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Genosensors as an alternative diagnostic sensing approaches for specific detection of virus species: A review of common techniques and outcomes

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ABSTRACT

Viral infections are responsible for the deaths of millions of people throughout the world. Since outbreak of highly contagious and mutant viruses such as contemporary sars-cov-2 pandemic, has challenged the conventional diagnostic methods, the entity of a thoroughly sensitive, specific, rapid and inexpensive detecting technique with minimum level of false-positivity or -negativity, is desperately needed more than any time in the past decades. Biosensors as minimized devices could detect viruses in simple formats. So far, various nucleic acid, immune- and protein-based biosensors were designed and tested for recognizing the genome, antigen, or protein level of viruses, respectively; however, nucleic acid-based sensing techniques, which is the foundation of constructing genosensors, are preferred not only because of their ultra-sensitivity and applicability in the early stages of infections but also for their ability to differentiate various strains of the same virus. To date, the review articles related to genosensors are just confined to particular pathogenic diseases; In this regard, the present review covers comprehensive information of the research progress of the electrochemical, optical, and surface plasmon resonance (SPR) genosensors that applied for human viruses' diseases detection and also provides a well description of viruses' clinical importance, the conventional diagnosis approaches of viruses and their disadvantages. This review would address the limitations in the current developments as well as the future challenges involved in the successful construction of sensing approaches with the functionalized nanomaterials and also allow exploring into core-research works regarding this area.

1. Introduction

Viral infections are one of the global concerns that threaten the health, social, and economic dimensions of the life of millions of people. The well-characterized and newly-emerged viruses caused dynamic epidemics and pandemics around the world. A wide range of viruses with special structural and pathogenicity features have been detected by scientists. For example, Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human immunodeficiency virus (HIV), Human papillomavirus (HPV), Influenza viruses, Arboviruses like Zika virus (ZIKV), Dengue virus (DENV), and Chikungunya are most important and studied infectious agents for human [1–7]. Although most viral diseases are asymptomatic or mild, however, remarkable number of viruses are caused by deadly acute or chronic illnesses. Hepatitis viruses (A to E and G) target the liver and are responsible for hepatic and extrahepatic failures such as chronic hepatitis, occult hepatitis cirrhosis, hepatocellular carcinoma, hematologic, and renal disorders [4]. According to the World Health Organization (WHO) report, 1/3 people in the world have been infected by either HBV or HCV and around 1.3 million people have died of viral hepatitis in 2015 [8]. Acquired immune deficiency syndrome (AIDS), tumors, aplastic and hemolytic anemia are fatal diseases are caused by Retroviruses [9–11]; for instance, 1.94 million new cases of HIV

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infection and 0.95 million deaths were reported in 2017 [12]. Arboviruses led to acute viral hemorrhagic fevers (VHFs) including; Dengue fever (DF), Dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), bleeding from organs, Marburg hemorrhagic fever (MHF), Ebola hemorrhagic fever (EHF), and yellow fever [13–15]. Different subtypes of influenza virus have resulted in acute and deadly pandemics in different nations during the 20thand earliest of the current century [16]. Meanwhile, severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) is responsible for the newly emerged viral pandemic named COVID-19 which is still killing millions of people [17]. In acute infections, clinical signs are nonspecific and time is a very important factor in the control of rapidly epidemiologically dangerous diseases such as Ebola, Dengue fever, Influenza, SARS-CoV-2, and MERS. On the other side, effective prevention of infection spread, clinical management, and successful therapeutic in chronic diseases including HIV and viral hepatitis depends on early diagnoses of the pathogen [18]. Consequently, detection tests of viral diseases should be applicable in the early stages of infection.

Generally, different ranges of old and newly-emerged diagnostic tests and toolkits including electron microscope (EM), virus isolation by cell culture, immunoassay-based tests, amplification-based approaches, next-generation sequencing (NGS), and mass spectrometry (MS) have been developed for recognition of viruses [19–24](Table 1). Each diagnostic test has its advantages and disadvantages which restrict its application in the detection of viral pathogens (Table 1). Since 1941, electron microscope (EM) was a useful tool for the direct detection of viruses in human specimens [25]. Although, EM is one of the direct diagnostic techniques for visualization and counting of the viral particles but now is seldom considered as a detection method [26,27]. Cell culture-based techniques are direct gold standard methods for virus propagation and isolation and also are used for assessing the cytotoxicity and cytopathic effects (CPE) of viruses [28]. Since the 1960s, virus culturing in the human and animal cell line was popular in clinical virology, nevertheless, low sensitivity, long time required for virus isolation, and need for experienced personnel limit the usefulness of this diagnostic assay in the clinical [27,29]. Thus, cell culture-based methods could not be considered as a rapid test for the diagnosis of viral illnesses at the onset of infection. Antibody production is one of the immune system responses to fight against viruses. Thus, testing the level of antibodies or antigens by immunoassay-based techniques is an effective approach for the diagnosis of viral pathogens in clinical specimens [30,31]. Up to now, different types of immunoassays were designed and tested for viruses (Table 1), however, The Enzyme-Linked Immunosorbent Assay (ELISA) and Radio-immunoassay (RIA) are well-known [20,32]. The formation of the antigen-antibody complex is a general basis of them [33]. Although they have acceptable sensitivity, specificity and are easily settled but the high cost of reagents, being time-consuming, false-positive or negative results are their drawbacks and -restrictions in clinical diagnostic [34,35].

