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# Ultrasensitive detection of pathogenic viruses with electrochemical biosensor: State of the art

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## ABSTRACT

Last few decades, viruses are a real menace to human safety. Therefore, the rapid identification of viruses should be one of the best ways to prevent an outbreak and important implications for medical healthcare. The recent outbreak of coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus which belongs to the single-stranded, positive-strand RNA viruses. The pandemic dimension spread of COVID-19 poses a severe threat to the health and lives of seven billion people worldwide. There is a growing urgency worldwide to establish a point-of-care device for the rapid detection of COVID-19 to prevent subsequent secondary spread. Therefore, the need for sensitive, selective, and rapid diagnostic devices plays a vital role in selecting appropriate treatments and to prevent the epidemics. During the last decade, electrochemical biosensors have emerged as reliable analytical devices and represent a new promising tool for the detection of different pathogenic viruses. This review summarizes the state of the art of different virus detection with currently available electrochemical detection methods. Moreover, this review discusses different fabrication techniques, detection principles, and applications of various virus biosensors. Future research also looks at the use of electrochemical biosensors regarding a potential detection kit for the rapid identification of the COVID-19.

## 1. Introduction

In late 2019, severe acute respiratory-related coronavirus (COVID-19) emerged in Hubei Province, China (Bai et al., 2020, p. 19). As of May 29, 2020, according to the World Health Organization (WHO), the pandemic has spread to 216 countries and infected more than 5 704 736 people, resulted in 357 736 deaths. It was found that the genome of COVID-19 had ~80%, ~50%, and ~96% similarity to SARS-CoV, Middle East respiratory syndrome virus (MERS-CoV), and bat coronavirus RaTG13, respectively (Zhou et al., 2020), (Lu et al., 2020a,b). The development of vaccines for the treatment of COVID-19 patients is yet to come, therefore sensitive, selective, and rapid diagnostic can play a vital role in the containment of this new virus.

During last few decades, virus borne infectious diseases causing outbreaks and become the major concern of today's world which includes influenza virus, Zika virus (ZIKV), human immune deficiency virus (HIV), Ebola virus (EBV), dengue virus (DENV), hepatitis virus and

most recently COVID-19 (Khater et al., 2017; Kwon et al., 2020; Lu et al., 2020a,b). These viruses are genetically stable and creating ongoing threats to public health due to their fast spreading capacity. Considering clinical point-of-care (POC) purposes, it is crucial to detect pathogenic viruses rapidly and accurately. Currently, polymerase chain reaction (PCR)-based tests are being used to diagnose COVID-19 infected patients (Chu et al., 2020). However, slow diagnostic (3–4h), rise to false-negative and false-positive results, the requirement of extra kits, lack of sensitivity, limit of flexibility make the PCR method vulnerable (An et al., 2020). To overcome these issues research focused has been driven to develop alternative approaches and devices. In light of this, the combination of PCR and lateral flow assay (LFA) technology, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) powered assays employing conventional LFAs (Sheridan, 2020), graphene-based field-effect transistors (FET) (Seo et al., 2020), optical biosensor combining plasmonic photothermal effect and localized surface plasmon resonance has been developed (Qiu et al., 2020). However,

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considering highly sensitive and selective, cost-effective, simple, label-free detection, POC testing, antibody seroprevalency, nucleic acid amplification-free, and rapid diagnosis, electrochemical biosensor might be potential for the detection of COVID-19 (McArdle et al., 2017), (Lai et al., 2018), (Christodouleas et al., 2018), (Sinawang et al., 2016), (Hajian et al., 2019). Development of electrochemical biosensor for COVID-19 detection is now in early stage. Therefore, a thorough review on electrochemical biosensor for virus detection will help biosensing communities as soon as to develop effective electrochemical biosensor platform for COVID-19.

At present, several kinds of virus detection tools can be found on the market based on biosensing techniques (Baek et al., 2019; (Nasrin et al., 2020), (Simão et al., 2020), (Kumar et al., 2020), (Wu et al., 2020), (Toldrà et al., 2020), (Mukama et al., 2020, p. 18), (Huang et al., 2020), (Faria and Zucolotto, 2019), (Siuzdak et al., 2019). Moreover, FET based biosensor, enabling portable devices, is considered for real-time detection of the EBV antigen (Chen et al., 2017), rotavirus (Liu et al., 2013), and recently graphene-based FET has been designed to determine COVID-19 viral load in clinical nasopharyngeal samples, using a specific antibody against its spike protein (Seo et al., 2020). Regardless of their accuracy, sensitivity and numerous favorable circumstances, the presently available biosensors possess essential demerits in terms of high cost, complex pre-treatment, time-consuming, and low stability. Presently, new recognition elements are envisaged as a competent technique and have been studied to improve nano biosensor technology attributed to rapid analysis capability, minimal sample pre-treatment and cost-effective to modernize virus diagnostic methods.

This review will look at the principle and types of electrochemical biosensors discussed recently according to the latest findings for rapid virus diagnosis. Furthermore, different approaches will be extensively discussed according to surface functionalization, biological recognition component (enzyme, antibody, DNA, etc.), and strategies for the labeling and immobilization. Moreover, several tables summarize detailed information about all diagnostic methods comparing related commercial biosensors and finally, a short conclusion is mentioned to sum up the review.

## 2. Principle of biosensor

A biosensor is an analytical device that combines a biological component with a physicochemical detector consist of a sensing bioreceptor, a transducer; and a detector with a digital output (Tsang et al., 2016). The transducer recognizes the analyte through a reaction and transforms the signal to translates molecular changes to a quantifiable signal (Li et al., 2010). To date, different types of biosensors have been developed including electrochemical, piezoelectric, optical, surface plasmon resonance, quartz crystal microbalance, fluorescence, magnetic etc. (Adam et al., 2010; Luo et al., 2008; Uliana et al., 2011; Xue et al., 2007; Zhu et al., 2009). In a biosensor, the target analyte of interest interacts with the pre-designed bioreceptor and produces a signal for the transducer. Depending on the interaction with target biomolecule to be detected, several types bioreceptors commonly used including enzymes/ligands, biomimetic materials, nucleic acids/DNA, antibodies/antigens etc. (Krejčova et al., 2013; Liu et al., 2011). Last decades, several biosensing methods have been exploited to detect proteins, cancer biomarkers, viruses, bacteria, nucleic acids and other distinctive analytes. These biosensors can detect changes at the microscopic level and several studies are reported that involves the detection of pathogens using biosensors. They provide better results in a short time with greater sensitivity and high selectivity and even at a small cost with easy processing in comparison with other conventional methods.

## 3. Electrochemical biosensors for virus detection

Electrochemical biosensors are the biosensing devices containing electrochemical transducer that transforms biochemical information

and possess advantages as simple instrumentation, high sensitivity, cost-effectiveness and the possibility of miniaturization (Silvestrini et al., 2015). Electrochemical biosensors commonly depend on the enzymatic catalysis reaction between the immobilized biomolecules and the targeted analyte that produces electrons and affects the electrical properties of the solution (Kamikawa et al., 2010). Depending on the detection principle and application, electrochemical biosensor devices can be of potentiometric, amperometric, conductometric, voltammetric, polarographic, impedimetric, capacitive, and piezoelectric (Silva et al., 2014). Moreover, depending on the biological element incorporated, different types of electrochemical biosensors are constructed, such as immunosensors, DNA sensors or microbial sensors (Krejčova et al., 2013; Liu et al., 2011). In this review, we will primarily focus on the recent development of impedance-based electrochemical methods, immunosensors and DNA based electrochemical sensors for the detection of various viruses recently reported. An electrochemical biosensor can monitor the activities of living cells or enzymes by measuring the interaction between an analyte and the bioreceptor. In recent years, electrochemical biosensors have shown great success in the fields of medical diagnostics due to their unique properties and easy-to-use platform. Fig. 1 represents the potential platform of electrochemical biosensor for the detection of pathogenic viruses.

