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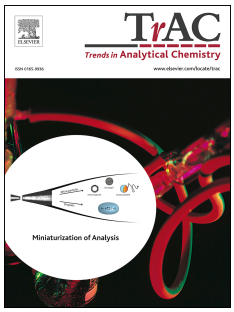

Advancements in electrochemical biosensing for respiratory virus detection: A review

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Advancements in electrochemical biosensing for respiratory virus detection: A review

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Abstract

Respiratory viruses are real menace for human health which result in devastating epidemic disease. Consequently, it is in urgent need of identifying and quantifying virus with a rapid, sensitive and precise approach. The study of electrochemical biosensors for respiratory virus detection has become one of the most rapidly developing scientific fields. Recent developments in electrochemical biosensors concerning respiratory virus detection are comprehensively reviewed in this paper. This review is structured along common detecting objects of respiratory viruses, electrochemical biosensors, electrochemical biosensors for respiratory virus detection and future challengesThe electrochemical biosensors for respiratory virus detection are introduced, including nucleic acids-based, immunosensors and other affinity biosensors. Lastly, for Coronavirus disease 2019 (COVID-19) diagnosis, the future challenges regarding developing electrochemical biosensor-based Point-of-Care Tests (POCTs) are summarized. This review is expected to provide a helpful guide for the researchers entering this interdisciplinary field and developing more novel electrochemical biosensors for respiratory virus detection.

Keywords: *Electrochemical; Biosensors; Respiratory viruses; Virus detection; COVID-19; SARS-CoV-2;*

Abbreviations

ACE2 Angiotensin-converting enzyme II
ALP Alkaline phosphatase
AP Auxiliary probe
APP 4-amino phenyl phosphate
Au NPs Gold nanoparticles
BDD Boron-doped diamond
bi-FMNs bifunctional fluorescence magnetic nanospheres
CNTs Carbon nanotubes
COVID-19 Coronavirus disease 2019
CP Capture probe
CV Cyclic voltammetry
DPV Differential pulse voltammetry
dsDNA Double strand DNA
E Envelope
EDOT 3,4-ethylenedioxythiophene
EIS Electrochemical impedance spectroscopy
ELISA Enzyme-linked immuno sorbent assay
Fab Fragment-antigen binding
FDA Food and Drug Administration
GO Graphene oxide
HA Hemagglutinin
HAU Hemagglutination unit
HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IC Immunochromatography
IFA immunofluorescence assay
IgG Immunoglobulin G
IgM Immunoglobulin M
ITO Indium tin oxide
K_D Dissociation constants
LFIA Lateral Flow Immunoassay
LOD Limit of detection
LP Label probe
M Membrane
M2 Matrix protein 2
mAb Monoclonal antibodies
MCH 6-mercapto-1-hexanol
MERS-CoV Middle East Respiratory Syndrome coronavirus
MNPs Magnetic nanoparticles
N Nucleocapsid
NA Neuraminidase
ORF Open reading frame
pAb Polyclonal antibodies
p-AP P-aminophenol

p-APP P-aminophenyl phosphate monohydrate
PCR Polymerase chain reaction
PDMS Polydimethylsiloxane
PNA Peanut agglutinin
POCTs Point-of-Care Tests
RBD Receptor binding domain
RGO Reduced graphene oxide
RSV Respiratory Syncytial Viral
RT–PCR Reverse transcription–polymerase chain reaction
S Spike
SAM Self-assembled monolayer
SARS-CoV Severe Acute Respiratory Syndrome coronavirus
SARS-CoV-2 Severe Acute Respiratory Syndrome coronavirus 2
scFv Single-chain Fv fragments
SELEX Systematic Evolution of Ligands by Exponential Enrichment
SERS Surface Enhanced Raman Scattering
SPCE Screen-printed carbon electrode
SPEs Screen-printed electrodes
SPR Surface Plasmon Resonance
ssDNA Single-stranded DNA
SWV Square wave voltammetry
upE RNA upstream of the E gene
UTR Untranslated Regions
WHO World Health Organization

1. Introduction

Respiratory viruses, well-known as influenza virus and coronavirus, usually result in viral respiratory infections through contact as well as airborne transmission [1]. The infected individuals generally present fever, dry cough, fatigue, sputum production and loss of smell, such acute respiratory virus illnesses symptoms. Though sounds like a mild cold, acute respiratory disease caused by respiratory viruses have brought death and pandemics over the past years [2-3]. Only Respiratory Syncytial Viral (RSV) could lead to 14,000 deaths among adults older than 65 years every year in the US [4]. Currently, Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) is the responsible culprits of the Coronavirus disease 2019 (COVID-19) pandemic. According to the data collected from the World Health Organization (WHO), there are totally over 6284,000 cases and 1465,000 death in 220 countries, areas or territories by 2 December 2020 [5]. The prevention and control have been taken depending on the features of the spread of the respiratory viruses, such as wearing the P2/N95 masks to prevent airborne spread, cleaning contaminated surfaces to avoid risky contact [6]. However, owing to the non-specific and comprehensive symptoms among the respiratory viruses and the silent transmission from positive asymptomatic, early accurate diagnosis and isolation of patients remain to be crucial for controlling the pandemic resulted by the respiratory viruses [7]. Thus, respiratory virus detection would be particularly decisive.

Conventional methods for respiratory virus detection are mostly based on lab-based techniques. From initial virus cultures, morphological observation, and serological tests to subsequent reverse transcription–polymerase chain reaction (RT–PCR) [8], isothermal amplification techniques [9], immunochromatography (IC) [10], enzyme-linked immunosorbent assay (ELISA) or an immunofluorescence assay (IFA) [11] and classical diagnostic methods have helped physicians to distinguish the causative agents with accuracy. Although, in clinical practice, cumbersome sample-preparation, high cost, professional operators and time-consuming equally become the drawbacks of most classical lab-based techniques [12]. There is still a demand to exploit rapid, simple, cheap assays with precision on respiratory virus detection. Biosensors, cooperating the bio-recognition elements with the sensor system, are capable of recognizing the targets with high sensitivity and selectivity [13]. Biosensors have arisen in numerous areas, including environment monitoring, food safety, drug control, disease diagnosis and so on [14]. Among them, many optical based techniques are proposed for virus detection such as Surface Plasmon Resonance (SPR) [15], Lateral Flow Immunoassay (LFIA) [16], Surface Enhanced Raman Scattering (SERS) [17].

Electrochemical biosensors have aroused burgeoning attention because of intrinsic strengths: simplicity, rapid response, flexibility, miniaturized instrumentation, excellent sensitivity and low cost [18], which have been emerging alternative tools for the quantitative or semi-quantitative analyzing respiratory viruses. Excellent reviews are accessible in the literature about the state-of-art of electrochemical biosensors for pathogen detection: Anusha et al. [19] highlighted various types of electrochemical biosensing techniques and the role of biorecognition molecules in sensing of dengue virus; Kaushik et al. [20] discussed the recent developments in developing intelligent sensing strategies to monitor Zika virus; Rasouli et al. [21] gathered the advancements in electrochemical DNA biosensors for the detection of human papillomavirus virus. However, these reviews are all restricted to include only a kind of virus, which lack of the summary of electrochemical detection methods for a class of viruses. Furthermore, there are other excellent reviews that present the current state of biosensors for respiratory virus detection: Ribeiro et al. [22] covered important advancements in the

biosensor field in terms of most current respiratory viruses, presenting the development in the assembly of the devices and figures of advantages. Samson et al [23] present all the novel types of biosensors that could be used for the rapid detection of COVID-19. Ruiz de Eguilaz et al. [24] reported on virus and antibody detection using electrochemical methods, focusing on recent key innovations which drive the progress of portable, high performance point-of-care technologies. Nevertheless, few articles cover and focus on both electrochemical biosensor background and respiratory virus detection, or their key aspects are a kind of special field. For example, Nelson et al. [25] provided a brief overview of currently available Point-of-Care Tests (POCTs) for the diagnosis of emerging and new respiratory viruses along with their merits and limitations, and discussed recently published methods and techniques with a potential use in future POCTs. Therefore, our review article aims to fills the blank by combining essential background information about electrochemical biosensors with the rapidly moving advancements of electrochemical biosensors for respiratory virus detection.

Hence, we reviewed the recent advances in electrochemical biosensors for respiratory virus detection. In this review, common detecting objects of respiratory viruses, electrochemical biosensors, electrochemical biosensors for respiratory virus detection and future challenges are discussed successively. When exploring the methods for testing a new virus, it is often worthy of reviewing the already existing methods for other congeneric virus in comparison. Therefore, it is anticipated that this review regarding respiratory viruses will provide a complete guide to develop novel COVID-19 diagnosis assays with prominent accuracy and sensitivity, thereby performing appropriate antiviral therapies for patients.

2. Common detecting objects of respiratory viruses

For respiratory virus detection, the whole virus, their structural proteins, gene sequences and antibodies could be the targets. Here we will give a comprehensive discussion regarding common detecting objects of representative respiratory viruses: influenza virus, MERS-CoV and SARS-CoV-2.

2.1 Whole virus and their structural proteins

Influenza virus. Basically, the whole influenza virus and the structural proteins, including M1 protein, hemagglutinin (HA) and neuraminidase (NA) all can serve as antigens for influenza virus detection. The type of influenza virus: A, B and C are classified according to the encoding proteins: matrix protein M1 and viral nucleoproteins. M1 protein is the only essential viral component for virus-like particles formation and suitable for all serotypes of influenza virus [26]. Besides, the virus can combine with the host cells through the contacts of HA and NA. There has been 18 HA and 11 NA variants so far owing to their high variety. The subtype of influenza virus is usually decided by the properties of HA and NA [27].