In the past years, virus testing methods have experienced significant developments and molecular procedures have been become inevitable methods for the detection of viruses, with accurate and rapid results [36,37]. Currently, gene amplification-based methods are considered as a reference method for the detection of many viruses [22]. The molecular diagnostic procedures have wide applications in virology and they are used for virus detection and co-infections, genotyping genome sequencing, measuring the viral load, and management of infection [38–43]. Up to now, several modifications of PCR (polymerase chain reaction) were designed and patented to increase the capability of molecular tests (Table 1) [29,44]. However, conventional PCR, real-time PCR, and reverse transcription-PCR (RT-PCR) are the most popular and routine variants of molecular tests for the detection of the virus genome in clinical laboratories [45,46]. Besides the strengths of molecular diagnostic approaches including great sensitivity and specificity, this method is very time-wasting and creates the need for the assessment of virus genome in a faster way. The importance

Table 1
A summary of diagnosis testing approaches of viral infections.

Diagnostic Test Methods	Advantages	Disadvantages	Ref
Electron microscope (EM)	TEM[a] SEM[b]	Good for direct morphology information and counting of viral particles	Expensive, time-consuming, required to substantial technical skills	[71]
Virus isolation	Conventional cell culture shell vial technique	Acceptable specificity and sensitivity	Low sensitivity, long time required for virus isolation, need for experienced personnel	[20]
Immunoassay-based tests	ELISA[c] RIA[d] CA[e] MEIA[f] CLIA[g] FPIA[h] HI[i]	High sensitivity and specificity, easily settled	High cost of reagents, time-consuming, false-positive or negative results,	[29], [72], [73], [74], [75],
Molecular techniques .	PCR[j] Real-time PCR RT-PCR[k] DNA Microarrays LAMP[l] NGS[m]	cost-effectiveness, reliability and accuracy of results	Need to experienced operators, risk of contamination, need to specific primers for every target	[22], [76], [77]

[a] Transmission electron microscopy.
[b] Scanning electron microscopy.
[c] Enzyme-linked immunosorbent assay.
[d] Radioimmunoassay.
[e] Immunochemiluminescent Assay.
[f] Micro-particle enzyme immunoassay.
[g] Chemiluminescent immunoassay.
[h] Fluorescence polarization immunoassay.
[i] Hemagglutination Inhibition.
[j] Polymerase chain reaction.
[k] Reverse Transcription-PCR.
[l] Loop-Mediated Isothermal Amplification.
[m] Next-Generation Sequencing.

of this issue prompted the bio-scientists to focus on fabricating and testing the smart biosensing assays to immediate detection of viruses with the highest sensitivity, accuracy, specificity, selectivity, and simplicity, and eventually, their attempts led to designing several nucleotide and immunoassay-based sensors for the detection of wide range viral pathogens in clinical samples [3,36,47–50].

However, there exists some challenges and direction towards developing genosensors including: sensitivity, repeatability, analysis time and non-specific signals. For example, small dimensions of the nucleic acids and the nature of the attachment of DNA/RNA probe onto the electrode surface and large amount of sequences required to sensitize the sensor, make more difficult repeatability, sensitivity and specificity of such types of sensing devices [51–53]. Hence in recent years, they have been partially overcome by the positioning of the reporter between the base mismatches and the electrode surface and appearance new sensors fabricated by nanotechnology. But to succeed in commercialization requires more effort [54–57].