### 3.1. Electrochemical impedance biosensor

Over a period of decades, viruses such as DENV, Avian influenza virus (AIV), Hepatitis virus, ZIKV, Enterovirus, HPV, Chikungunya virus (CHIKV), Rabies virus (RABV), Human norovirus, Japanese encephalitis virus (JEV), HIV, and Coronavirus cause outbreak of infectious diseases. Fast spreading and fatalities by these viruses lead to either a global epidemic or pandemic. Therefore, it is a crying need to establish smart biosensors. Electrochemical impedance spectroscopy (EIS) is considered as an efficient technique, which even detects any tiny changes that occur at the solution–electrode interface. Hence, EIS is widely used to characterize materials, and to monitor binding. Not only rapid response, low limit of detection (LOD), and low cost but also real-time monitoring of the samples attracts extensive research attention towards EIS method rather than using a traditional method like enzyme-linked immunosorbent assay (ELISA). Another benefit of impedimetric methods is that the necessity of enzymatic labels is not required to detect samples. On the other hand, EIS is a well-known technique to investigate the electrical properties of nanomaterials. While interfaces tend to expose to electronically conducting electrodes (Tlili et al., 2006) and (Hleli et al., 2006). Moreover, EIS has emerged as a powerful tool to quantify signals, because of its capability to separate surface binding from the solution resistance. It is worthy to note that, in the case of biological interaction, small amplitude perturbation does not reveal any negative effect (Bogomolova et al., 2009).

Santos et al. reported the first dual marker dengue electrochemical assay and showed that dengue antigen assay times could be reduced to a few seconds in serum by moving to an immittance assay from capacitance. Bifunctional self-assembled monolayer (BSAM) formed on electrode surfaces that contain PEG moieties and a tethered redox thiol (See Fig. 2 (A-D)). The approach leads to the rapid sensitive quantification of NS1 and IgG. 100 Hz optimized frequency was found, and the target-responsive data is shown in Fig. 2E (Santos et al., 2018). Nguyen et al. used serotype 2 (Denv2) as an analyte in the EIS system and explored that resistance and capacitance are effective to detect Denv2. The monitoring of capacitive signals plays a significant role in order to develop biosensors (Nguyen et al., 2012). Previously, Cheng et al. developed an electrochemical membrane-based nano biosensor, which showed a specific response of the nano biosensor towards DENV rather than flaviviruses and nonflaviviruses. The decrease in normalized nano biosensor signal response  $\{1 - (I_{\text{virus}}/I_{\text{virus}} = 0)\}$  toward DENV-2 is  $\sim 2.5$  times higher than the other nonspecific viruses according to Fig. 2F. Signal response  $\{1 - (I_{\text{virus}}/I_{\text{virus}} = 0)\}$  toward three different

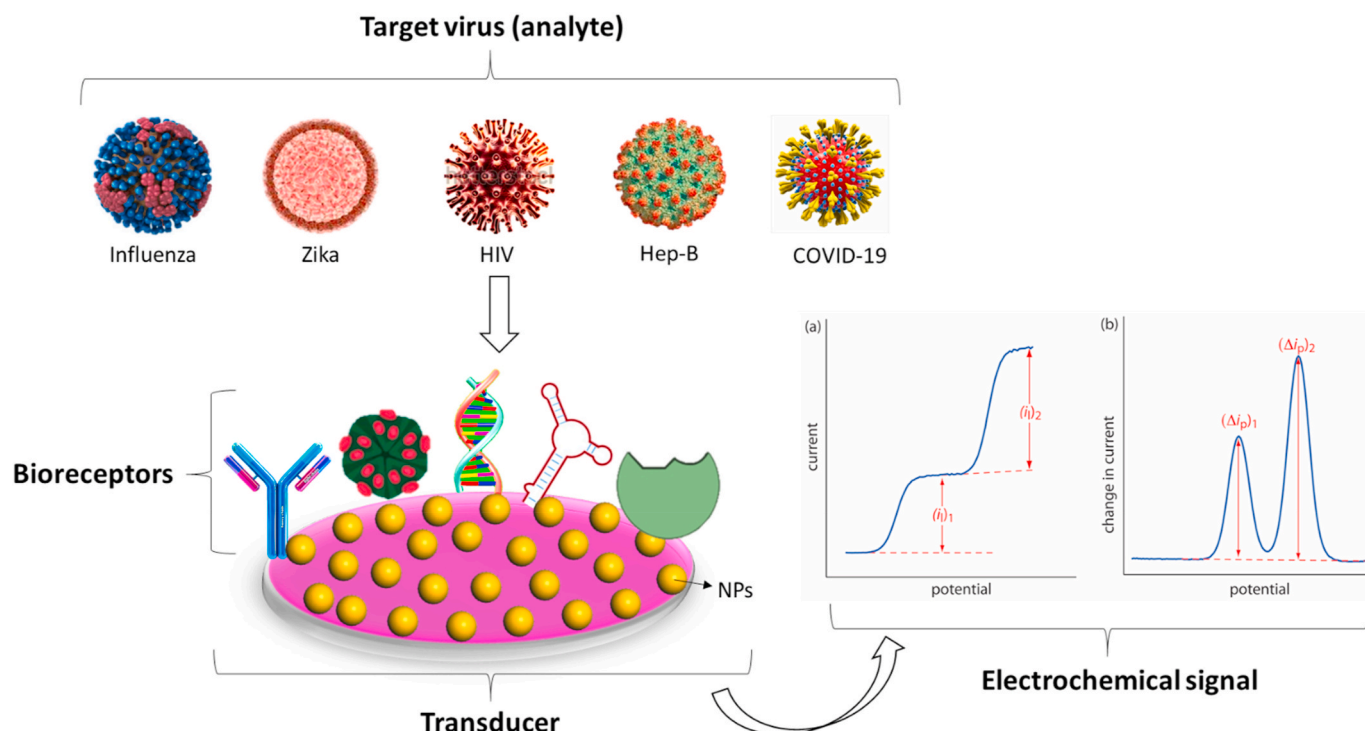


Fig. 1. Potential electrochemical biosensor platforms for the detection of various pathogenic viruses including COVID-19.

viruses was investigated. Where Ivirus depicts signal response after the virus adopts from  $102 \text{ pfu mL}^{-1}$  solution. On the other hand, Ivirus = 0 refers to the signal response of the same nano biosensor after 30 min exposure to antibody and BSA immobilized virus-free medium (Cheng et al., 2012). In another interesting study, Tung et al. exemplified electrochemical biosensor for confirming of weak interaction between DENV and its receptor (C-type lectin domain family 5, member A (CLEC5A)). Anodic aluminum oxide (AAO) substrate modified with gold nanoparticles (AuNPs) was used to immobilize CLEC5A followed by specific pre-treatments. The hybridization interaction between CLEC5A-DV was intensively investigated by EIS. (Tung et al., 2014).

The introduction of carbon nanoparticles onto electrode surfaces leads to the high surface. As a result, excellent sensitivity and reasonable detection of JEV on CNPs incorporated screen-printed carbon electrode (SPCE) is achieved by using the EIS method. According to EIS analysis, Unmodified SPCE and CNPs modified SPCE electrochemical biosensor showed  $764 \Omega$  and  $660 \Omega$  electron transfer resistance, respectively. JEV positive serum used as analytes and examined by EIS, which further proves the useful application of CNPs modified SPCE biosensor strip in the clinic. Fig. 3A depicts a schematic diagram of the SPCE modification procedure. The scanning electron microscopic (SEM) image (Fig. 3B) shows carbon nanoparticles (CNPs) on the SPCE strip. CNPs provide a large specific surface area for JEV antibody immobilization. Which improves electron transfer efficiency. In addition, the amino group on the surface of CNPs enable the attachment of antibody (Lai et al., 2017). Later, AgNPs was used to modify SPCE, and investigated by EIS analysis concerning JEV. Ag NPs modified SPCE showed a smaller semi-circular diameter than pristine SPCE, due to quick electron transfer kinetics of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  on the as modified sample. EIS result showed  $2.60 \text{ ng mL}^{-1}$  (at  $S/N = 3$ ) LOD against JEV antigen (Chin et al., 2019).

Baek and his team developed electrochemical biosensor by assembling eight peptides severally on Au electrode. Following that, sensitivity towards human noroviruses was conducted. Affinity strength of norovirus on the gold electrode was investigated by EIS analysis. It has been postulated that NoroBP-nonFoul(FlexL)2-coated Au electrode acts as an effective electrochemical biosensor. Fig. 3C shows a schematic

illustration of norovirus detection using novel peptide-coated electrochemical biosensor (Baek et al., 2019b). In 2009 Meiri et al. correlated immunosensor functionalized gold electrode with EIS method to detect Enterovirus (leads to human diseases), with 10 viral particles (vp) LOD (Meiri et al., 2009). Later, Hafaiedh et al. explored the EIS method for C-reactive protein detection (Hafaiedh et al., 2013).