The Middle East Respiratory Syndrome Corona Virus (MERS-CoV). Belonging to coronavirus, MERS-CoV owns four structural proteins: spike (S) protein, envelope (E) protein, membrane (M) protein and nucleocapsid (N) protein. The S protein is involved in the binding process between the virus and the host cell surface receptors. The E protein is the smallest protein in the major structural proteins, mediating virus assembly and budding. The M protein is able to decide the shape of the virus envelope. The N protein is the only protein binding to the RNA genome [28]. Among these, the S protein is the most-frequently used antigens because of its significant role in the attachment of the

virus to the host cells. However, there are still few published articles about the detection of the whole MERS-CoV virus.

SARS-CoV-2. The whole SARS-CoV-2 and their four structural proteins: S, E, M and N could be used as targets for SARS-CoV-2 detection. M and E protein are essential proteins when occurring viral assembly, while S and N proteins are the most significant biomarkers in terms of COVID-19 early diagnosis. The S protein can mediate the fusion of the virus and the host cell membrane, making the virus more easily enter the host cells [29]. Besides, the highly immunogenic S protein could promote producing neutralizing antibodies as well as T-cell responses in the SARS-CoV-2 patients [30]. Moreover, the S1 subunit of the S protein exhibits the receptor binding domain (RBD) with strong binding affinity for the host angiotensin-converting enzyme II (ACE2) receptor on the human cells [31]. Therefore, the RBD protein of SARS-CoV-2 could also be selected as the targets.

2.2 Gene sequences derived from viruses

Influenza virus. The origins of the derived gene are generally classified into two groups: (i) deriving from the biomarkers of the influenza virus. The most frequently-used RNA transcripts and DNA oligonucleotides when diagnosing influenza virus are the HA gene of them. (ii) sequences of DNA derived from influenza virus then amplified by polymerase chain reaction (PCR). Although some electrochemical biosensors are able to detect gene sequences in the pure samples, there is still distance before their application to real samples owing to the high background responses from matrix effects [32]. Therefore, researchers begin to detect the amplified products from PCR to solve the problems from real samples. Nevertheless, for amplified products, the efficiency will decrease when the targets and probe hybridize because of interference factors [33]. In fact, it is the ideal that the electrochemical biosensors do not rely on the PCR technique or less, which may increase workload. Unluckily, the electrochemical biosensors independent on PCR are chiefly suitable for abundant DNA targets. The low-abundance DNA analytes even if not depending on PCR, still involve quantitative real-time PCR [34].

MERS-CoV. The genome of MERS-CoV includes 30,119 nucleotides and 11 open reading frames (ORF). The first open reading frames (ORF 1a and 1b) at the 5′-Untranslated Regions (UTR) (278 nucleotides) have become essential detecting objects in the MERS-CoV specie identification, which are predicted to encode nonstructural proteins [35]. The genes downstream to ORF1ab encode for structural proteins and accessory proteins (**Fig. 1**). The RNA upstream of the E gene (upE) has also been recommended by WHO for MERS-CoV detection [36]. Besides, with a sensitivity of ≤10 copies/reaction, identifying the MERS-CoV N gene is an alternative method complementing upE and ORF 1a approaches, recommended by the US Food and Drug Administration (FDA) [37].

SARS-CoV-2. Similar to MERS-CoV, the 5'-terminal genome ORF1a/b encode two large polyproteins, the other ORFs on the genome encode four main structural proteins and accessory proteins. ORF 1a, ORF 1b, non-structural RNA-dependent RNA polymerase, S gene, N gene of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) are the preferred targets for nucleic acid tests [38]. Owing to the 79% similarity of the whole-genome between SARS-CoV and SARS-CoV-2, unique primers or guide RNAs are required for distinguishing SARS-CoV-2 with no cross-reactivity for SARS-CoV [39]. To avoid the "false negative" result, multiple gene sequences are usually detected simultaneously in the COVID-19 diagnosis.

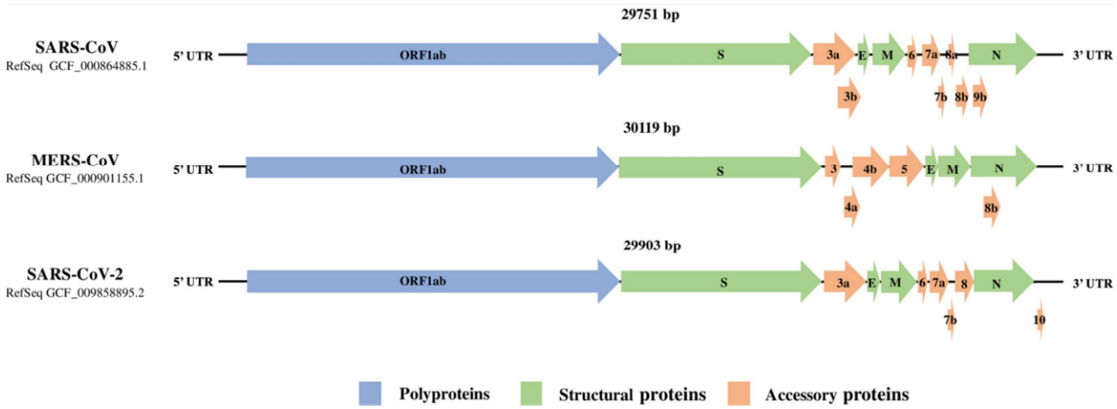


Fig. 1. The genome of SARS-CoV, MERS-CoV and SARS-CoV-2, all of which consist of conserved replicase domain (ORF 1ab) (blue). The structural genes (green) S, E, M and N encode the structural proteins: spike (S) protein, envelope (E) protein, membrane (M) protein and nucleocapsid (N) protein, respectively. Different coronaviruses have different accessory genes (orange). Reproduced with permission from Ref. [35].

2.3 Antibodies

Influenza virus. It is well known that the immunoglobulin M (IgM) presents in patients' blood after 3–6 days, and immunoglobulin G (IgG) presents after 8 days [40]. Moreover, the specific antibodies of structural protein are also alternatives for influenza virus detection, such as the antibodies of HA and NA. The vaccines could induce the increase of virus-specific antibodies about virus invasion [41].

MERS-CoV. Generally, antibodies to proteins S, 3a, N, and 9b could be detected in the serum samples of convalescent-phase patients [42]. Anti-S and anti-N are detectable until week 30, and anti-N appears earlier than anti-S, so anti-S may be preferable with convalescent sera comparatively [43]. Whereas, for early diagnosis of diseases related to respiratory viruses, detecting relative specific antibodies is not appropriate, which may be useful for treating convalescent patients [44].

SARS-CoV-2. For SARS-CoV-2 infection, IgG against N protein is detectable as early as 4 days after infection. Zhang et al. [45] have confirmed that IgG and IgM could be detected by enzyme-linked immuno sorbent assay (ELISA) in the serum of the patients after 5 days of infection. After SARS-CoV infection, it has been proved that the sensitivity of N-based IgG ELISA (94.7%) is significantly higher than that of S-based IgG ELISA (58.9%) [46], but there is still no report to present the sensitivity of SARS-CoV-2 IgG/IgM.

3. Electrochemical biosensors

The development of chemical and biosensors is one of the most active fields in current analysis and research. Biosensors are small devices including bio-recognition elements and signal transducers, which can be used for the direct detection of objects in samples [47]. Electrochemical sensors, using electrodes as energy exchangers, are the important branch of biosensors. Electrochemical sensors occupy an important position in current biosensors, widely applied in the clinical, industrial, environmental and agricultural analysis [48-49]. Therefore, we discussed the electrochemical biosensors utilizing the framework upon the working principles, merits and defects of electrochemical

biosensors, electrochemical transduction, bio-recognition elements and nanomaterials. The components and principle as to electrochemical biosensors used for the detection of the respiratory viruses are displayed in the **Fig. 2**.

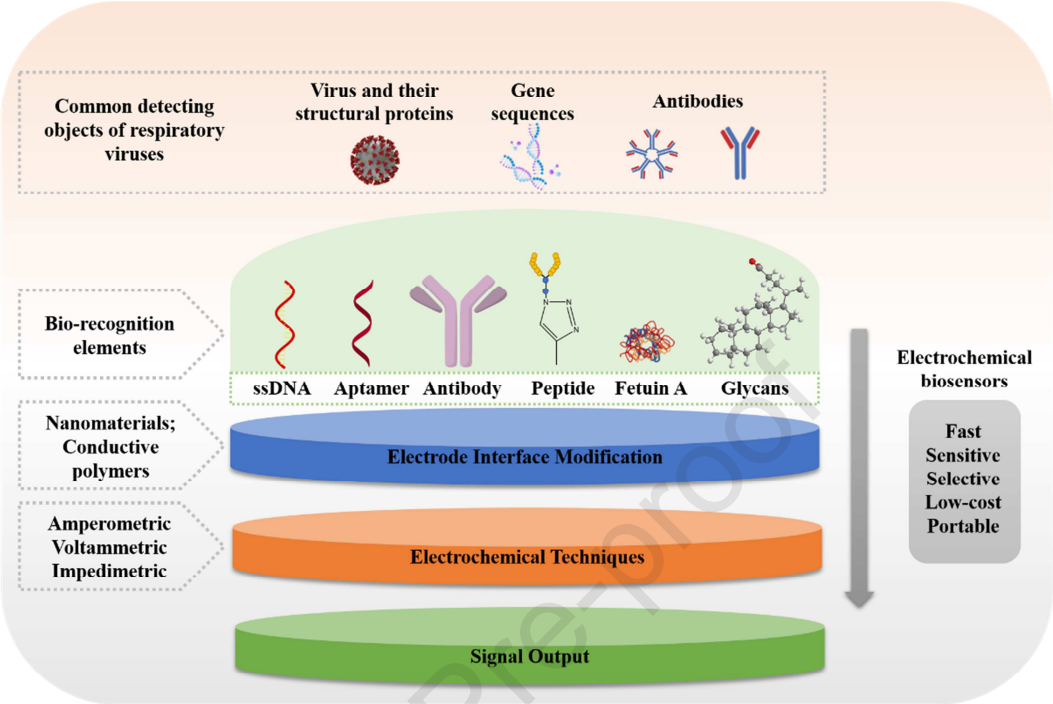


Fig. 2. Schematic description of components and principle for electrochemical biosensors used in detection of respiratory viruses. (ssDNA: Single-stranded DNA.)