There are various review articles concerning the biosensor's application in health care and medical laboratory [58–62]. These articles include studies about a particular type of biosensor, e.g. electrochemical, for detecting none-human and human-infecting viruses [63–65] or pathogens in general [66–69] and implementation of biosensor in diagnosis of a specific infectious disease like human papilloma virus (HPV) [70]. Hence, in this review, we have contributed comprehensive information through utilized genosensors in virus detection. First clinical approaches for recognition of various kinds of viruses will be reviewed. Afterwards, different sensing platforms based on genosensing assays for highly sensitive determination of viruses will be discussed. Finally, a summary and future outlook of the current limitations and upcoming challenges in analyzing of various kinds of viruses will be explained as broadly as possible.

2. Genosensors

Biosensors have become very well-known in the last two decades and a wide range of them have increasingly been utilized in the field of food, forensics, bioterrorism, environmental, and in the therapeutic and diagnostic area of medicine [78–80]. Generally, biosensors are small analytical tools used for quantification and/or detection of biological analytes. In these bio-sensing platforms, biologically active elements including DNA, RNA, enzyme, antibody, protein, etc produce a measurable signal using the transducers [81–83]. The surprising advances in the biosensor branch and integration of this field with medicine, and also the inability of conventional diagnostic techniques to monitor the infectious agents with ultra-sensitivity lead to the development of diagnostic methods for recognition of pathogenic microorganisms (viruses, bacteria, fungi) in the clinical samples [84–86]. Although, nucleic acid, immuno, and protein-based biosensors could recognize and measure the genome, antigen, or protein level of microorganisms and the triggered immune responses against a pathogen, however, nucleic acid-based sensing techniques because of having ultra-sensitivity and applicability in the early stages of infections are preferred to the others [84].

Genes are genomic distinct region that contains the information for the synthesis of proteins. Gene-based recognition approaches such as nucleotide-based sensors rapidly developed for the detection of genes illnesses, especially viral infections in recent years [87]. The nucleotide-based sensing (NABSs) or so-called “genosensors” are biological devices that could recognize the target nucleic acids (DNA or RNA) based hybridization reaction [88]. The single-strand DNA (ssDNA) sequences called probes and target nucleic acid sequence are recognition elements of genosensors which their hybridization is monitored by a direct format, but, sometimes, the ss-DNA probe-target DNA complex on the surface of the sensor could not produce desirable changes in the transduction values, so, to improve the detection limits, sandwich, and competitive formats are preferred to the direct ones [89–92]. The principle of direct format is based on label-free detection by immobilization of ssDNA probe on a transducer surface, while in the sandwich and competitive formats, a mixture of DNA target and incubated ssDNA probe on the surface of the sensor through a specific label is used for recognition [93].

The biorecognition of oligonucleotides by genosensors is more useful and more valuable than the other types of biosensors because the complementarity of oligonucleotides is very specific, accurate, and potent. As the immobilization of single-stranded DNA sensor on the surface of recognition layer, such oligonucleotide binds to the target DNA based on base-pairing interaction. The gathering of target DNA molecules on the recognition surface gives rise to the generation of signals followed by their amplification [94]. The selection of transducer elements has a significant influence on the sensitivity and specificity of genosensors and it depends on the physio-chemical alterations of the biochemical reaction that happens in the biorecognition-layer. Genosensors have been divided into electrochemical (amperometric, voltammetric, and impedimetric), optical (surface plasmon resonance, colorimetric, fluorescent, luminescent, and interferometric), calorimetric, mass-based (magnetic, piezoelectric, quartz crystal microbalance, and acoustic-wave) based on transducer element [95],and, newly, they mostly considered for the detection of viruses genome based on hybridization ability of oligonucleotides [91,96–98].

Electrochemical genosensors are most popular because of their high sensitivity and specificity, rapid, and low-cost bio-sensing platforms for viral infections detection [99]. Fig. 1 represents the structure of an electrochemical genosensor developed for a DNA virus genome diagnosis; in these sensors, a hybridization event between an immobilized ssDNA probe onto the surface of the electrode and its complementary DNA produces a detectable quantitative signal, and eventually signal detection is done in two ways; i: direct detection (label-free) based on nucleotide oxidation in the DNA probe and ii: indirect detection (label-based) through using an indicator of the hybridization process [100]. Although, increasing the immobilization and DNA probe accessibility for DNA target is the main disadvantage of these genosensors but this foible is easily improvable by using form metal and oxide nanomaterials and nanoparticles including silver [101], gold [48], osmium [102], palladium [103], and platinum [104]; among them, gold nanoparticles are first choice to increase the sensitivity of electrochemical genosensors for the detection of DNA [105].