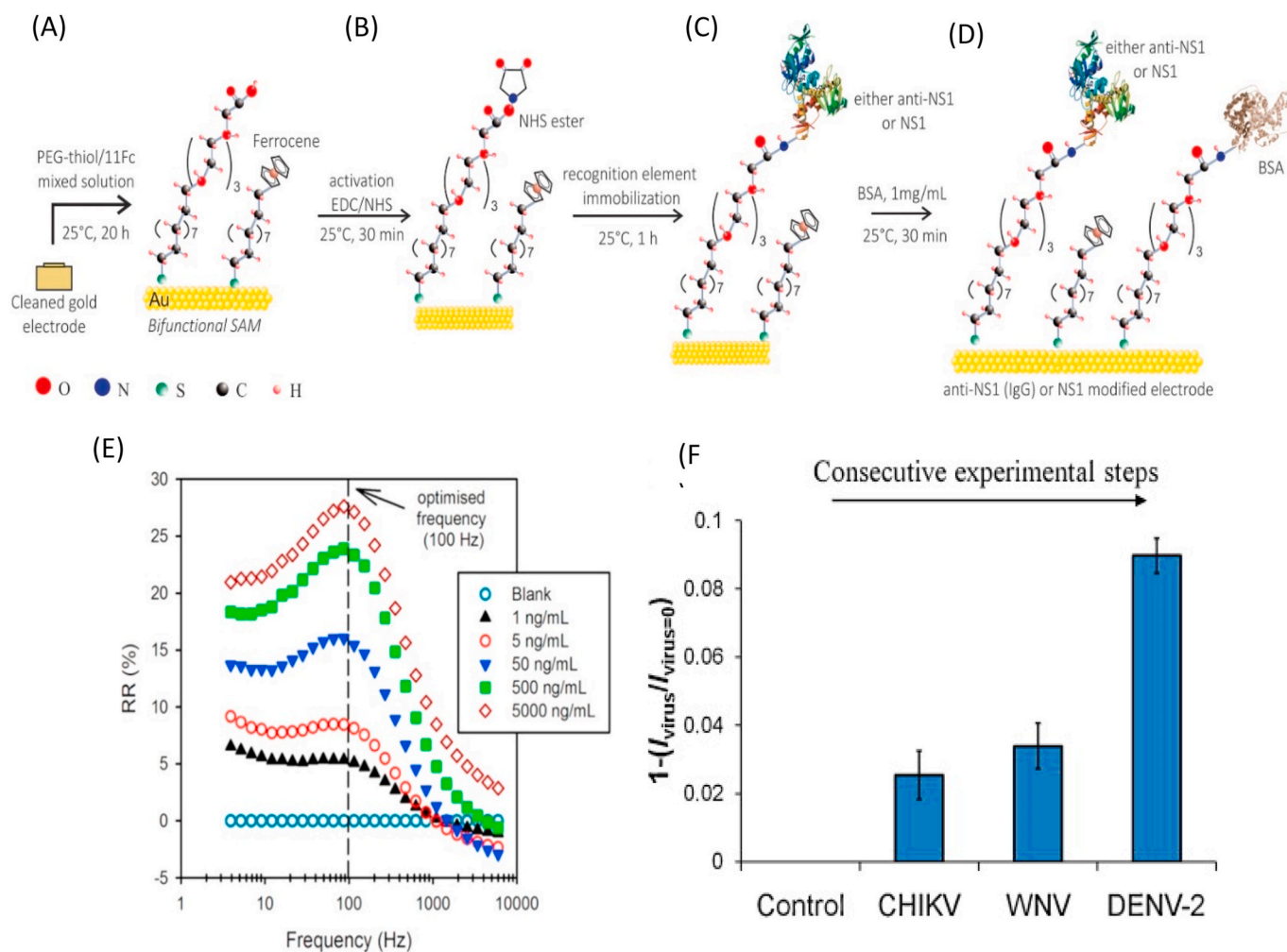
George et al. detected CHIKV-nsP3, which causes alphavirus replication, using the EIS method. Anti-CHIKV-nsP3 immobilized on Au surface by a self-assembled monolayer (SAM) of 11-mercaptoundecanoic acid and 6-mercaptohexanol. Charge transfer resistance was investigated for each stage of EIS based immunosensor development. EIS study revealed that electrode with SAM exhibits higher impedance than the bare electrode. SAM was activated by N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) to form NHS ester. As a result, impedance decrease effectively (George et al., 2019). It should not be left unmentioned that the study is consistent with previously reported literature (Radhakrishnan et al., 2016).

Mishra et al. studied increased impedance while measuring CD4+ (human cells) on the micro-electrode. Moreover, impedance also increased with increasing the number of captured cells (Mishra et al., 2005). On the other hand, EIS as a sensing tool to detect and quantify virus or drug-induced cell death in cell cultures was investigated by other researchers. For instance, Madin-Darby Canine Kidney (MDCK) cells infected with influenza A virus (McCoy and Wang, 2005), pancreatic and hematopoietic necrosis virus (Campbell et al., 2007b).

Heo et al. postulated a label-free electrochemical method to detect the hepatitis B virus (HBV) on the Au electrode surface based on immobilization strategy. The study indicates the potentiality of commercialization of a point-of-care testing (POCT) tool for viral diagnosis (Heo et al., 2012). Table 1 shows the analytical performance of EIS-based biosensors reported recently for the detection of different viruses.

Impedimetric detection of COVID-19 is at an early stage. Due to rapid response, low LOD, label-free detection, cost-effective, and real-time monitoring of the samples, the EIS method could be potential for the





**Fig. 2.** (A–D) Schematic construction of the anti-NS1 and NS1 modified electrode surfaces, (E) Example of  $1/C''$  immittance function investigation for NS1 detection in PBS, the optimized frequency, 100 Hz, corresponds to the most sensitive response with  $R^2 > 0.96$ . Adapted from (Santos et al., 2018). (F) Consecutive experimental steps using the same nano biosensor, investigated after 30 min incubations of CHIKV, WNV, and DENV-2 respectively. Adapted from (Cheng et al., 2012). Reproduced with permission, copyright @ Elsevier.

detection of COVID-19. For example, functionalized graphene-wrapped silica electrode materials were reported for viral RNA detection. Specific DNA primers were immobilized for hybridization with viral (dengue) RNA. Further, the EIS test explored the significant difference in interfacial RCT upon non-complementary DNA hybridization and complementary DNA hybridization (S.-A. Jin et al., 2016). Following the article, We assume that modifying this dengue RNA detection platform, design of specific COVID-19 DNA primers, specific hybridization between the probe (DNA primers) and spike surface glycoprotein (S)/small envelope protein (E)/matrix protein (M) or nucleocapsid protein (N) can play a significant role for COVID-19 detection.

### 3.2. Electrochemical immunosensors

Immunosensors are biosensing devices that are applied to detect particular antigens or antibodies through immunochemical reactions in body serum and many other media (Luppa et al., 2001). Immunosensors are mainly comprised of a bioreceptor and a transducer. A bioreceptor is used to recognize the target antigen or antibody that converts the producing biological signal into the desired signal by a transducer. It is an effective technique for the detection of pathogens because antibodies are naturally bound with antigens to form an antigen-antibody complex which is the main principle of immunosensor for detecting antibody or antigen (Pollap and Kochana, 2019). Four types of immunosensor can be

categorized according to the transducer, e.g., electrochemical, thermometric, optical and magnetic (Patris et al., 2016). In electrochemical immunosensor, the biological signal is converted into an electrical signal when the antigen-antibody complex is formed (Yadav et al., 2019). Besides, electrochemical immunosensors exhibit significant interest in clinical diagnostics (Gómez et al., 2010). Based on the transducing mechanisms, electrochemical immunosensors can be performed as non-labeled or labeled immunological biosensors (Oswald et al., 2000). Both types of immunosensors had been successfully used for the detection of particular viruses. Different viruses like, *Plum pox virus* (Jarocka et al., 2013), *DENV* (Darwish et al., 2015), *Fig mosaic virus* (Haji-Hashemi et al., 2019) etc. are detected by electrochemical immunosensor recently.