3.1 Working principles, merits and defects

The biosensor is an analytical system composed of three essential parts: the bio-recognition element, the transducer and signal output [50]. The diagnosis molecules process could be summarized as: the targets firstly are recognized by the specific bio-recognition elements via amounts of interaction like the covalent bond or non-covalent bond; then the changes could be felt by the transducer and further translated into the digital detector; finally, the digital signals are output by the digital device such as computers and phones [51]. Particularly, the transducers of the electrochemical biosensors are a variety of electrodes, such as glassy carbon electrodes, gold electrodes, screen-printed electrodes (SPEs) and carbon paste electrodes. The electrodes in the electrochemical biosensors provide the platform for kinds of modification, which aim at improving the property of analytical system: sensitivity, selectivity, stability, reproducibility and so on [52]. Thus, the well-designed electrochemical biosensors exhibit abundant advantages: low-cost, quick-response, simple, high sensitivity with the help of electrode fabrication and the bio-recognition element design [53].

On the one hand, compared with other transduction processes, that of the electrochemical biosensors could be completed at the electrochemical workstation at least, which reduce the cost of test greatly. This is because the electrochemical detection is based on the result of direct electronic signals, like amperometric, voltammetric and impedimetric changes. Therefore, the detecting process could be over in a short time [54]. Moreover, the electrochemical biosensors are capable of realizing label-free

detection without the incorporation with any label, making POCTs possible [55]. In addition, the high sensitivity of electrochemical biosensors could be ensured by applying the bio-recognition elements with high specificity and affinity or decorating the electrodes with special materials with excellent electronic performance [56]. Over the past few years, the electrochemical biosensors have gained numerous progresses in the analytical field owing to the advantages, especially in the diagnosis of the pathogens, offering a kind of new possibility for healthcare. The electrochemical biosensors have been utilized to monitor the virus particles during virus outbreaks in epidemic areas.

On the other hand, even if most of electrochemical biosensors are successfully tested in buffered solutions or diluted real samples spiked with targets, matrix effects always influence the analytical performance of the biosensors in practice. Therefore, the stability and accuracy of electrochemical biosensors remain to be the biggest limitations, especially after repeated usages and long storage. Besides, owing to some interaction between the biorecognition elements and targets is irreversible, thus these electrochemical biosensors could only be used once, increasing the cost of testing.

3.2 Electrochemical transduction

There have been a variety of electrochemical biosensors fabricated for respiratory virus detection, the most commonly used electrochemical techniques are chronoamperometry, cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV) and the electrochemical impedance spectroscopy (EIS) whose principles are described in reference [57]. Voltammetric biosensors (CV, DPV and SWV etc.) have been widely implemented for respiratory virus detection owing to their fast response, less sample, simple preparation and excellent reproducibility. However, on account of the requirement of the extra electroactive species, its application is limited in some degree for respiratory virus detection [58]. EIS technique is attractive for biomedical and biological fields in accordance with the ability of revealing the weak interaction between different species. Moreover, the EIS is the only research method for studying the interactions between biolayers, which have active effect on the designing rapid, stable, sensitive and portable electrochemical biosensors for respiratory virus detection.

3.3. Bio-recognition elements.

Bio-recognition element is the key component of the electrochemical biosensors. Only when the recognition of the targets is guaranteed, the later steps can start. Bio-recognition element in the electrochemical biosensors could be divided into biocatalytic and biocomplexing. Biocatalytic elements, such as enzymes, cells and tissues, are based on the catalytic reactions for recognizing targets. For example, enzymes are involved in various chemical sensing applications, which are primarily served as signal labels in the respiratory virus detection. Enzymes are usually introduced during the secondary binding process. Biocomplexing elements are the most-frequently used bio-recognition elements in the respiratory virus detection, which rely on the interaction of targets with macromolecules or organized molecular assemblies. Antibodies, aptamer and peptide are common bio-recognition elements in the respiratory virus detection. Some researchers also used imprinted polymers as bio-recognition elements in the electrochemical biosensors.

3.4. Nanomaterials.

The modification of the working electrode is very important in the fabrication process of the electrochemical biosensors, resulting in the link between analytes in the bulk solution and sensing interface. The affinity of the biosensors is usually improved by modifying with bio-recognition elements, and the sensitivity of the biosensors is often enhanced by realizing signal amplification through the addition of nanomaterials. The common nanomaterials and their properties utilized in electrochemical biosensing are briefly introduced as followed:

(i) Gold-based nanomaterials. Metallic nanoparticles, owning unique optical/electrical properties, especially gold nanoparticles (Au NPs) have been served as stable immobilizer for bio-recognition elements without distorting their bioactivity, meanwhile facilitating excellent electron transfer between the targets and sensing interface. Both various functional groups (−SH, $-NH_2$, −CN) and amine or thiol linkers could coordinate Au NPs attachment forming multilayered bionanocomposite-film on the interface [59].

(ii) Carbon-based nanomaterials. Graphene oxide (GO), reduced graphene oxide (RGO) and carbon nanotubes (CNTs) are used under other circumstance in designing biosensors with high sensitivity. The main advantage of the carbon-based nanomaterials is increasing the electron transfer rates. Additionally, by chemically functionalizing the surface architecture, both the electrical conductivity and the surface area could be enhanced and result in the improvement of the sensitivity of the biosensors [60].

(iii) Magnetic nanoparticles (MNPs). Their handling and the large variation of surface allow them to be employed as coating support for further modification, and its high surface energy and large surface area allow electrons transfer more efficiently at the same time. Moreover, owing to being controllable by external magnet, when attached with labels and bio-recognition elements simultaneously, the MNPs are able to realize the reproducible magnetic virus separation and further signal amplification in the real clinical samples [61].

4. Electrochemical biosensors for respiratory virus detection

According to the type of bio-recognition element, we divided the electrochemical biosensors for respiratory virus detection into three groups: nucleic acid-based, immunosensors and other affinity biosensors. Their advantages and limitations when applied for respiratory virus detection are summarized in **Table 1**. Next, we would review the recent electrochemical biosensors for respiratory virus detection in terms of the classification.

Table 1 Advantages and limitations of common bio-recognition elements applied for respiratory virus detection.

Type of electrochemical biosensors	bio-recognition elements	Advantages	limitations
Nucleic acids-based	ss-DNA	Detection of ssDNA PCR products, easy to produce and more	Limited for gene sequence detection, strict hybridization

		stable	conditions and expensive
	Aptamer	Size-smaller, low-cost, more stable, easy-to-produce and of lower immunogenicity	The strict hybridization conditions, long-term SELEX process and sometimes need complex steps
Immunosensors	Monoclonal antibodies (mAb)	More specific than pAb, avoiding the cross reaction	Expensive, instable and complexity-to-synthesis
	Polyclonal antibodies (pAb)	Less expensive, more epitopes and mass-productive	Instable and easily appearing cross reaction
	Antibody single-chain Fv fragments (scFv)	Highly customizable, low variability and smaller size compared with whole antibody	Slow synthesis, lower affinities compared with whole antibodies and can't be produced for small molecules
Others affinity biosensors	Fetuin A	Low-cost, selective and lower limit of detection	Limited to influenza virus
	Peptides	Easily being designed and prepared	Less specific compared with aptamers and antibodies
	Glycans	Storing more code information	Limited to a few viruses, the affinities need to be proved further

4.1 Nucleic acids-based

Electrochemical biosensors based on nucleic acids as recognition element generally used DNA or RNA. The DNA or RNA sequences are usually immobilized on the sensing interface. Owing to the specific binding between probes and targets, the formation on the electrode, like double strand DNA (dsDNA), could trigger the properties change of the electrode surface, which can be detected via electrochemical techniques. The electrochemical signals are generally from the electron transfer of redox-active probe with the electrode, and the common redox-active probes are $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+}$ complexes [62]. Nucleic acid-based electrochemical biosensors own various merits: high specificity, stability, possibilities for miniaturization, which are very attractive for the fabrication of biosensors [63]. The nucleic acid-based electrochemical biosensors for respiratory virus detection are summarized in **Table 2**.