Piezoelectric bio-sensing devices or more precisely piezoelectric crystals are label-free monitoring methods that work based on oscillations change because of a mass bound on the transducer like quartz crystal disks. Good sensitivity and low price are the benefits of these types of sensors, however they are not completely commercialized and still applied in experimental studies [106,107]. Surface plasmon resonance (SPR) sensors are other types of are optical sensors that work based on internal reflection in a glass prism. In SPR genosensors polarized plasmon between analytes and biomolecules are online detectable without any need for labeling process. Besides these advantages, in the conventional SPR method, expensive sensor chips are used in biosensing tools and protein or ligand immobilization requires complicated chemistry, as well [108,109].

The quartz crystal microbalance (QCM) is a type of platform for biosensors that working on piezoelectric principle. Even though QCM-based biosensors are not as common as other biosensing methods like optical or voltametric ones, they are very popular in

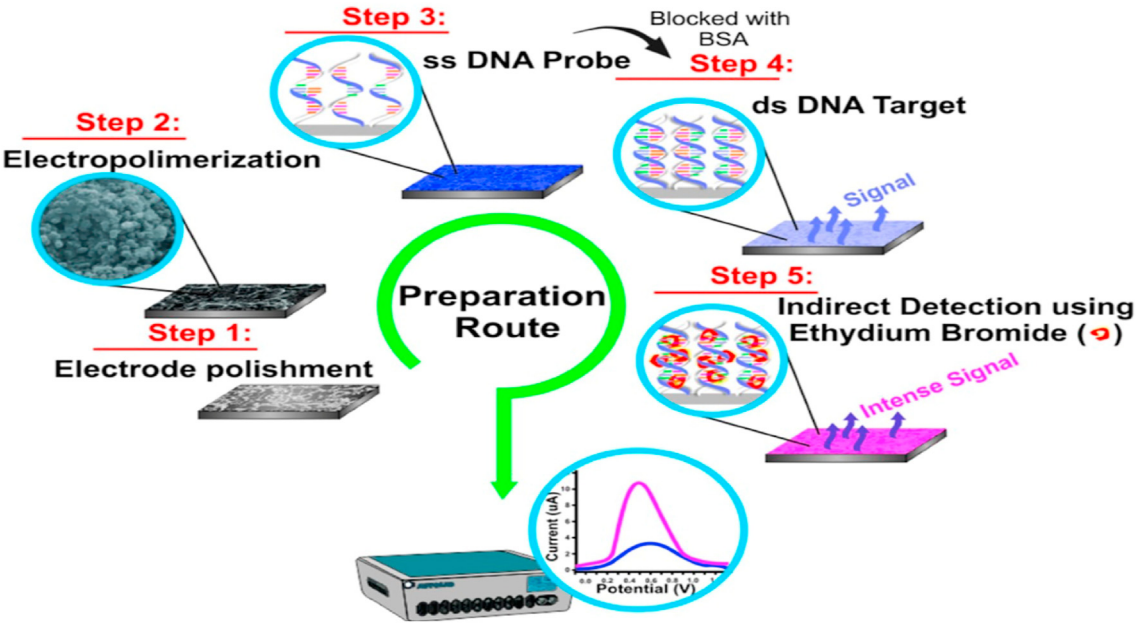

Fig. 1. Schematic presentation of an electrochemical genosensor developed for detection of a DNA virus. Step 1) Preparation and modification of graphite electrode with poly(4-aminophenol) on the sensitive surface, Step 2) Immobilization of oligonucleotide probes (ssDNA) by dropping poly (GA) or HepB1 onto the modified graphite electrodes, Step 3) Hybridization Of negative and positive (ds DNA) serum samples with poly (CT) or HepB2, Step 4)The electrode rinsing by immersion in phosphate buffer solution for 30 s under agitation, and Step 5) Analysis and detection of transduced electrochemical signal in sera samples after upon adding ethidium bromide to the electrode surface. This figure is obtained with permission from Ref. [100].

the field of pathogen detection because this method is highly sensitive, cheap and able to detect any types of biomolecules via a label-free method as well as quick response [110,111]. This system provides a selective and sensitive scheme to detect viral infections, and can be applied to an early screening of epidemic situations; further, provide effective methods for outbreak management and public health management [112].

3. Investigation of the various genosensing platforms for detection of different viral pathogens

3.1. Hepatitis B virus (HBV)

The most important member of viral hepatitis causes is HBV. This virus is a small DNA virus that could expose the liver of an infected person to several horrible liver-related diseases such as liver cancer or hepatocellular carcinoma. The infection is a world-wide health challenge that involves approximately 300 million people around the world with various manifestations from simple liver failure to cancer. The thing which deteriorates this infection is to become chronic if the virus, not is cleared from the patient's body by the immune system. Furthermore, the routine molecular and immune-based detection methods used for HBV monitoring are applicable only after several times upon infection [2]; hence, to overcome this limitation, abundant types of genosensors have been designed and used to diagnose this virus genome in the early days post-infection from clinical specimens during recent years [4,48,105]. Table 2 gives an overview of different types of genosensors designed and used for HBV detection.