Non-labeled electrochemical immunosensors possess a very simple and low-cost technique because it requires easy sample preparation, not too long detection procedure and no need of secondary antibody compared to labeled technique (Mazloum-Ardakani et al., 2015). The detection efficiency of label-free electrochemical immunosensors depends on the precise alignment of antibodies during the immobilization of antibodies (Shen et al., 2015). There are various immobilization techniques of antibodies, such as straight adsorption on the electrode surface, magnetic beads (MBs) or polymer matrices, SAMs, etc., that can be used. Among these techniques, SAMs of alkanethiols are widely applied techniques because this technique provides an easy way to

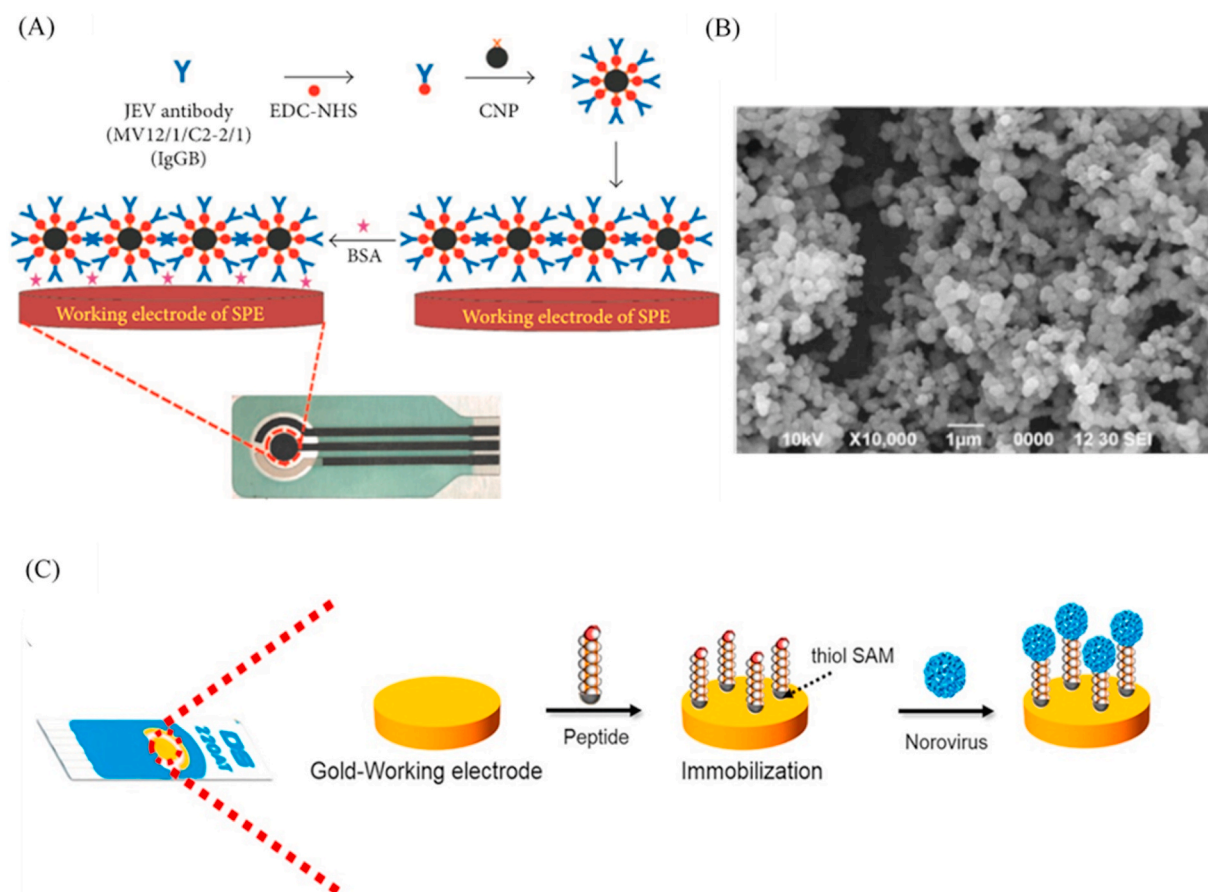


Fig. 3. (A) Schematic diagram of the SPCE modification procedure, (B) SEM image of carbon on the SPCE strip. Adapted from (Lai et al., 2017). (C) Schematic illustration of norovirus detection using novel peptide-coated electrochemical biosensor (Baek et al., 2019). Reproduced with permission, copyright @ Elsevier.

**Table 1**

Electrochemical impedance spectroscopy-based biosensor for the detection of a virus with respect to the comparison of analytical performance.

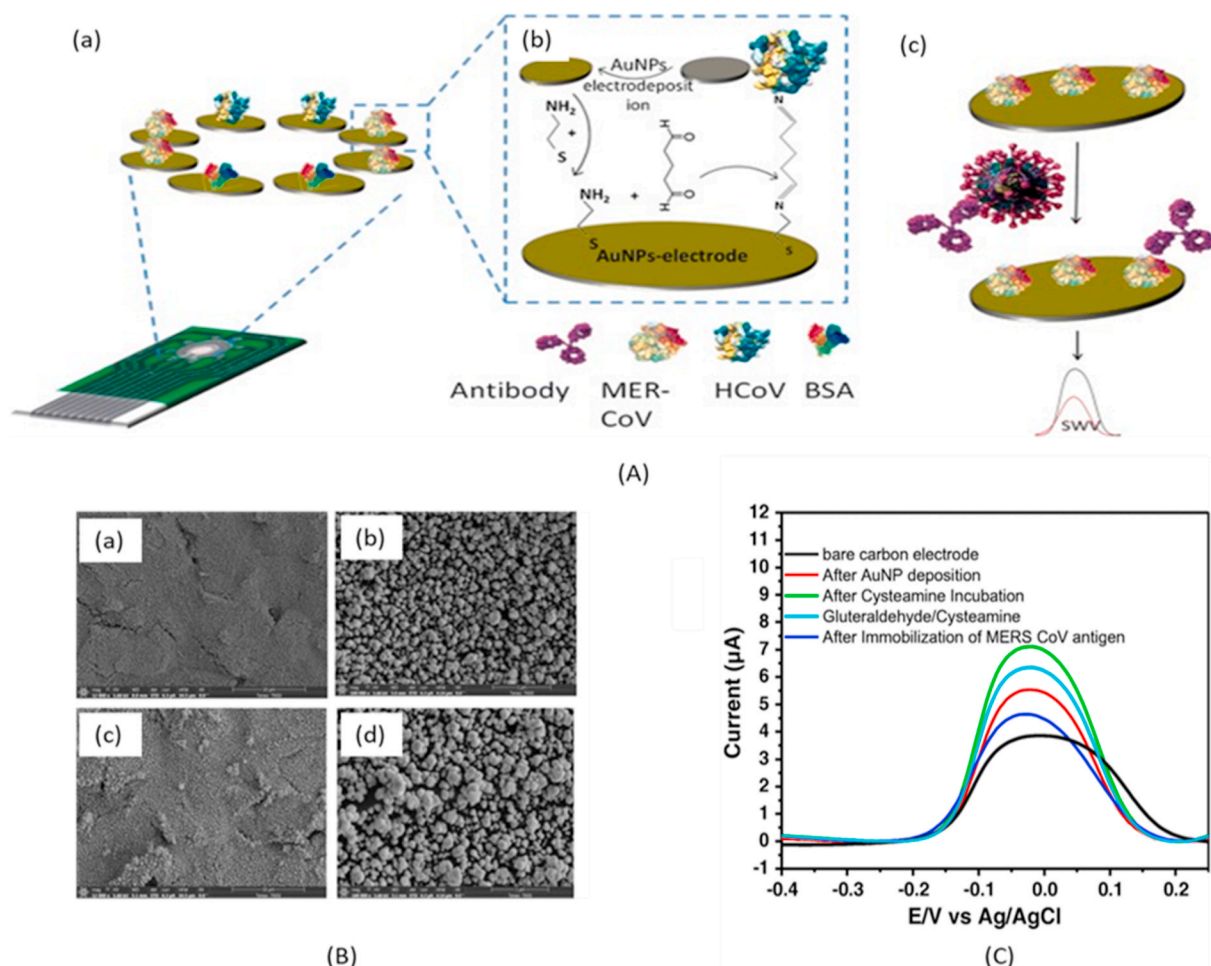
Analyte	Limit of detection (LOD)	Detection time	Linear range	References
serotype 2 (Denv2)	1 pfu mL <sup>-1</sup>	-	1 to 900 pfu mL <sup>-1</sup>	Nguyen et al. (2012)
HBV, antigen	0.14 ng mL <sup>-1</sup>	2h	1 ng mL <sup>-1</sup> to 50 µg mL <sup>-1</sup>	Heo et al. (2012)
JEV, antigen	0.75 µg mL <sup>-1</sup>	20 min	1–10 µg mL <sup>-1</sup>	Huy et al. (2011)
VSV (vesicular stomatitis virus), whole virus	10 <sup>4</sup> pfu mL <sup>-1</sup>	-	5 × 10 <sup>4</sup> to 5 × 10 <sup>6</sup> pfu mL <sup>-1</sup>	Muharemagic et al. (2012)
JEV positive serum	0.36 ng mL <sup>-1</sup>	10 min	1–20 ng mL <sup>-1</sup>	Lai et al. (2017)
JEV antigen	2.60 ng mL <sup>-1</sup>	20 min	0.1–20.0 ng mL <sup>-1</sup>	Chin et al. (2019)
Norovirus	1.7 copies mL <sup>-1</sup>	1.5 h	0 to 10 <sup>5</sup> copies mL <sup>-1</sup>	Baek et al. (2019)
RABV	0.5 µg mL <sup>-1</sup>	1 h	0.1–4 µg mL <sup>-1</sup>	Adnane (2011)
CHIKV-nsP3	8 ng mL <sup>-1</sup>	1.5 h	25 ng mL <sup>-1</sup> to 1 µg mL <sup>-1</sup>	George et al. (2019)
A/H7N1 Influenza virus	5 µg mL <sup>-1</sup>	-	-	Diouani et al. (2008)
HPV, cDNA	1 aM	-	1 aM to 1pM	Wang et al. (2013a,b)