Table 2 Nucleic acid-based Electrochemical biosensors for respiratory virus detection

Single-stranded (ss) DNA, hairpin DNA, peptide nucleic acid, and locked nucleic acid are the

Type	Virus	Recognition element	Linear range	LOD	Electrochemical method	Ref
Detection of proteins or whole virus	H1N1	aptamer	10^1 PFU mL^{-1} - 10^4 PFU mL^{-1}	3.7 PFU mL^{-1}	DPV	[45]
	H5N1	DNA probe	1 pM - 100 nM	HEPES buffer: 1 pM chicken serum: 1 pM	CV	[64]
	AIV	anti-AIV NP aptamer	2 nM - 2 μM	1.13 nM	CV	[65]
	H7N9	DNA tetrahedral probe and ssDNA aptamer	1 pM - 100 nM	100 fM	amperometry	[66]
	H5N1		100 fM - 10 pM	100 fM	DPV	[67]
	H1N1	aptamer against inactivated intact H1N1	/	0.3 ng mL^{-1}	EIS	[68]
Detection of PCR ssDNA products	H5N1	thiolated ssDNA probe	/	RNA transcripts: 10 pM DNA oligonucleotides: 1 pM	SWV	[69]
	H5N1	ssDNA probe	1 - 10 pM	1.39 pM	SWV	[70]
	Influenza A	DNA probe	1.0 fM - 1.0 nM	84 aM	DPV	[71]
	H1N1	HA gene specific ssDNA probe	0.1- 400 ng in 6 μL	0.004 ng in 6 μL	EIS	[72]

probe often used in the electrochemical biosensors [73]. The most common probe in kinds of nucleic acid-based electrochemical biosensors is ssDNA. Specially, aptamer, a kind of ssDNA with high affinity and selectivity toward targets has been widely utilized in ssDNA-based electrochemical biosensors. The aptamer is selected from Systematic Evolution of Ligands by Exponential Enrichment (SELEX), which could combine with targets via interaction like hydrogen bonds, van der Waals forces [74]. The targets of aptamer can be proteins, nucleic acid or chemical substances. Comparing with antibodies, the aptamer is size-smaller, low-cost, more stable, easy-to-produce and of lower immunogenicity, which has considerable potential for developing novel electrochemical biosensors with high specificity [75].

i) Detection of proteins or whole virus

Bhardwaj et al. [45] selected an ssDNA aptamer against stem region of HA protein of influenza A virus by five rounds of SELEX. Simultaneously, mini-HA protein and whole H1N1 virus could be recognized by this aptamer. The dissociation constants (K_D) of the developed aptamer are higher than the average K_D of the influenza virus antibodies, which means the affinity of aptamers is superior to relative antibodies. The specific aptamer was adsorbed on the working areas of the ITO/glass strips previously functionalized by a polyethylenimine solution, the final aptasensor achieved a H1N1 virus limit of detection (LOD) of 3.7 plaque-forming units (PFU) per mL. More importantly, six strains of

H1N1 influenza A viruses could be identified by the aptamer-modified electrode, indicating the possibility of the rapid subtyping of H1N1 and diagnostic applications. Apart from single aptamer as recognition element, the most usual detecting assay in aptamer-based biosensor is the aptamer-target-antibody sandwich method. The dual recognition pattern greatly improves the accuracy and selectivity of the detection process, decreasing the LOD of biosensors. Diba et al. [67] fabricated an amperometric bio-affinity electrochemical sensor for avian influenza virus proteins detection with aptamer modified Au NPs decorated on carbon chips. The electrochemical signals were from the reaction between alkaline phosphatase (ALP) and 4-amino phenyl phosphate (APP). The current generated from the Au NPs-aptamer/H5N1/anti-H5N1-ALP sandwich complex with the enzyme substrate increased with the concentration of H5N1. Differential pulse voltammetry was used for detection with a linear dynamic range of 100 fM-10 pM. The 100 fM LOD of the aptamer-antibody sandwich platform compares favorably with commercial antibody ELISA kits. The proposed biosensor has been used in the detecting H5N1 protein for diluted human serum samples.

However, to immobilize the aptamer, the fabrications of the electrode often involve labeling and anchoring operation, which required complex steps. In order to solve the problem, Lee et al. [65] introduced a multi-functional probe which consists of recognition part, signal producing part and combining part. It was immobilized on the porous Au NPs modified electrode for avian influenza virus detection. The recognition part was based on the specific aptamer of HA protein. The DNA 3 way-junction probe could realize three steps: recognizing, immobilizing and generating without additional process and loss of functionality. Besides, the multifunctional DNA probe could also insert redox probe, functional groups and other aptamers. The multi-functional probe-based electrochemical biosensor showed the LOD of HA protein at 1 pM in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) solution and 1 pM in diluted-chicken serum, respectively. Although the proposed biosensor didn't own the lowest LOD, the redox probe labeling step and signal amplification step were both reduced compared to previous works.

ii) Detection of PCR ssDNA products

Alafeef et al. [76] reported using antisense oligonucleotides directed electrochemical biosensor chip for realizing the digital diagnosis. The sensing chip was based on the paper-based electrochemical sensor chip modified with Au NPs. The highly specific antisense oligonucleotides towards viral N gene were served as bio-recognition element, yielding a nucleic-acid-testing device with a readout presented by a hand-held reader. The samples collected from Vero cells infected with SARS-CoV-2 virus and clinical specimens have been tested for the device, whose incubation time was less than 5 min, with a sensitivity of 231 (copies μL^{-1}) $^{-1}$and LOD of 6.9 copies μL^{-1} without further amplification. For most nucleic acid-based electrochemical biosensors, the nucleic acid-probes are generally immobilized on the sensing interface through the attachment between points. The density of the recognition elements couldn't be ensured to be homogeneous, resulting in the additional process to block the unspecific adsorption [77], the DNA nanotechnology has been as the solution to solve the problem. The DNA with different structures is designed to control the recognition, such as DNA tetrahedra. The three vertices of the DNA tetrahedra are usually modified with thiol groups, the DNA tetrahedra will attach to the electrode surface via Au-S bond thus one signal probe could be immobilized on one DNA tetrahedra with the fourth vertex [61]. Comparing to the conventional point-tethered signal probe, the signal anchored by DNA tetrahedra present 5000-fold greater affinity [78]. Essentially, because of the high mechanical rigidity of the DNA tetrahedra, the signal probes will keep an upright orientation on the electrode surface even without the help of 6-mercapto-1-hexanol (MCH). Latest advances have also

extended the applications of DNA tetrahedra in nucleic acid-based electrochemical biosensors. Dong et al. [66] developed a DNA tetrahedra-based electrochemical biosensor for H7N9 virus ssDNA detection, the amperometric signals were recorded from the interaction between the avidin-horseradish peroxidase attached to bio-ssDNA (biotin-labeled ssDNA) and 3,3′,5,5′-tetramethylbenzidine substrate. Before testing, H7N9 virus cDNA was employed to conducting asymmetric PCR for obtain H7N9 virus ssDNA targets, the dependence degree study of the developed biosensors on PCR is also proceeded, the results showed ssDNA products from only one cycle of asymmetric PCR could be identified by the proposed sensor platform. The detection limit of the biosensor for asymmetric PCR ssDNA products was determined to be 97 fM. The asymmetric PCR ssDNA products and PCR-free samples both could be distinguished from zero samples by DNA biosensor. It is also the first time that the DNA tetrahedra-based electrochemical biosensor was proposed to be tested in the clinical samples, which potentially verified the practicability of DNA tetrahedra probe (**Fig. 3**).

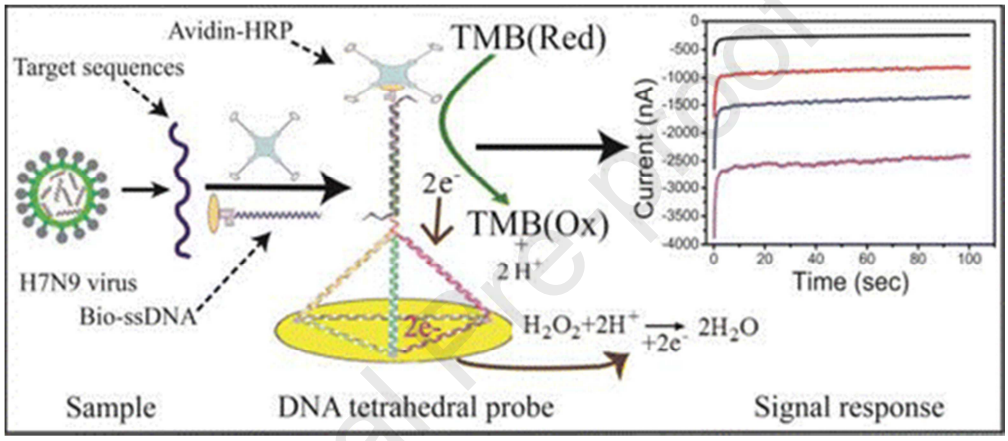


Fig. 3. A DNA tetrahedral nanostructure-based electrochemical biosensor was developed to detect avian influenza A (H7N9) virus through recognizing a fragment of the hemagglutinin gene sequence. Biotin-labeled (bio)-ssDNA was the bio-recognition element toward targets, which also could combine with avidin-horseradish peroxidase (HRP) probes through biotin-avidin interaction. The DNA hybridization hence was transformed into the redox reaction of TMB (enhanced K-blue substrate) and H_2O_2. Reproduced with permission from Ref. [66].

Zhao et al. [79] firstly proposed supersandwich-type electrochemical biosensor regarding SARS-CoV-2 from COVID-19 patients by a smartphone (**Fig. 4**). The supersandwich-type electrochemical biosensor included: capture probe (CP), auxiliary probe (AP), label probe (LP), and target sequence. The 5′- and 3′-terminals of target sequence are complementary to CP and LP, respectively. The 5′- and 3′-regions of AP have complementary sequences with two LP regions. The detection was based on using CP and LP, AP and LP to hybridize frequently for producing long concatemers, resulting in high sensitivity. Besides, p-sulfocalix[8]arene functionalized graphene was utilized to enrich toluidine blue, which was an approach of facilitating of LP with signal probes for selectivity enhancement. The detectable ratios (85.5% and 46.2%) were rather higher than those that were obtained using RT-PCR (56.5% and 7.7%) according to the testing for 88 RNA extracts from 25 SARS-CoV-2-confirmed patients and eight recovery patients.

