et al., MXene-Graphene Field-Effect Transistor Sensing of Influenza Virus and SARS-CoV-2, ACS Omega (2021). http://doi.org/10.1021/acsomega.0c05421.

[14] Mahari, S., Roberts, A., Shahdeo, D., & Gandhi, S. (2004)eCovSens-ultrasensitive novel in-house built printed circuit board based electrochemical device for rapid detection of nCovid-19 antigen, a spike protein domain 1 of SARS-CoV-2. bioRxiv;.

[15] B. Mojsoska, S. Larsen, D.A. Olsen, J.S. Madsen, I. Brandslund, F.A. Alatraktchi, Rapid SARS-CoV-2 detection using electrochemical immunosensor, Sensors (Switzerland) 21 (2021) 1–11. http://doi.org/10.3390/s21020390.

[16] Z. Rahmati, M. Roushani, H. Hosseini, H. Choobin, Electrochemical immunosensor with Cu2O nanocube coating for detection of SARS-CoV-2 spike protein, Microchim. Acta 188 (3) (2021) 105. http://doi.org/10.1007/s00604-021-04762-9.

[17] M.Z. Rashed, J.A. Kopechek, M.C. Priddy, K.T. Hamorsky, K.E. Palmer, N. Mittal, et al., Rapid detection of SARS-CoV-2 antibodies using electrochemical impedance-based detector, Biosens. Bioelectron. 171 (2021) 112709. http://doi.org/10.1016/j.bios.2020.112709.

[18] A. Raziq, A. Kidakova, R. Boroznjak, J. Reut, A. Öpik, V. Syritski, Development of a portable MIP-based electrochemical sensor for detection of SARS-CoV-2 antigen, Biosens. Bioelectron. 178 (2021) 113029. http://doi.org/10.1016/j.bios.2021.113029.

[19] G. Seo, G. Lee, M.J. Kim, S.-H. Baek, M. Choi, K.B. Ku, et al., Rapid Detection of COVID-19 Causative Virus (SARS-CoV-2) in Human Nasopharyngeal Swab Specimens Using Field-Effect Transistor-Based Biosensor, ACS Nano 14 (4) (2020) 5135–5142. http://doi.org/10.1021/acsnano.0c02823.

[20] W. Shao, M.R. Shurin, S.E. Wheeler, X. He, A. Star, Rapid detection of SARS-CoV-2 antigens using high-purity semiconducting single-walled carbon nanotube-based field-effect transistors, ACS Appl. Mater. Interfaces 13 (2021) 10321–10327. http://doi.org/10.1021/acsami.0c22589.

[21] R.M. Torrente-Rodríguez, H. Lukas, J. Tu, J. Min, Y. Yang, C. Xu, et al., SARS-CoV-2 RapidPlex: A graphene-based multiplexed telemedicine platform for rapid and low-cost COVID-19, Diagnosis and Monitoring Matter 3 (2020) 1981–1998.

[22] B.S. Vadamani, T. Uppal, S.C. Verma, M. Misra, Functionalized tio2 nanotube-based electrochemical biosensor for rapid detection of SARS-CoV-2, Sensors (Switzerland) 20 (20) (2020) 1–10. http://doi.org/10.3390/s20205871.

[23] Vezza, V. J., Butterworth, A., & Lasserre, P. et al. (2020). An uncomplicated electrochemical sensor combining a perfluorocarbon SAM and ACE2 as the bio-recognition element
to sensitively and specifically detect SARS-CoV-2 in complex samples. ChemRxiv. doi: http://doi.org/10.26434/chemrxiv.13416272.v1

[24] A. Yakoh, U. Pimpitak, S. Rengpipat, N. Hirankarn, O. Chailapakul, S. Chaiyo, Paper-based electrochemical biosensor for diagnosing COVID-19: Detection of SARS-CoV-2 antibodies and antigen, Biosens. Bioelectron. 176 (2021) 112912. http://doi.org/10.1016/j.bios.2020.112912.

[25] H. Yousefi, A. Mahmud, D. Chang, J. Das, S. Gomis, J.B. Chen, et al., Detection of SARS-CoV-2 viral particles using direct, reagent-free electrochemical sensing, J. Am. Chem. Soc. 143 (4) (2021) 1722–1727. http://doi.org/10.1021/jacs.0c10810.

[26] J.A. Zakashansky, A.H. Imamura, D.F. Salgado, H.C.R. Mercieca, R.F.L. Aguas, A.M. Lao, et al., Detection of the SARS-CoV-2 spike protein in saliva with Shrinky-Dink© electrodes, Anal. Methods 13 (7) (2021) 874–883. http://doi.org/10.1039/d1ay00041a.

[27] H. Zhao, F. Liu, W. Xie, T.-C. Zhou, J. OuYang, L. Jin, et al., Ultrasensitive supersandwich-type electrochemical sensor for SARS-CoV-2 from the infected COVID-19 patients using a smartphone, Sens. Actuators B 327 (2021). http://doi.org/10.1016/j.snb.2020.128899.
Abstract
Pathogen detection is an essential application of electrochemical biosensors. Through the integration of selective biorecognition elements with sensitive transducers, electrochemical biosensors have enabled the rapid, sensitive, and selective detection of viruses. While various studies have achieved impressive detection limits, in some cases a single virus or tens to hundreds of viral RNA molecules, the developed approaches for electrochemical detection of virus particles significantly vary in regard to device and measurement approach, such as the electrode, biorecognition element, electrochemical method utilized for transduction of target binding, and measurement format (e.g., sample collection, preparation, and handling protocols). Thus, the reagents, materials, and measurement approach must be carefully considered to accurately assess the utility and time-to-results (TTR) for a given electrochemical biosensor-based assay for pathogen detection in a pandemic setting.

Keywords
Biorecognition; Biosensor; COVID-19; SARS-CoV-2; Pathogen