produce strong covalent bonds, controlled arrangement, ultrathin,

oriented and ordered monolayer on the surface of the electrode (Haji-Hashemi et al., 2017). Monolayers of metals like gold, silver with the sulfur compound are able to give a proper immobilization of biomolecule. In the fabrication of electrochemical biosensor, SAMs functionalized carboxylic-antibodies group with succinimide and carbodiimide are also widely used (Chinnadayala et al., 2019). Several reports have been made for the label-free detection of the antibody-antigen complex by using electrochemical immunosensors. Among them, some applications have been discussed in this review paper.

Recently, an electrochemical immunosensor has been developed for the detection of highly pathogenic coronavirus associated with the MERS-CoV (Layqah and Eissa, 2019). Many serious respiratory illnesses even death of human-caused by this coronavirus. In this work, carbon array electrodes were fabricated with AuNPs electrodeposition to increase the sensitivity of the sensor. The authors applied recombinant spike protein S1 as a biomarker for MERS CoV. Fig. 4A illustrates the immunosensor preparation for the detection of MERS-CoV. The SEM images for the carbon array electrode surface modified with 20 CV scans and 30 CV scans at various magnifications were presented in Fig. 4B. SEM image shows the homogenous layer of spherical gold particles onto the electrode surface with an average of 50 nm diameter was obtained for 20 CV scans. The stepwise modification of the antigen modified electrodes was characterized by square wave voltammetry (SWV) as shown in Fig. 4C. This immunosensor showed a LOD of 1.0 pg/mL and a satisfying recovery percentage. The proposed sensor also exhibited high selectivity against Influenza A and B.

A highly sensitive as well as selective label-free electrochemical immunosensors for the detection of Fig mosaic virus (FMV) has been developed with a LOD of 0.03 nM (Haji-Hashemi et al., 2019). In this paper, a polyclonal antiserum (anti-FMV) against the virus nucleocapsid



**Fig. 4.** (A) Schematic drawing of COV immunosensor array chip (a), the fabrication steps of the immunosensor (b), application of the immunosensor for the virus detection (c). (B) SEM images of AuNPs deposited on electrodes using 20 CV scans at 12000x (a) and 100,000x magnification (b); the AuNPs deposited using 30 CV scans at 12000x (c) and 100,000x magnification (d). (C) SWV in ferro/ferrocyanide redox couple of the bare carbon array electrodes (black), after AuNPs electrodeposition using 20 CV scans (red), after cysteamine attachment (green), after glutaraldehyde activation (cyan) and after immobilization of MERS-CoV (blue) antibody (Layqah and Eissa, 2019). Reproduced with permission, copyright © Springer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was immobilized at the surface of the gold electrode modified with 11-mercaptopundecanoic acid (MUA) and 3-mercaptopropionic acid (MPA) through carbodiimide coupling reaction. Differential pulse voltammetry (DPV) was performed in ferri/ferrocyanide solution to evaluate the electrochemical detection of FMV.

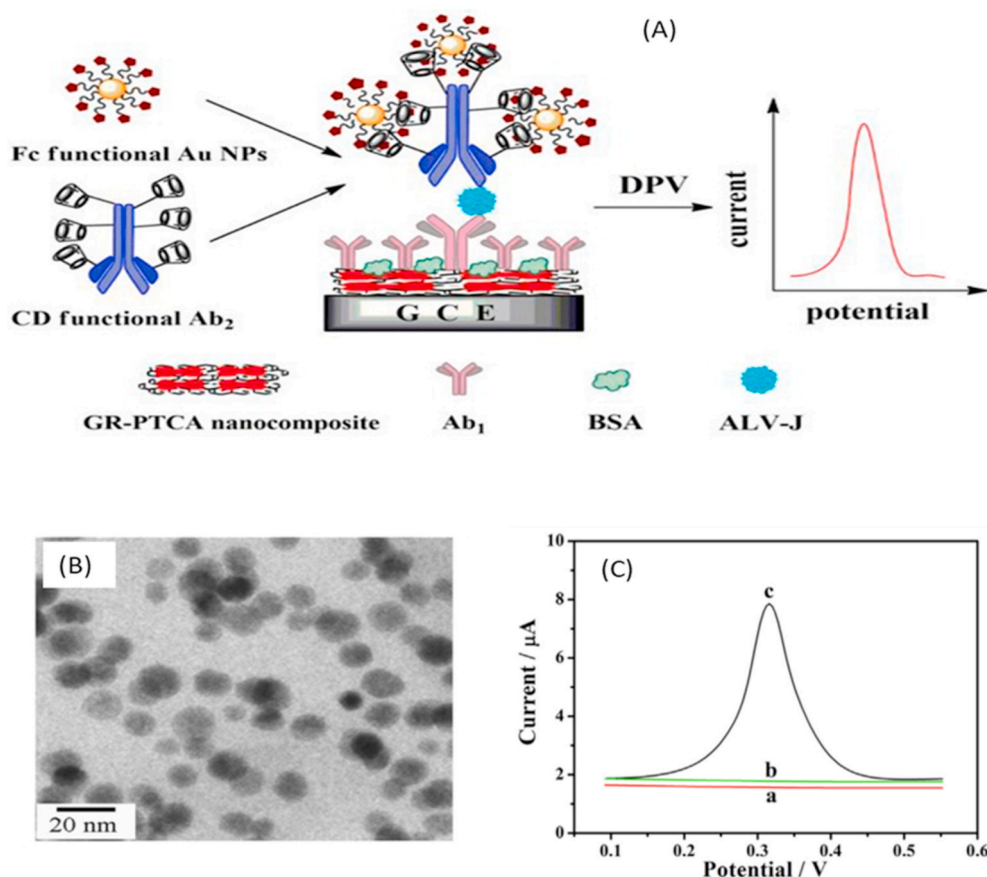
Recently, Ning et al. have designed a highly sensitive sandwich-type electrochemical immunosensor for the detection of avian leukosis virus subgroup J (ALV-J) (Ning et al., 2019). Avian leukosis virus is an endogenous retroviral pathogen that can cause the tumor, cancer etc. in poultry, doves, birds and mammals. Infections with avian leukosis virus caused huge financial losses to the poultry industry since the early of the 20th century. Although humans are not directly infected by this virus, it can pass into humans because the commercially available chicken and eggs carry this virus (Johnson, 1994). Authors have developed the sensor using a perylene-3,4,9,10-tetracarboxylic acid functional graphene nanocomposite (GR-PTCA) as the sensor platform and a -cyclodextrin-nanogold-ferrocene (CD-AuNP-Fc) host-guest complex as the label. The fabrication of the immunosensor is depicted in Fig. 5A. Firstly, -CD was directly immobilized on the secondary antibody (Ab<sub>2</sub>) through covalent bonding and then 6-ferrocenylhexanethiol functionalized Au NPs (AuNP-Fc) were induced via host-guest interactions. In this work, AuNPs decorated with 6-ferrocenylhexanethiol to increase the signal of immunosensor. Fig. 5B represents a TEM image of the Fc-AuNP composite. The diameter of the Au NPs used to prepare the composite was