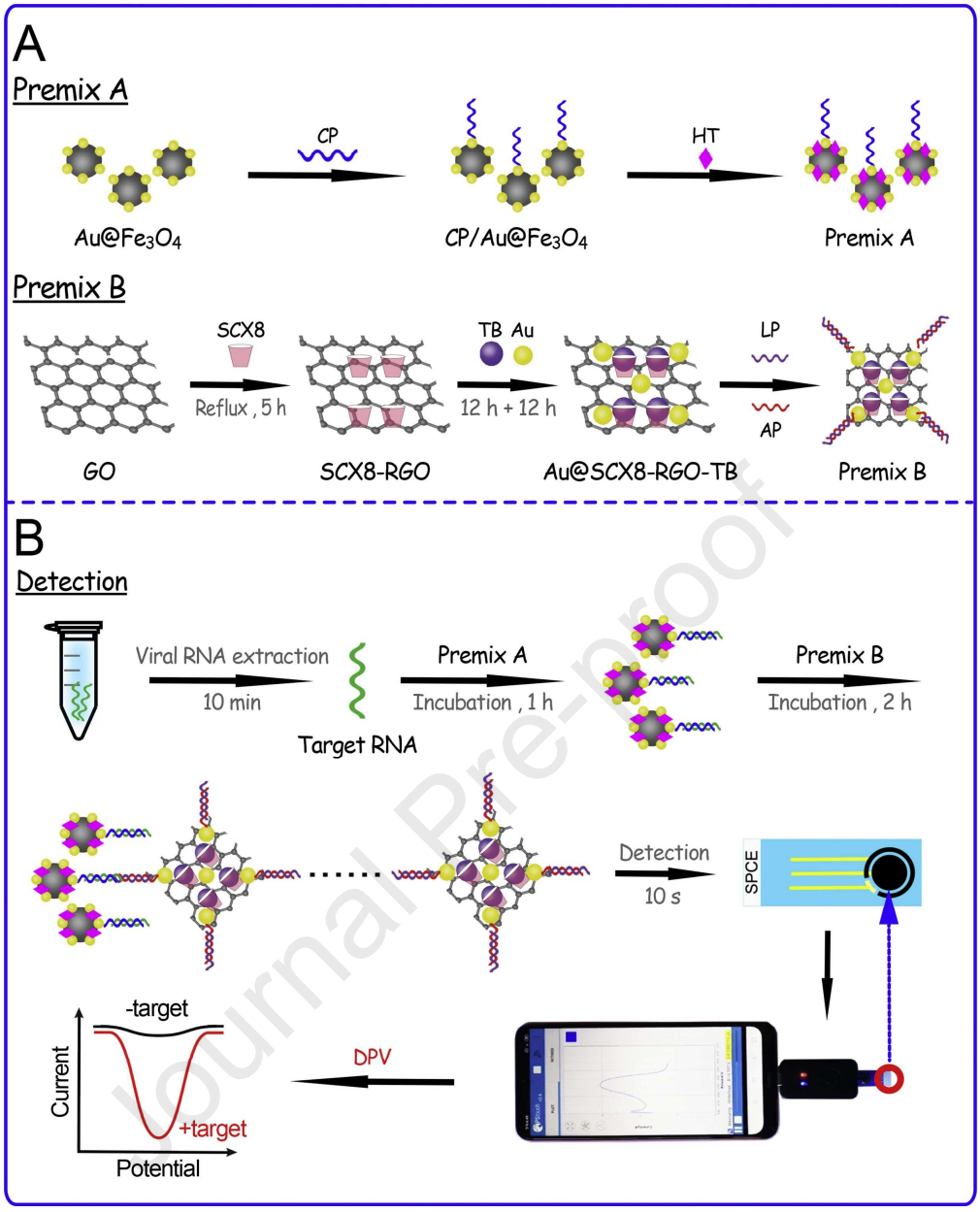


Fig. 4. Schematic representation of SARS-CoV-2 detection using the electrochemical biosensor. (A) Prepare of premix A and B; (B) Process of electrochemical detection using a smartphone. Reproduced with permission from Ref. [79].

Totally, for nucleic acids hybridization assays, electrochemical biosensors based on nucleic acids probe is the first choice, and aptasensors are suitable for both nucleic acids and other small molecules. The affinity of the probe depending by the sequences selection of the probe mostly decides the specificity of the electrochemical biosensors. The conditions of the hybridization such as the buffer composition and temperatures are also the influencing factors. Therefore, the design of electrochemical biosensors based on nucleic acids probe are comparatively strict. Besides, when the sensitivity of the biosensor is insufficient, it is common to use tagged hairpin probes or hybridized tapered sequences as amplification steps, which may add additional experimental steps.

4.2 Immunosensors

Antibodies are the bio-recognition elements of the electrochemical immunosensors. Antibodies are a series of serum proteins produced by B-lymphocytes and plasma cells, which could recognize and bind the targets (antigens). The antibody contains two antibody fragment-antigen binding (Fab) that are held by the key hinge disulfide bridges. The disulfide-termed Fab fragments are named Fab' which allow the binding with the sensing interface via the covalent bond [80]. Antibodies are the workhorse in commercial and lab bioanalytical assays due to their high specificity, extreme affinity and great sensitivity, showing interesting applications for detecting virus, proteins, and cancer cells [81]. The antibodies could be obtained by amounts of methods, natural or recombinant, as monoclonal or as polyclonal. Nevertheless, comparing to the nucleic acid elements, the defects of the antibodies are high-cost, instability, complexity-to-synthesis, the affinity of which may be affected by adding the signal tags, and can't be used for small molecules, drugs and metal ions [82].

The most prominent antibodies in respiratory virus detection are monoclonal antibodies (mAb), polyclonal antibodies (pAb) and antibody single-chain Fv fragments (scFv). mAb are more specific than pAb because mAb could only combine with single epitope hence avoiding the cross reaction, and the pAb are produced towards various epitopes on a single antigen [83]. While the pAb are less expensive and mass-productive providing the widespread application in biosensors construction. The scFv fragments include one light chain and one heavy chain with a molecular weight of 30 kDa, with smaller size compared with whole antibody and low variability, the scFv fragments are brilliant for antigen capture [84]. The merits of the antibody-antigen reaction are high specificity, reversible binding between surface chemical groups, suitable ratio and concentration and staged reaction. The special properties of antibody-antigen reaction make antibody-based electrochemical biosensors being one of the most versatile and available detection tools for respiratory virus. The antibody-based electrochemical biosensors for respiratory virus detection are summarized in **Table 3**.

Table 3 Antibody-based electrochemical biosensors for respiratory virus detection

Type	Label	Virus	Recognition element	Linear range	LOD	Assay time	Electrochemical method	Ref
Label-free	/	H1N1, H3N2	Anti-M1 antibody	/	50 fg mL^{-1}	0.1 h	EIS	[85]
	/	MERS-CoV, HCoV	Anti-recombinant spike protein S1 antibody	MERS-CoV: 1.0 pg mL^{-1} HCoV: 0.4 pg mL^{-1}	MERS-CoV: 0.001 - 100 ng mL^{-1} HCoV: 0.01 - 10,000 ng mL^{-1}	20 min	SWV	[86]
	/	H5N1	scFv against HA H5	The short fragment: 0.6 pg mL^{-1} The long fragment: 0.9 pg mL^{-1}	The short fragment: 4.0 - 20 pg mL^{-1} The long fragment:	/	SWV	[87]

					1.0 - 8 pg mL^{-1}			
	/	H1N1	mAb	0.5 PFU mL^{-1}	1 - 10^4 PFU mL^{-1}	/	Chronoamperometry	[88]
	/	AIV H7	H7-mAb and H7-pAb	1.6 pg mL^{-1}	1.6 pg mL^{-1} -16 ng mL^{-1}	/	LSV	[89]
	/	H1N1	Goat anti-influenza A antibody	113 PFU mL^{-1}	10 - 10^4 PFU mL^{-1}	30 min	DPV	[90]
	/	Influenza virus	Anti-M1 antibody	1 fg mL^{-1} in saliva bu・er	/	5 min	EIS	[91]
	/	H5N1, H1N1	mAb against the HA proteins	H5N1: 9.4 pM H1N1: 8.3 pM	25 – 500 pM	1 min	Chronoamperometry	[92]
	/	H1N1	Anti-H1N1 antibody	Phosphate-buffered saline: 26.04 PFU mL^{-1} diluted saliva: 33.11 PFU mL^{-1}	10 – 10^4 PFU mL^{-1}	/	EIS	[93]
	MNP	H9N2	Anti-M2 antibody	8-128 HAU	8 HAU	160 s	Chronoamperometry	[94]
	HRP	H1N1, H5N1 and H7N9	Anti-H1N1, H5N1 and H7N9 antibodies	1 pg mL^{-1} - 10 ng mL^{-1}	1 pg mL^{-1}	/	Amperometry	[95]
	MNP	H5N1	Anti-H5N1 antibody	0.0025-0.16 HAU	0.0022 HAU in 6 μL	/	CV	[96]
Label-based	HRP	H1N1	Anti-influenza A HA antibody	/	5 PFU mL^{-1} for saliva samples	6 min	EIS	[97]
	MNP	H7N9	mAb and biotinylated antibody	0.011 ng mL^{-1}	0.02 - 50 ng mL^{-1}	1.5 h	LSV	[98]
	Fluorescence MNP	H7N9	mAb and pAb	7.8 fg mL^{-1}	0.01 - 1.5 pg mL^{-1}	/	LSV	[99]
	MNP	H7N9	mAb and rabbit derived pAb	6.8 pg mL^{-1}	0.01 – 20 ng mL^{-1}	/	LSV	[100]

According to different antibodies application, usually mimicking ELISA, the antibody-based electrochemical biosensors consist the following patterns: standard (non-competitive), competitive

direct, competitive indirect and sandwich. Generally, the specific antibodies are immobilized on the transducer surface in the respiratory virus detection, hence standard (non-competitive) and sandwich are the most used antibody formats in the respiratory virus detection with electrochemical biosensors. Competitive direct and competitive indirect are less commonly used, because respiratory viruses are usually small-sized, and difficult to be attached on the electrode surface. Furthermore, depending on if the labels are used, the antibody-based electrochemical biosensors could be divided into label-free immunosensors and label-based immunosensors.