Electrochemical genosensors are commonly designed biosensing platforms for HBV detection [99]. Until now, various types of electrochemical genosensors based on transducer elements including amperometric, voltammetric, and impedimetric [113] sensors with variable detection of limit (LOD) were developed for detecting of HBV genome [114–119]. For example, in 2020, M Shariati et al., have fabricated a highly sensitive impedimetric electrochemical genosensor based on tin-doped WO_3/In_2O_3 nanowire (WO_3/indium tin oxide (ITO) NW) for HBV diagnosis. In this bio-sensing method, tin-dopedWO_3/In_2O_3 nanowires have synthesized through physical vapor deposition and then four DNA sequences including thiolated ssDNA probe, complementary (a sequence of HBV genome), non-complimentary, and mismatch targets were used for bio-recognition aims on the surface of these nanowires. The label-free designed genosensor detected the hybridization among probe and HBV DNA through the oxidation reactions at very low concentrations of DNA with liner range at 0.1×10^{-12} M to10^{-5} M. This is an electrochemical bio-detection under laser emission led to releasing of more charged carriers and their interaction with DNA on the electrode surface and achieved the detection limit of 1 fM. Applying the laser amplification, photogeneration process, and electron-hole recombination rate processes are unique features of this study, which all of them led to distinguishable detection of complementary HBV DNA from the mismatch and non-complementary target sequences with ultra-high sensitivity, high selectivity, and 96% stability of its initial activity after six weeks [120].

In an investigation in 2017, an electrochemical genosensor platform was constructed for the diagnosis of HBV genome in the clinical samples by the cascade signal amplification based on a molecular beacon (MB) mediated circular strand displacement (CSD) and rolling circle amplification (RCA) strategies. In this label-free genosensor, target DNA was dropped on the surface of a gold electrode modified with molecular beacon (MB/GE), then MB mediated CSD was performed and eventually viral genome biosensing was followed by adding the circular template onto the electrode surface. To complete the detection, methylene blue was added onto the surface of gold electrodes to increase the differential pulse voltammetric (DPV) response of their sensor (Fig. 2). The platform's LOD reached 2.6×10^{-18} M with a liner range of 1×10^{-17} to 7×10^{-16} M of target DNA [115].

Table 2
The overview of various reported genosensing platforms for the detection of HBV.

Genosensor type	Method electrode assay time linear range detection limit Ref
Electrochemical	Impedimetric Gold−1 × 10^{-18} M − 1 × 10^{-6} M 1 × 10^{-18} M [126]
Piezoelectric	Voltammetric Cobalt phenanthroline−281−563 × 10^{-6} M 2.46 × 10^{-8}M [127]
SPR	Impedimetric Glassy carbon−0.0005−0.5 × 10^{-9} M 0.082 × 10^{-12} M [128]
	Impedimetric Gold ~ 1 h 3.48285−15.00996 × 10^{-15} M0.33,705 × 10^{-15} M [129]
	Voltammetric Gold ~170 min 1.10 × 10^{3}−1.21 × 10^{5} copies/mL 1100 copies/mL [130]
	Voltammetric Gold−4.00 × 10^{-13}M to 4.00 × 10^{-9}M 3.00 × 10^{-13}M [131]
	Voltammetric Gold−1.0 × 10^{-12}−10.0 × 0−6 M 2.0 × 10^{-12}M [132]
	QCM Gold 30 min - 1.6 × 10^{-18} M [133]
	QCM Gold 1 h 0.8−38.8 × 10^{-3}M 0.8 × 10^{-3}M [134]
	QDs ~ 4 h 10^{6} IU/mL to 10^{1} IU/mL. 10^{3} IU/mL [135]
	LSPR Silver 70 min0.5−100 × 10^{-15}M 5 × 10^{17} M [136]
	FRET Gold 50 min 0.045−6.0 × 10^{-9} M15 × 10^{-12}M [137]

QDs; Quantum Dots, LSPR; localized-SPR, FRET; Fluorescence resonance energy transfer.