near to the Fc-AuNP ( $13 \pm 3$  nm). DVP response was used to characterize the electrochemical signal of the modified electrodes as shown in Fig. 5C. DPV response of Ab1/GR-PTCA/GCE (curve a) and ALV-J/BSA/Ab1/GR-PTCA/GCE (curve b) exhibited negligible electrochemical response which indicates the biomolecules and GR-PTCA both are not electroactive. On the other hand, ALV-J/BSA/Ab1/GR-PTCA/GCE (curve c) exhibited a strong electrochemical response at the time of introducing to the electrode of Fc-AuNP-b-CD labeled Ab<sub>2</sub>. A linear concentration of ALV-J, ranging from  $10^{2.0}$  to  $10^{4.0}$  TCID<sub>50</sub>/mL and a limit of detection  $10^{1.93}$  TCID<sub>50</sub>/mL were achieved by the developed sensor.

Moreover, an electrochemical biosensor has been developed for POC detection of human enterovirus 71 (EV71) (Hou et al., 2018). Detection of EV71 by dual-labeled magnetic nanobeads (DL-MBs) based on enzyme catalytic reaction amplification has been reported by authors. In this work, horseradish peroxidase (HRP) enzyme and monoclonal antibody (mAb) was immobilized on magnetic nanobeads to prepare DL-MBs. High sensitivity with a  $0.01 \text{ ng mL}^{-1}$  detection limit was achieved by this developed sensor. Table 2 shows the analytical performance of electrochemical immunosensor for the detection of different viruses.

In March 2020, Xiang et al. reported two different immunoassays ELISA and colloidal gold-immunochromatographic assay (GICA) which are simple, fast, easy, and clinically adaptable for novel coronavirus





**Fig. 5.** (A) Schematic representation of the fabrication of electrochemical immunosensor. (B) TEM image of Fc-AuNP. (C) the DPV signal for different modified electrodes towards 102.3 TCID50/mL ALV-J: (a) Ab<sub>1</sub>/GR-PTCA/GCE, (B) ALV-J/BSA/Ab<sub>1</sub>/GR-PTCA/GCE, (C) Fc-AuNP-b-CD-Ab<sub>2</sub>/ALV-J/BSA/Ab<sub>1</sub>/GR-PTCA/GCE (Ning et al., 2019). Reproduced with permission, copyright @ Elsevier.

COVID-19 diagnosis. They reported that a combination of ELISA IgM and ELISA IgG produced detection sensitivity of 87.3% (55/63) whereas merged GICA IgM and GICA IgG produced 82.4% (75/91). Notably, both kits showed a negative response against controls (healthy samples) with 100% specificity (Xiang et al., 2020). Additionally, Li and his team have reported a rapid and POC adoptable lateral flow immunosensor which can diagnose both immunoglobulin M (IgM) and IgG antibodies simultaneously against COVID-19 in human's blood within 15 min (Li et al., 2020). The prescribed device was designed and manufactured by Jiangsu Medomics Medical Technologies, China, which was investigated for clinical validation and showed sensitivity and selectivity of 86.7% and 90.6% respectively against 397 positive and 127 negative PCR confirmed patients. They also concluded that IgM-IgG combined assay can produce better performance over only of their single choice (Li et al., 2020). Moreover, Zhao et al., in 2020 have revealed another immunosensor based on ELISA to detect total antibodies (Ab), IgM and IgG against COVID-19 from human serum. They diagnosed plasma of 173 patients with seroconversion rate for Ab, IgM and IgG was 93.1%, 82.7% and 64.7%, respectively (Zhao et al., 2020). The principle of Ab diagnosis is based on double-antigens sandwich immunoassay (Ab-ELISA) whereas IgM and IgG detection methods were based on IgM  $\mu$ -chain capture method (IgM-ELISA) and recombinant nucleoprotein respectively. Kit response to Ab, IgM and IgG from healthy samples (before the outbreak) was recorded as 99.1% (211/213), 98.6% (210/213) and 99.0% (195/197) respectively (Zhao et al., 2020).

### 3.3. DNA based biosensor

DNA based biosensors have the same assembly as a basic biosensor

with a receptor, a detector element that transformed readable signal from generated hybridization, and a processor to show the output. DNA based biosensors become the most efficient applicant in gene demonstration with sequencing diagnostic behavior. It exceeds the barrier of sampling size & synthesis complications (Stoughton, 2005). DNA biosensors are used in various ways to point out nucleotide sequences. Compared to traditional hybridization assessment, DNA biosensors provide rapid homo and bacterial nucleic acid information which broaden molecular diagnostics by capturing analyte and readout accurate biological responses. The targeted amount and hybridization intensity is the basis of a DNA based biosensors (Beattie et al., 1995). In the main, single-strand DNA (ssDNA) along with target (nucleic acid) provides speedy DNA sequence detection which makes it a considerate sensing element in medical diagnosis (Wang et al., 2011) as well as in the food industry and environmental analysis by measuring differential hybridization sensing from DNA probes and compatible sDNA. Due to all these features, it has been viewed more seriously in the area of research. Different types of DNA based biosensors like Electrochemical (Sun et al., 1998), Strip type (Glynou et al., 2003), Surface Plasmon Resonance (SPR) type (Wang et al., 2004), Optical (Minunni et al., 2005), Quantum dot-based (Kokkinos et al., 2015) biosensors have been introduced by many researchers in the field of applications.

Teengam et al. designed an electrochemical DNA sensor by anthraquinone-labeled peptide nucleic acid probe (AQ-PNA) using an inkjet printing technique for the determination of HPV-16 (Teengam et al., 2017). They electrostatically immobilized the DNA probe on graphene-polyaniline (G-PANI) modified electrode as shown in Fig. 6A. Peptide nucleic acid (PNA) with repeating N-(2-aminoethyl)-glycine units provide strong affinity & higher specificity for target DNA



**Table 2**  
Analytical performance of electrochemical immunosensor for different virus detection.

Virus	Recognition Element	Dynamic Range	Detection Limit	Reference
ZIKV	Specific envelop protein antibody	10 pM to 1 nM	10 pM	Kaushik et al. (2018)
MERS-CoV	Recombinant spike protein S1	0.001 ng mL <sup>-1</sup> to 100 ng mL <sup>-1</sup>	1.0 pg mL <sup>-1</sup>	Layqah and Eissa (2019)
Newcastle diseases virus (NDV)	Chicken egg yolk antibodies (IgY)	10 <sup>2</sup> EID <sub>50</sub> /mL to 10 <sup>6</sup> EID <sub>50</sub> /mL	10 <sup>2</sup> EID <sub>50</sub> /mL	Tran et al. (2019)
NS1 protein	Monoclonal anti-NS1 IgG antibody	5 ng mL <sup>-1</sup> to 4000 ng mL <sup>-1</sup>	5 ng mL <sup>-1</sup>	Darwish et al. (2015)
Fig mosaic virus (FMV)	Polyclonal antiserum	0.1 nM to 1 μM	0.03 nM	Haji-Hashemi et al. (2019)
Plum pox virus (PPV)	Anti-PPV IgG polyclonal antibody	10 pg PPV/mL to 200 pg/mL	10 pg/mL	Jarocka et al. (2011)
Avian leukosis virus (ALV-J)	Purified serum specimen with ALV-J and ALV-J antibodies (Ab)	10 <sup>2.0</sup> TCID <sub>50</sub> /mL to 10 <sup>4.0</sup> TCID <sub>50</sub> /mL	10 <sup>1.93</sup> TCID <sub>50</sub> /mL	Ning et al. (2019)
Human immune deficiency virus (HIV-1)	Horse radish peroxidase [HRP] enzyme and anti-p24 monoclonal antibodies	0.01 ng mL <sup>-1</sup> to 100 ng mL <sup>-1</sup>	0.008 ng mL <sup>-1</sup>	Zheng et al. (2012)
Point-of-care of human enterovirus 71 (EV71),	Horse radish peroxidase (HRP) and EV71 monoclonal antibody (mAb)	0.00 ng mL <sup>-1</sup> to 1000 ng mL <sup>-1</sup>	0.01 ng mL <sup>-1</sup>	Hou et al. (2018)
Avian influenza virus (H5N1)	Anti-H5 HA monoclonal antibody (Mab 6-9-1)	4 pg/mL to 20 pg/mL	2.1 pg/mL	Jarocka et al. (2014)
Epstein-Barr virus (EBV)	Mouse monoclonal antibody and rabbit polyclonal antibody	0.01 ng mL <sup>-1</sup> to 40 ng mL <sup>-1</sup>	0.62 pg mL <sup>-1</sup>	Zhang et al. (2016)
Citrus tristeza virus (CTV)	Purified rabbit polyclonal anti-CTV IgG antibody	1 nM to 5 μM.	0.27 nM	Haji-Hashemi et al. (2017)