Label-free Immunosensors. Standard is the representative antibody format in label-free immunosensors. The virus particles are captured by the antibodies modified electrode, generating the properties change of sensing interface. The signals could be detected directly with the electrochemical workstation. Label-free electrochemical biosensors are the fastest and simplest with high selectivity and non-cross-reactivity, widely used in the rapid and stable monitoring of respiratory viruses. EIS is the most commonly used electrochemical techniques in the label-free immunosensors, the change from before and after binding to the targets are directly transferred into the change of the interfacial impedance or the change in charge transfer resistance to electroactive probe dissolved in electrolyte. Nidzworski et al. [91] employed the boron-doped diamond (BDD) electrode functionalized with polyclonal anti-M1 antibodies for influenza virus detection. The BBD electrode was dealt with 4-aminobenzoic acid for forming self-assembled monolayer (SAM), then anti-M1 antibodies could be immobilized on the SAM. Hence, the M1 protein was captured onto the BBD electrode, of which changed the impedance spectra. The electrochemical biosensor has a LOD of 1 fg mL^{-1} M1 protein in saliva bu·er within 5 minutes, per sample which corresponds to 5-10 virus particles. Besides, the assay has been verified by applying into different strains of influenza A virus. Meanwhile, as label-free electrochemical biosensors need more simple sensing protocol, they have been integrated with portable devices. Singh et al. [88] reported a novel label-free RGO-modified electrochemical immunosensor, cooperated with a microfluidic platform for influenza A H1N1 virus detection (**Fig. 5**). The three microelectrodes were fabricated on the glass substrate, then modified with RGO and mAb, and encapsulated with a polydimethylsiloxane (PDMS) microchannel finally. The amino groups on antibodies could form the direct linkage with amounts of carboxyl groups on RGO surface in absence of linker or spacer. Moreover, the large surface area of RGO presents lots of defects and electroactive sites, hence improving the sensitivity. The microfluidic label-free immunosensor presented excellent linear range of 1 to 10^4 PFU mL^{-1} and improved LOD (0.5 PFU mL^{-1}), exhibiting the potential of being handheld multianalyte sensing devices for clinical diagnosis. Label-free methods do not integrate any amplification step which could limit their sensitivity.

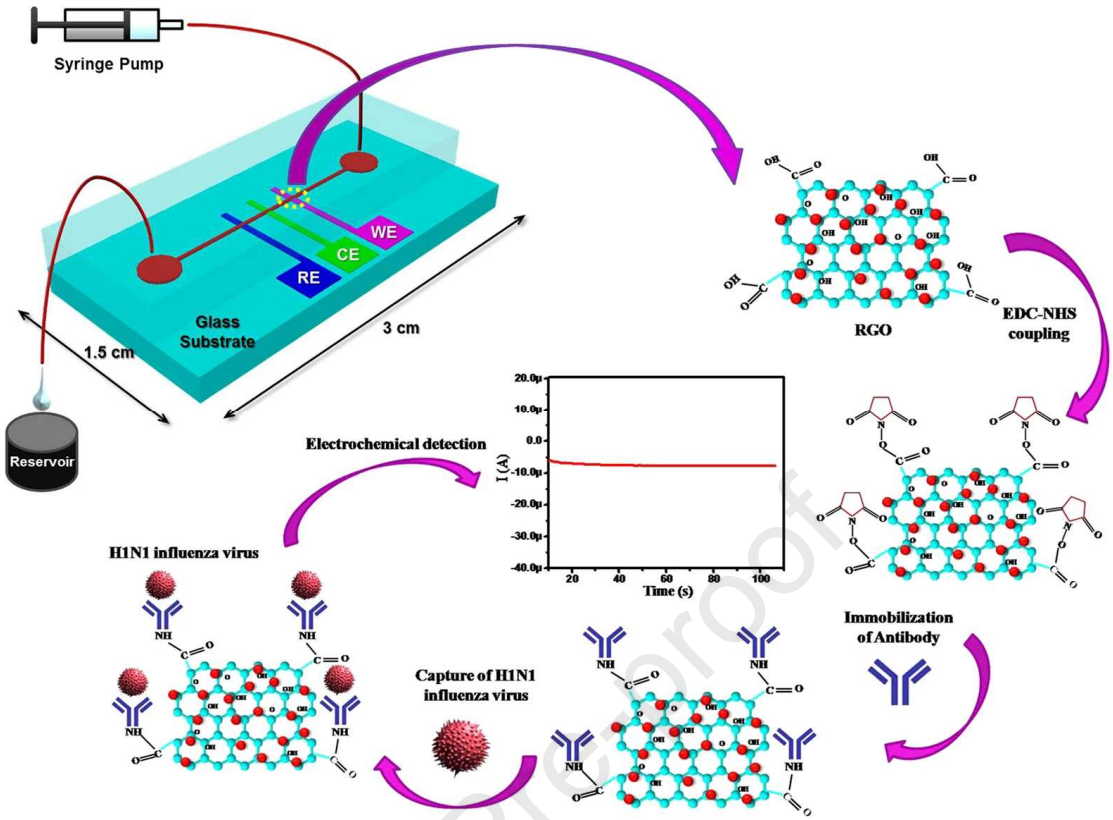
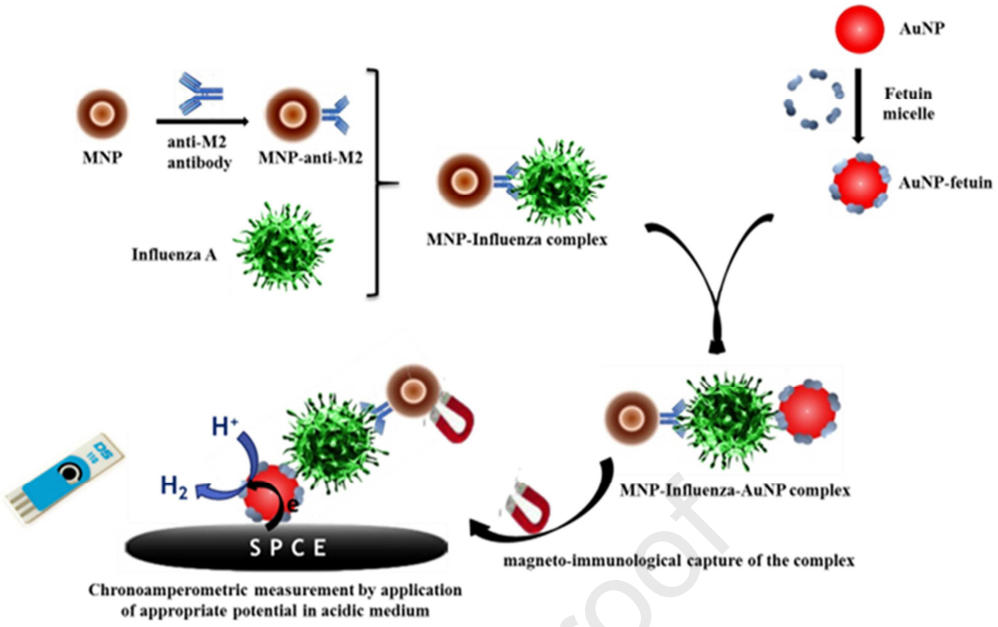


Fig. 5. Schematic illustration of the microfluidics-integrated electrochemical immunosensing chip coated with RGO, followed by antibody immobilization using EDC/NHS coupling for the detection of influenza virus H1N1. Reproduced with permission from Ref. [88].