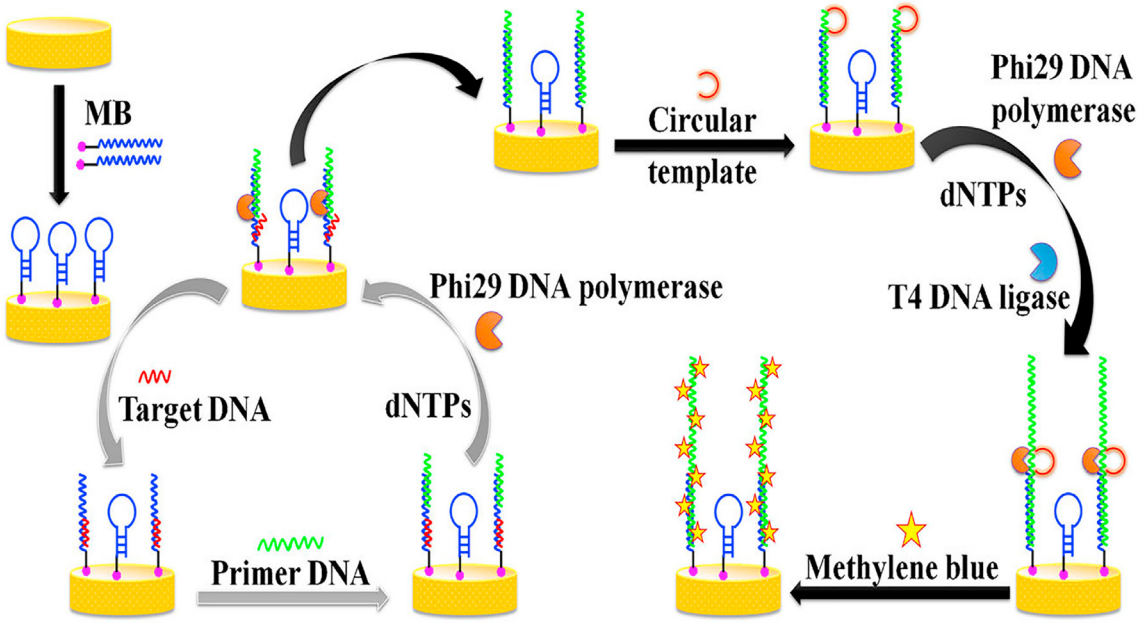

Fig. 2. The scheme illustrates the detection of the HBV genome via a voltammetric electrochemical genosensor preparation process. Present diagram represents the preparation and modification processes of gold electrode modified with molecular beacon (MB/GE) using bare GE and MB to bio-sensing of the ssDNA sequence derived from the genome of hepatitis B virus (HBV) by circular strand displacement (CSD) and rolling circle amplification (RCA) strategies mediated by a molecular beacon (MB). Hence, the target ssDNA hybridizes with the loop section of the MB/GE, while the rest of partial DNA sequences of MB hybridizes with primer DNA. In the following steps, the RCA initiates in the presence of dNTPs, Phi 29 DNA polymerase, and T4DNA ligase to produce a long DNA strand with multiple tandem-repeat sequences and this results led to detection of the induced voltammetric signals in the presence of electrochemical probe Methylene Blue. This figure is obtained with permission from Ref. [115].

Besides the electrochemical assays, piezoelectric genosensors were designed for HBV monitoring [36,121]. In the last two decades, three types of these label-free bio-sensing platforms such as quartz crystal microbalance (QCM) [122], microcantilever biosensors [123], and surface acoustic wave (SAW) [2] were tested for detecting HBV DNA in clinical samples; some of them are listed in Table 2. For real-time detection of HBV-specific sequences genome, Ya et al., have constructed a peptide nucleic acid (PNA) piezoelectric genosensor. In the PNA QCM platform, the biotin-avidin method was applied to deposition of PNA and RecA protein-coated ssDNA probe on the surface of a crystal disk following a common traditional procedure. The data showed that hybridization was done in less than 1-h time with 19.3242 × 10^{-18} M limit of detection rate and 94.44% specificity in comparison with real time-PCR assay. The constructed PNA probe could able to distinguish sequences of target DNA that were different only in one base, hence, improving the efficiency sensitivity of the sensor and reducing time needs for doing the test were the main reasons for using this protein-coated probe [122]. In another piezoelectric sensing platform, a silica nanoparticle (SiNPs)-improved dynamic microcantilever platform was tested for HBV DNA detection. Conjugation of detection probe with silica nanoparticles and capture probe with the surface of microcantilever disks were conducted to detection of 243 base pair (bp) of the core region of HBV target DNA. The sequence 243 bp of target DNA was diagnosed up to pM level without using of nanoparticles (NPs) and up to fM level in silica NPs-improved microcantilever platform. As depicted in Fig. 3, a linear correlation was seen among resonant frequency alterations and HBV target concentrations, which was due to the increased mass arising from the binding of the probe to the HBV DNA and SiNPs to the HBV DNA to improve the signal. The detection limit of 2.3 × 10^{-15} M and around 2−3 folds of enhancement in sensitivity were salient features of this label-free procedure [124].