sequences. EIS & SWV confirm the AQ-PNA immobilization & hybridization (Fig. 6B) of target DNA HPV-16 with a linear range between 10 and 200 nM & gave a LOD of 2.3 nM. As shown in Fig. 6C, seven gold electrode assembly was attached to the screen-printed Ag/AgCl reference electrode which allows thiolated hairpin-DNA probes sequentially onto the sensing surface. The multi-electrode array has an advantageous property of good reproducibility. The hybridization process was monitored by SWV (Fig. 6D) showing higher peak currents with the immobilized condition. Optimum accumulation time for methylene blue indicator was observed (5 min) for high reproducibility. Excluding cross-interference for multiplexed DNA sequence determination, this biosensor shows linear current over ranges from 20 to 100 nM for HIV-1 and HIV-2 with the same detection limit of 0.1 nM (S/N = 3). Furthermore, Cao et al. used the piezotronic effect of ZnO NPs nanowire template to design a sensitive biosensor for the detection of HIV. The ZnO nanowire provided an in-situ selective DNA detection of HIV (Cao et al., 2016).

Recently, a PGE based biosensor was found to be effective to diagnose both influenza A and influenza B in PCR amplified samples with detection limits of 35 nM and 21 nM respectively. DPV technique was utilized to monitor real-time hybridization between the probe and target DNA in the presence of acetate buffer solution as an electrolyte (Subak and Ozkan-Ariksoysal, 2018). Later, many research groups have

reported different Nps modified electrode-based biosensors focusing on improvement in the diagnosis process for several viruses.

Hepatitis B virus (HBV) becomes a major infectious disease that leads to liver failure or progress to liver cirrhosis. Based on the need to early diagnose this disease, Meric et al. developed electrochemical DNA biosensors for voltammetry detection of the DNA sequence of HBV (Meric et al., 2002). They targeted the immobilized oligonucleotide (21- or 24-mer single strand) and hybridized on the carbon paste electrode (CPE). After that, the target sequence for infection diseases was determined from hybridized and probe modified CPE by SWV using methylene blue as a hybridization indicator with PCR amplification of real sample. In 2008, Hassen et al. presented another DNA biosensor based on streptavidin functionalized magnetic nanoparticles for HBV detection by using EIS strategy (Hassen et al., 2008). Firstly, they modified the Au electrode with biotin following functionalized magnetic nanoparticles (biotin-streptavidin) and & immobilized DNA probes using 300 mT magnet. Magnetic particles enhanced specific cell identification sensitivity. They visualize & characterize nanoparticle layer before detection by Atomic force microscopy (AFM) & Cyclic voltammetry. After investigating hybridization by non-faradic impedance spectroscopy for different HBV DNA target concentrations they found excellent immobilization, which allows them to detect 50 pmol of HBV DNA on a sample of 20 μL without PCR amplification. Further, an electrochemical DNA biosensor was developed to detect HBV with high conductive gold nanorods (GNRs) (Shakoori et al., 2015). They used GNRs except for other emerging nanoparticles like colloidal gold (Cai et al., 2001; Zhang et al., 2004) to increase surface area. They placed GNRs on a modified 1, 6-hexanedithiol (HDT) electrode and used [Co(phen)<sub>3</sub>]<sup>3+</sup> as an indicator to study the immobilization of different DNA sequences. After characterization, they found good specificity with LOD of 2.0 × 10<sup>-12</sup> mol/L in a linear range from 1.0 × 10<sup>-12</sup> mol/L to 10.0 × 10<sup>-6</sup> mol/L for target DNA. Additionally, L. Ahangar and M. Mehrgardi in 2017 have reported an DNA-based biosensor where SiO<sub>2</sub> electrode was modified with a nano-porous layer of gold (working electrode) and ssDNA probe were covalently immobilized to trace complementary target element characteristic of HBV. The device was found linearly dynamic and sensitive in the range of 0.4–10 nmol of altered DNA showing reliable reproducibility confirmed by EIS and DPV (Ahangar and Mehrgardi, 2017).

Recently, a novel electrochemical based biosensor for the detection of deadly EV-DNA had been successfully developed (Ilkhani and Farhad, 2018). In this work, the DNA biosensor was fabricated using a thiolated DNA capture probe sequence immobilized onto the Au surface of screen-printed electrodes functionalized with thiolated single strand capture probe DNA (1 μM). It was used to capture biotinylated target sDNA (0.5M) and provides maximum hybridization in 60 min. The biotinylated target sDNA was labeled with a streptavidin-alkaline phosphatase conjugate for electrochemical detection of the enzymatic product. The hybridization process on the electrode was performed by the EIS and differential pulse voltammetry (DPV) methods. This fabricated electrochemical DNA biosensor showed a low detection limit of 4.7 nM using the standard deviation of blank solution for EBV complementary strand.

Rai et al. have fabricated an electrochemical nanoporous membrane-based DNA biosensor for DENV RNA detection by the immobilization of aminated DNA probes onto the alumina channel walls, which improves the sensitivity of the sensor (Rai et al., 2012). The electrochemical anodization was used to imprint alumina nanoporous membrane-like structure over the platinum wire electrode. DNA capture probe sequence was immobilized on the electrode and hybridized with complementary target DNA for the fabrication of DENV RNA -sensing devices. Later, Oliveira et al. have developed graphite-based probe DNA specific biosensor to diagnose DENV3 serotype in human serum having LOD of 3.09 nM (Oliveira et al., 2015). A silicon nanowire-based biosensor was proposed by Nuzaihan et al. for detection of the target DNA sequence of DENV with LOD ~2.0 fM and sensitivity 45.0 μA M<sup>-1</sup> was achieved (Nuzaihan et al., 2016). In another work, Odeh et al. have