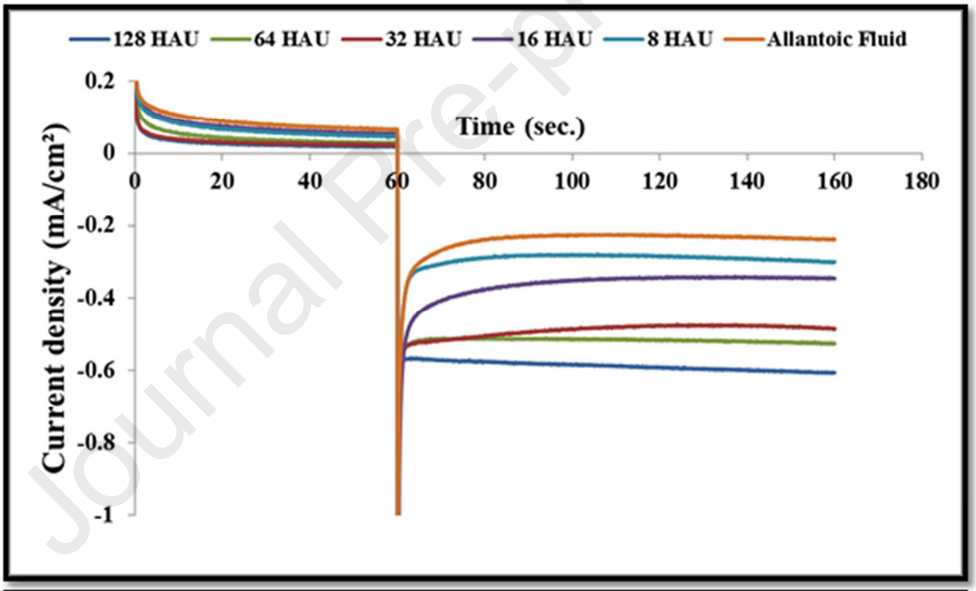
Label-based Immunosensors. Sandwich is the common antibody format in the respiratory virus detection with label-based immunosensors. The detected antigen is sandwiched between two antibodies, one of which are attached on the transducer surface, called capture antibody. The other one is the detection antibody, which is usually labeled with enzyme, nanomaterials or biotin, it can directly measure the amount of antigen. The dual-recognition consolidate the specificity of the biosensors and own better label availabilities. The pAb and mAb are the most frequently used antibody combination in the sandwich-based immunosensors. Owing to the capture antibody will be attached to the electrode, the multi-site binding of antibody and antigen is restricted, so if pAb served as capture antibody, the advantage of high affinity cannot be exerted well. In addition, some pAb may occupy the epitope of mAb, resulting in less binding amount of detection antibody. Therefore, the mAb is often as capture antibody, and pAb is as detection antibody. For instance, Wu et al. [99] according to the ELISA designed the ultrasensitive electrochemical biosensors for H7N9 virus counting. The microelectrode array was modified with Au NPs and mAb, the MNPs decorated with Fe_3O_4 nanoparticles and quantum dots were incubated with pAb and ALP, forming bifunctional fluorescence magnetic nanospheres (bi-FMNs). The fact that pAb could conjugated with modified MNPs was supported by the color change of the fluorescence. Firstly, a single virus could be separated from the complex samples by one bi-FMNs at most, which is controllable by the proportion of bi-FMNs to virus concentration. Then abundant complexes were transferred into the electrolyte, captured by the mAb modified microelectrode assay. Because the ALP on the bi-FMNs can catalyze the dephosphorylation of

p-aminophenyl phosphate monohydrate (p-APP) to produce p-aminophenol (p-AP), hence inducing the reduction from Ag^{+} to Ag^{0} on the sensing interface. The changes from the Ag deposition could be recorded by linear sweep voltammetry. Finally, signals are counted as “0” or “1” depending on digital analysis, the virus concentrations could be estimated through the probability of “0”. The LOD of the label-based immunosensor was 7.8 fg mL^{-1}, which was 1-3 orders of magnitude more sensitive than previous research. Not all sandwich-based immunosensors use pAb and mAb as receptors, other bio-recognition elements are also suitable for sandwich format. Sayhi et al. [94] employed anti-Matrix protein 2 (M2) antibody attached to MNPs and fetuin modified with Au NPs for electrochemical detection of H9N2 virus, the sandwich conformation was finally separated from real samples by applying a permanent magnetic field (**Fig. 6**). After the treatment in acid solution, the sandwich conformation was destroyed, the MNPs were removed by magnet. Because Au NPs can catalyze the hydrogen ions reduction in acidic medium under an appropriate potential, the Au NPs were deposited on the electrode and generated current signals, which was also proportional to the virus titer. The proposed immunosensor displayed the linear relationship between the virus titer in range 8-128 hemagglutination unit (HAU) and cathodic current, with LOD of less than 16 HAU titer. Although the LOD is higher than already published immunosensors, the approach with short detection time leaves out pretreatment steps and overcomes the difficulty of the virus separation from the bulk phase. Generally speaking, the sandwich-based immunosensors are of high sensitivity, high specificity, whose antigen without prior purification. Undeniably, the label-based detection procedures are time-consuming and an antigen must have at least two antibody binding sites.

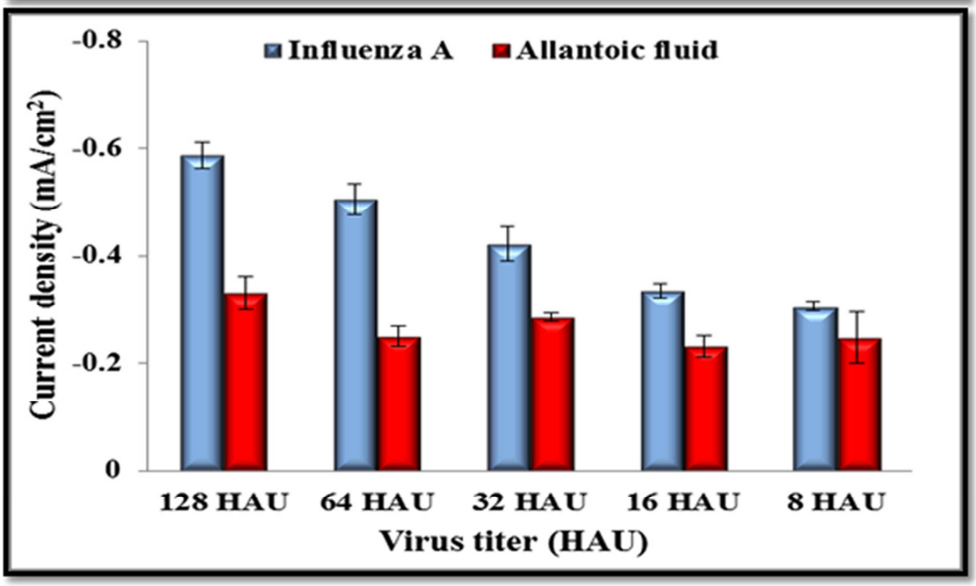
(A)


AuNP
Fetuin micelle
AuNP-fetuin
MNP
anti-M2 antibody
MNP-anti-M2
Influenza A
MNP-Influenza complex
MNP-Influenza-AuNP complex
H+
H2
e
SPCE
magneto-immunological capture of the complex
Chronoamperometric measurement by application of appropriate potential in acidic medium

(B)


128 HAU
64 HAU
32 HAU
16 HAU
8 HAU
Allantoic Fluid
Time (sec.)
Current density (mA/cm²)
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
0
20
40
60
80
100
120
140
160
180

(C)


Influenza A
Allantoic fluid
Current density (mA/cm²)
-0.8
-0.6
-0.4
-0.2
0
128 HAU
64 HAU
32 HAU
16 HAU
8 HAU
Virus titer (HAU)

607

Fig. 6. (A) Schematic illustration of the strategy used to develop the gold nanoparticle-based chronoamperometric magneto-immunosensor for influenza virus detection. The influenza virus could be recognized by anti-Matrix protein 2 (M2) antibody modified magnetic nanomaterials (MNP) and fetuin decorated Au NPs. (B) Chronoamperometric curves obtained without influenza virus (Allantoic fluid) and with 8; 16; 32; 64 and 128 hemagglutinin Units (HAU) of the virus (upper panel). (C) Diagrams (lower panel) correspond to the response of the magneto immunoassay to various influenza virus titers ranging from 8 HAU to 128 HAU (blue) and to various concentration of non-infected allantoic fluid in 1 M HCl solution (red). SPCE: Screen-printed carbon electrode. Reproduced with permission from Ref. [94].

4.3 Other affinity biosensors

Except for nucleic acid, antibodies, there have been other kinds of bio-recognition element presented in the electrochemical biosensors for respiratory virus detection: fetuin A, peptides and glycan.

Fetuin A. Fetuin A is a kind of glycoprotein derived from fetal calf serum, every fetuin A has terminal 12-14 sialic acid residues. Fetuin A is diffusely cooperated with peanut agglutinin (PNA) lectin [101]. Owing to the fact that fetuin A could combine with different influenza virus via NA protein, it could serve as bio-recognition element in influenza A detection with lower cost and high selectivity. For example, Anik et al. [102] developed an electrochemical biosensor based on graphene-Au hybrid nanocomposite for recognizing influenza A. The biosensor utilized fetuin A as bio-recognition element: Firstly, the fetuin A was immobilized onto the electrode surface for NA protein capture, and PNA specific binding sites would display after the interaction, then washed the NA protein on the SPEs, because the sugars from fetuin A have been masked by NA protein, the PNA lectin hence could bond to the N-acetylgalactosamine galactose-(Gal β1-3GalNAc). The resistance changes on the electrode surface were recorded by electrochemical impedance spectroscopy. The biosensor has a linear range between 10^{-8} U mL^{-1} and 10^{-1} U mL^{-1}, which has been applied into H9N2 detection in real samples. Besides, the biosensor's LOD of 10^{-8} U mL^{-1} is lower than LOD values of ELISA assays relying on NA activity or antibody-antigen interaction.

Peptides. Easily being designed and prepared, peptides are theoretically favorable for antigens and drugs measurement. Previous studies showed the pentapeptide Ala-Arg-Leu-Pro-Arg is available to combine with the binding sites of all kinds of HA protein [103]. Surely, the corresponding N-stearoyl derivatives and carbosilane-based dendrimers could inhibit the activity of seasonal H1N1 and H3N2 except for H1 and H3 HAs [104]. Therefore, Matsubara et al. [105] modified the BDD electrode with a sialyloligosaccharide receptor-mimic peptide, the density of the peptide and dendrimer generation terminated on the electrode could affect the probability that the respiratory virus were captured by the functionalized electrode. Electrochemical impedance spectroscopy was used for the virus identification according to the resistance variation. The proposed electrochemical biosensor could isolate the avian virus particles from H5N3, H7N1 and H9N2, presenting the satisfactory specificity and practicability. Faced with the antigenic drift and new subtypes of the respiratory, the designed peptide dendrimer has great potential as antibodies candidates. Besides, Tara Bahadur et al. [106] developed an electrochemical biosensor toward influenza virus particles based on the selection of electrosensitive

peptide ligand in vitro (**Fig.7**). The electrochemically sensitive 3,4-ethylenedioxythiophene (EDOT) moiety was modified with a peptide ligand then worked as electro-polymerization monomers. In the scheme, the real samples were mixed with the solution including the peptide ligand-EDOT monomers. The presentation of the virus particles would influence the electro-polymerization of the peptide ligand-EDOT monomers on the electrode surface, consequently affecting the efficiency of the electron transfer between the redox molecules and the electrode. The LOD of the detection system was found to be 12.5 μg mL^{-1}, which is 2.5-fold more sensitive than the dot blot immune assay or conventional rapid diagnosis test. The "turn-on system": the current increases when there is influenza virus doesn't need negative control measurement for practical application.