Furthermore, SPR sensors were utilized for HBV genome detection [105] (Table 2). These NABSs sensors are optical sensors that work based on internal reflection in a glass prism. In SPR

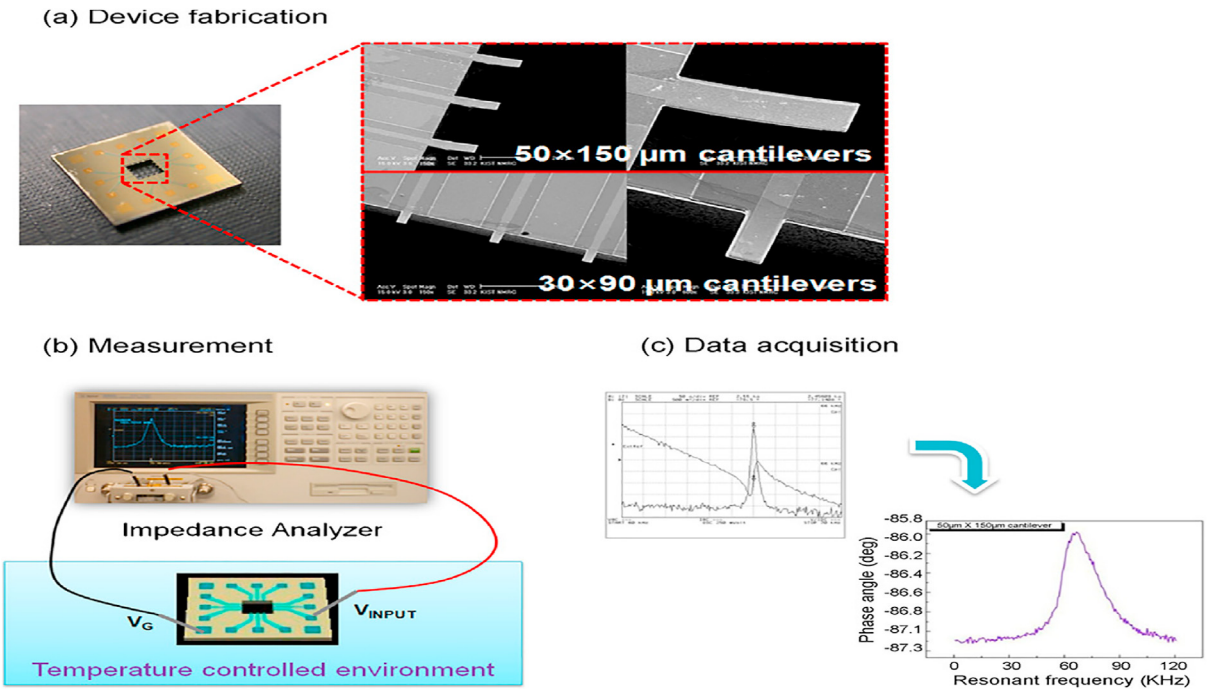

Fig. 3. Measurement of the resonant frequency and data receiving from a dynamic microcantilever: (a)is a picture of a device containing 12 microcantilevers and SEM photographs of 2 kinds of microcantilevers, (b)is a diagram of equipment utilized for estimation of the frequency resonant from the dynamic microcantilevers, which uses form an impedance analyzer, and (c) results of frequency resonant which is achieved the impedance signal. This figure is obtained with permission from Ref. [124].

genosensors polarized plasmon between analytes and biomolecules are online detectable without any need for the labeling process [108,109]. In a research work, Chuang and co-workers, a simple and inexpensive SPR genosensor platform were constructed based on a loop-mediated isothermal amplification (LAMP) way to the determination of HBV DNA. Their sensor was a new SPR based LAMP geno-sensing cartridge containing a polycarbonate (PC) prism and a polymethyl methacrylate (PMMA) surface. The limit of detection 4.494×10^{-18} M and almost 0.0011 refractive indexes (RI) were achieved by the SPRLAMP genosensing system for HBV DNA templates in only 17 min [125].