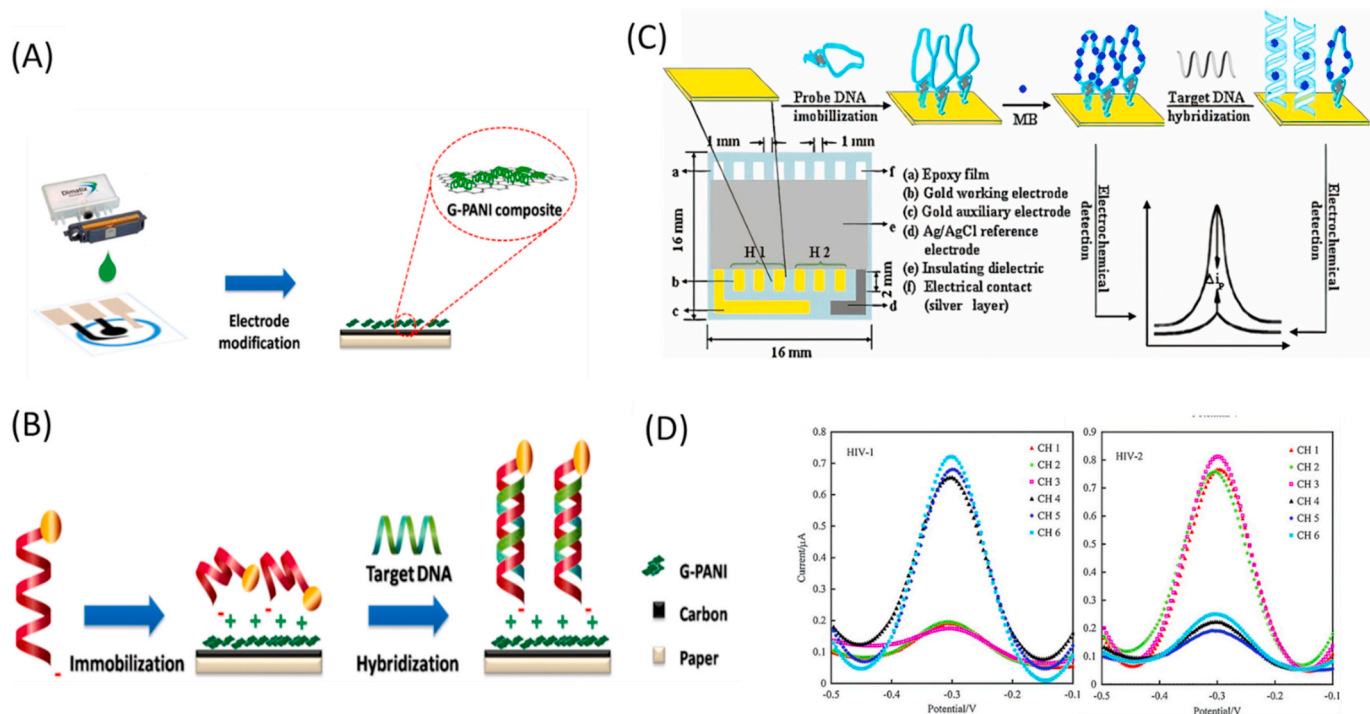


Fig. 6. (A) Schematic illustration of electrode modification with G-PANI. (B) immobilization and hybridization procedures of target DNA. (C) Cross-interference assays of the biosensor array for HIV-1 and HIV-2 detection. (D) Simultaneous detection of the biosensor array in the 100  $\mu$ L of electrolyte buffer solution (CH1~CH3 with sample solution & CH4~CH6 in immobilized condition). (Teengam et al., 2017, Zhang et al., 2010).

reported an amperometry biosensor for DENV2 serotype detection with LOD of 17 nM. The sensor is comprised of an immobilized dengue-specific DNA probe on  $\text{Cu}_2\text{CdSnS}_4$  quaternary alloy nanostructures deposited on an oxygen-etched silicon substrate ( $\text{O}_2/\text{Si}$ ) (Odeh et al., 2017).

Lee et al. reported a sensing platform for norovirus detection by using multi functionalized carbon nanotubes (Lee et al., 2018a,b). They fabricated the target DNA probe with Au/MNP-CNTs which is magnetically aligned on Pt-integrated electrode (PtIDE). A carbon chain (C6) based spacer was employed in the hybridization process. Under the magnetic field, nanomaterials can spread out well on the PtIDE electrode and enhanced sensing performance. Compared to other non-aligned platforms this method shows increased sensing behavior with different concentrations of target DNA (1 pM–10 nM) and confirmed the LOD around 8.8 pM. They also performed the selectivity test on influenza and ZKV virus DNA but among them, norovirus DNA shows an excellent response.

Recently, a promising DNA-based biosensor incorporated with silsesquioxane-functionalized gold nanoparticles (AuNPs-SiPy) modified glassy carbon electrode (oxidized) was introduced and showed selective performance for early-stage ZIKV diagnosis with LOD of 0.82 pM within 2 h (Steinmetz et al., 2019). Device performance was afterward intensely investigated by CV, EIS and AFM analysis which confirmed selective detection of ZIKV. The prescribed biosensor was also found stable and reusable after each use and performance restored around 98.0% of its initial response after 90 days.

Sun et al. in 2008 have reported a biosensor to detect cauliflower mosaic virus (CaMV) with LOD of  $4.38 \times 10^{-12}$  mol/L (3r) (Sun et al., 2008). In this method, target sequence ssDNA of CaMV was covalently immobilized on the surface of the mercaptoacetic acid-modified self-assembled Au electrode. On the other hand,  $\text{HNO}_3$  was used to dissolve lead sulfide (PbS) NPs capped probe oligonucleotide sequence (CaMV 35 S). Afterward three electrodes systems emerged inside and a sensitive differential pulse anodic stripping voltammetry method applied to observe hybridization between target ssDNA and complementary probe

ssDNA. Table 3 shows the analytical performance of DNA based electrochemical biosensors for the detection of different viruses.

In terms of COVID-19 detection, electrochemical transduction could be a potential approach. COVID-19 is an RNA virus and having single-strand RNA instead of ssDNA. By utilizing the corresponding immobilization of the single-stranded DNA probe nucleotide on to the biosensor, a specific viral RNA sequence of COVID-19 can be detected. During the outbreak, researchers have already reported different detection strategies focusing on DNA–RNA hybridization. Recently, Qiu et al. proposed a dual-functional plasmonic photothermal biosensor for highly sensitive and accurate COVID-19 detection by testing on SARS-CoV (Qiu et al., 2020). They made a DNA probe with functionalized gold nanoisland (AuNI) chips to match specific viral RNA sequences through nucleic acid hybridization. Combining the plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) sensing transduction significantly enhances the sensing stability, sensitivity, and reliability. Moreover, DNA hybridization can be considered as a portable electrochemical sensor for point mutation detection of COVID-19-specific viral RNA/cDNA (Tripathy and Singh, 2020). Future studies on the specific binding configuration of ssDNA on several viruses genomes could help to reduce the dependency of PCR-based tests and can be used to prevent future pandemics.

#### 4. Concluding remarks and future prospects

Since the coronavirus COVID-19 pandemic is the defining global health crisis of the present time and the greatest challenge the world is facing now, the development of rapid and high-sensitivity assays for its detection has tremendous importance. During past decades, extensive investigation has been conducted for the sensitive detection of viruses and it is clear from the literature discussed in this review that biosensing methodologies have achieved huge improvements for virus detection in terms of selectivity, sensitivity, specificity, and response time. Herein, we have critically discussed different electrochemical biosensors to detect pathogenic viruses and their modification methods as well.

Table 3

DNA based electrochemical biosensors for the detection of different viruses.

Virus/Sample	Recognition Element	Dynamic Range	Detection Limit	Reference
Human Papillomavirus (HPV-16)	AQ-PNA DNA probe	10–200 nM	2.3 nM	Teengam et al. (2017)
Human immunodeficiency virus (HIV-1)	ss-oligonucleotide DNA probe	–	$4 \times 10^{-9}$ M	Wang et al. (1996)
Human immunodeficiency virus (HIV-1, HIV-2)	self-assembled hairpin-DNA probe	20–100 nM	0.1 nM	Zhang et al. (2010)
Avian influenza A virus (H7N9)	Biotin-labeled ssDNA	2.5 nM–100 nM	100 fM	Dong et al. (2015)
Influenza A virus	Immobilized DNA	1–10 nM	0.5 nM	Tam et al. (2009)
Hepatitis B DNA	Streptavidin	2.53–50.6 nmol/mL	50 pmol	Hassen et al. (2008)
Hepatitis B DNA	HBV DNA	$1.0 \times 10^{-12}$ – $10.0 \times 10^{-6}$ mol/L	$2.0 \times 10^{-12}$ mol/L	Shakoori et al. (2015)
Ebola (DNA)	Biotinylated target strand DNA	0–5 nM	4.7 nM	Ilkhani and Farhad (2018)
Dengue (DENV3)	Specific DNA probe	$1 \times 10^{-6}$ – $1 \times 10^{-12}$ M	$9.55 \times 10^{-12}$ M	Rai et al. (2012)
Norovirus (DNA)	DNA	1 pM–10 pM	8.8 pM	Lee et al. (2018)
ZKV	Primer DNA	54–340 nM	25 nM	Faria and Zucolotto (2019)

Moreover, the details of immobilization procedures, electrochemical response mechanism and their applications have been also mentioned.

Although biosensor technologies are highly promising, they present many challenges in order to move from the bench to their use in the point of care. The immobilization method of the concerned nano-materials and biological elements is critical to minimize the risk of mistakes and errors in virus detection. Another important issue is the lifetime of the assay, which is sometimes considerable. Indeed, great effort is required to provide portable and reusable devices capable of discriminating for viruses with high selectivity and sensitivity levels.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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