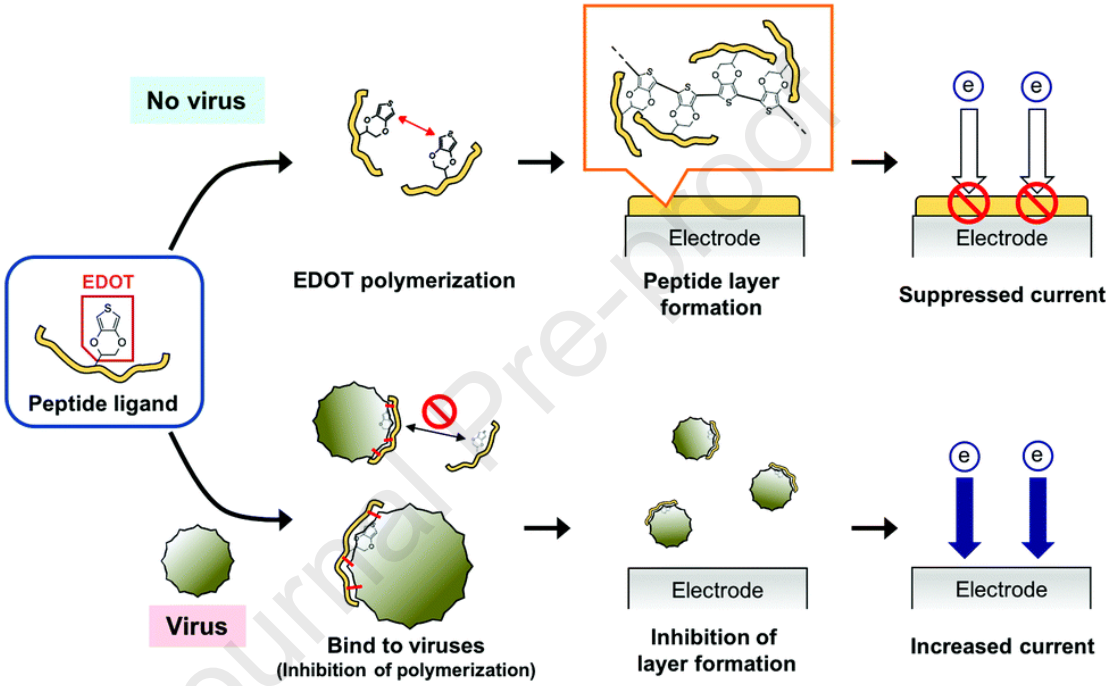


Fig. 7. Strategy for detection of influenza virus using an electrosensitive peptide ligand. Reproduced with permission from Ref. [106].

Glycans. Glycans are a kind of complicated carbohydrates which usually form the dense sugar layer on the numerous cell surface. The cell-cell recognition and host-pathogen interactions are both realized through the glycan coat [107]. For instance, in the influenza A virus infection process, HA protein interact with host glycans terminated in sialic acid firstly. Compared with DNA and proteins, the glycans could store more code information as there are over 10 million glycan molecules on the cell surface [108]. The function of glycan bio-recognition has been applied into the development of the diagnosis approaches and vaccines design. Hushegyi et al. [109] utilized glycans as natural viral receptors in the impedimetric biosensor design for inactivated, but intact influenza virus H3N2 detection. The gold electrode surface functionalized with thiols bearing oligoethylene glycol moieties formed a mixed SAM layer (self-assembled monolayer) for glycan immobilization. The biosensor could detect at least 13 virus particles in 1 μL real samples, revealing a LOD of 5 aM. It was the lowest LOD for influenza virus detection compared with published glycan-based electrochemical biosensors at that time. However, the application of glycans is limited to a few respiratory viruses, and the affinities

of glycans need to be proved further.

5. Future challenges

The global health crisis of the COVID-19 pandemic defines the greatest challenge the world is faced with at the present time, with the most important focus being the sensitivity and specificity enhancement, to which current innovations should pay attention for early detection of COVID-19 disease or future pandemic strains. Simple, low cost, easy to operate and fast-response electrochemical biosensors exactly meet the potential to be integrated into POCTs for COVID-19 diagnosis. Although efforts have been put to design electrochemical biosensors for COVID-19 diagnosis, few portable electrochemical biosensors were produced. There still exists numerous challenges to move from the bench to their use in POCTs.

The sample preparation. A large number of interferers, such as proteins, antibodies, DNA, cells, etc in various complex samples can disrupt the detection process of the targets. The sample pretreatment requirement before analysis to exclude the influence of matrix effects is a main impact factor for specificity and sensitivity improvement. So how to isolate the viruses from the real samples is the key step during the sample preparation. The viruses usually only occupy a small volume of the whole volume, so there is always a small possibility for virus to be captured by the receptor on the transducer when the whole volume is very small. Obviously, the viruses couldn't be concentrated without any preparation. At present, the use of magnetic nanoparticles and selecting most perfect bio-recognition elements are the two main approaches for solving the problem. For example, the specific receptors are coupled with MNPs to capture and separate the targets from complex sample; the association constant of the antibody should be maximized during the antibody selection.

The immobilization of the bio-recognition elements. The immobilization process of the bio-recognition elements is vital to reduce mistakes and errors during virus detection. Currently, the key recognition interaction in many electrochemical biosensors is often irreversible, hence the initial properties couldn't be restored after every detection, the biosensor part should be disposable, which is the rule for medical consumables. Moreover, during the modification process, the affinity of bio-recognition elements is related to the immobilization process, and the efficiency of the immobilization can influence that of detection. How to ensure that the receptor distribution on the electrode surface is uniform and roughly the same between the same batch, without affecting the efficiency of receptor recognition is the question for most portable electrochemical biosensors to be considered.

The miniaturization of the system. Basically, a whole research process of the available electrochemical biosensor-based POCTs includes: optimization of the operation condition, integrating a sensing chip with micro-/nanoelectronics, and interfacing of the sensing platform with a wireless device, transforming into on-site analytical devices, big data analytics and result output [79]. The development of the whole smart sensing system is a multidisciplinary project and need public−private participation. POCTs aim to be carried out close to the patients, the sample volumes, reagent use, transducer and power all need to be miniaturized without reducing the current density and transfer characteristics, and the whole system needs to be wearable and wireless. Most of the electrochemical biosensors own excellent properties and could easily be miniaturized and then should be associated with the whole system.

The reproducibility and stability. To guarantee the accuracy of the POCTs, the reproductivity

and stability of electrochemical biosensors should be improved dramatically. In a whole fabrication process of the electrochemical biosensor, there are many influencing factors: environmental conditions, operating procedures, performance of the instrument. Among these, the most difficult to be automatized is the manual steps for the preparation of the biosensor. Besides, the stability of the POCTs is also supposed to be excellent, because the storage conditions are often difficult to achieve at the laboratory level, before its use.

Environment-friendly and the cost. The environment-friendly and cheap POCTs are often the last hurdle before a biosensor is implemented for POCTs. With the development of material science, numerous nanomaterials have been introduced into the electrochemical biosensors. The potential health impacts and environmental pollution from the widespread usage of the nanomaterials could not be ignored. Besides, the cost of the POCTs should be affordable for primary medical institutions. Therefore, the materials used in the equipment manufacturing process should be as low-cost as possible meanwhile without affecting the performance. Now, paper-based microfluidic devices are relatively environmentally friendly and low-cost, therefore having been the most frequently used substrate platform. Carbon-based nanomaterials are also the excellent green alternative with less pollution.

It is evident that great effort is still required to overtake above challenges in the portable electrochemical biosensor design for SARS-CoV-2 POCTs detection, but we still believe that with the increasing trend in multidisciplinary integration, the ideal POCTs for COVID-19 diagnosis will be produced just around the corner.

6. Conclusion

Overall, we have presented the common detecting targets of the respiratory viruses, key parts of electrochemical biosensors design and discussed different bio-recognition element-based electrochemical biosensors. Future challenges in electrochemical biosensors for respiratory virus determination, especially for application in POCTs are discussed. In every section, several examples were explained, and all the analytical performance of recent developments are gathered in tables with their detection limits. We believe that the advancements from core technologies at multiple-disciplines areas will offer great potential of a next generation of highly specific, sensitive, selective, and reliable electrochemical biosensors for respiratory virus detection. More urgently, the developed electrochemical biosensors could make for better surveillance and control of SARS-CoV-2 infection in populations.

Declaration of interests

None

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Highlights

1. This review provides the recent advancements in electrochemical biosensing for respiratory virus detection.
2. The common detecting objects of representative respiratory virus: influenza virus, MERS-CoV and SARS-CoV-2 are concluded.
3. The electrochemical biosensors based on various bio-recognition elements are reviewed in the respiratory virus detection field.
4. The latest reports and future challenges on the electrochemical biosensors for COVID-19 diagnosis are presented.

Declaration of interests

☒ I, corresponding author of the submitted manuscript, declare on behalf of the author of the manuscript, that the authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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