3.2. Hepatitis C virus

Hepatitis C virus (HCV), a positive-sense single-stranded RNA virus from the *Flaviviridae* family, is responsible for blood-borne non-A and non-B hepatitis [138]. The clinical manifestations of this infectious agent are variable from hepatic findings such as chronic cirrhosis and hepatocellular carcinoma to non-specific extrahepatic signs including cryoglobulinemia, renal, and dermatologic disorders. HCV could infect ~130–170 million individuals around the world by transmission through contact with infected blood, organs, and body fluids [139]. Generally, PCR-based methods are known as the gold standard detection tests of HCV infection in the clinical laboratories, however, they are costly, time-consuming, and are applicable by specialized equipment and technician several months post-infection [140]. On the other hand, HCV patients have a low cure chance. So, due to the aforementioned restrictions of conventional HCV monitoring techniques, different types of electrochemical, Piezoelectric, andSPR-NABSs were developed to HVC RNA detection in the early stages of infection and increase the efficiency of treatment [139,141–144]. Although the earlier applied DNA-based sensors for HCV detection require PCR technology for cDNA production from viral RNA [139,140,143], fortunately, using nanoparticles and nanomaterials improved the PCR-based genosensors disadvantages with increased portability, accuracy, and reliability [139]. For example, a rapid, simple, and cost-effective genosensor for direct detection of unamplified HCV RNA using gold nanoparticles with the sensitivity of 92%, specificity of 88.9%, and detection limit of 50 copies/reaction within 1 min was designed for HCV genome screening in the clinical specimens [140,145]. Albeit, HCV infection is detectable by both immunosensors and genosensors, but immunosensors are unable to discriminate between current and previous infection, and also detect the pathogen in the absence of antibody during the acute phase. Hence, NABSs with the highest sensitivity and selectivity are the best choices for HCV genome screening, viral load measurement, and HCV genotyping in clinical specimens [146,147]. Table 3 represents some examples of various types of genosensors developed for HVC detection.

Electrochemical sensors are the most popular bio-sensing devices for HCV RNA monitoring and could detect this virus genome directly (label-free) or indirectly (label-based) [147,148]. In the direct method, electroactivity of HCV cDNA bases results in detectable electrochemical signal changes [149,150]. While in the indirect method, target cDNA is detectable after labeling with a nanoparticle or a redox-active enzyme [142,151]. An in-vitro electrochemical diagnostic platform with a highly sensitive and selective were constructed for early detection of HCV-genome in the clinical samples. The immobilization of gold nanostructures modified indium tin oxide (ITO) substrate as a probe with the untranslated regions (5′-UTR) of HCV-RNA was monitored using Raman spectra and square wave voltammetry (SWV) techniques (Fig. 4A). The designed genosensor showed the limit of detection 264.5 IU/mL and ~101.5 IU/mL based Raman spectroscopy and SWV results, respectively [152]. Another simple label-free genosensor for detection of HCV genotype 1a (HCV1a) was designed using poly (L-glutamic acid) (PGA) modified pencil graphite electrode (PGE) by Donmez and co-workers in 2015. Inosine-substituted 20 per probes were immobilized on the surface of PGA modified PGE surface by covalent linking. During the hybridization of probe and

Table 3
List of some nucleic acid-based biosensors (NABSs) for HCV genome detection investigated in present article.

Genosensor type	Method	Electrode	assay time	linear range	detection limit	Ref
Electrochemical	Amperometric	Graphite	30 min	-	50 IU/mL	[158]
Piezoelectric	Impedimetric	Silica	-	100-10^6 copies/mL	90 copies/mL	[150]
SPR	Voltammetric	Pencil Graphite	30 min	0.05–0.75 × 10^{-3} M	6.5 × 10^{-9} M	[148]
	Voltammetric	Gold	60 min	1.5–2 × 10^{-6} M	0.02 × 10^{-6}M	[159]
	Voltammetric	Gold	-	0.05–4.0 × 10^{-6}M	0.5 ± 0.2 × 10^{-9} M	[160]
	Voltammetric	Ag/AgCl	10 min	2.57 × 10^{-21}-3.16 × 10^{-22}M	1.82 × 10^{-21}M	[161]
	Voltammetric	pencil graphite	60 min	0.05–0.75 × 10^{-6} M	54.9 × 10^{-9} M	[162]
	Impedimetric	Gold	20 min	1.36–4.53 × 10^{-9} M	1.36 × 10^{-9} M	[163]
	QCM	Gold	10 min	-	4.494 × 10^{-7}M	[143]
	SPR	Gold	30 min	25–2000 copies/reaction	50 copies/reaction	[140]

PGA/PGE; poly(L-glutamic acid)-modified pencil graphite electrode.

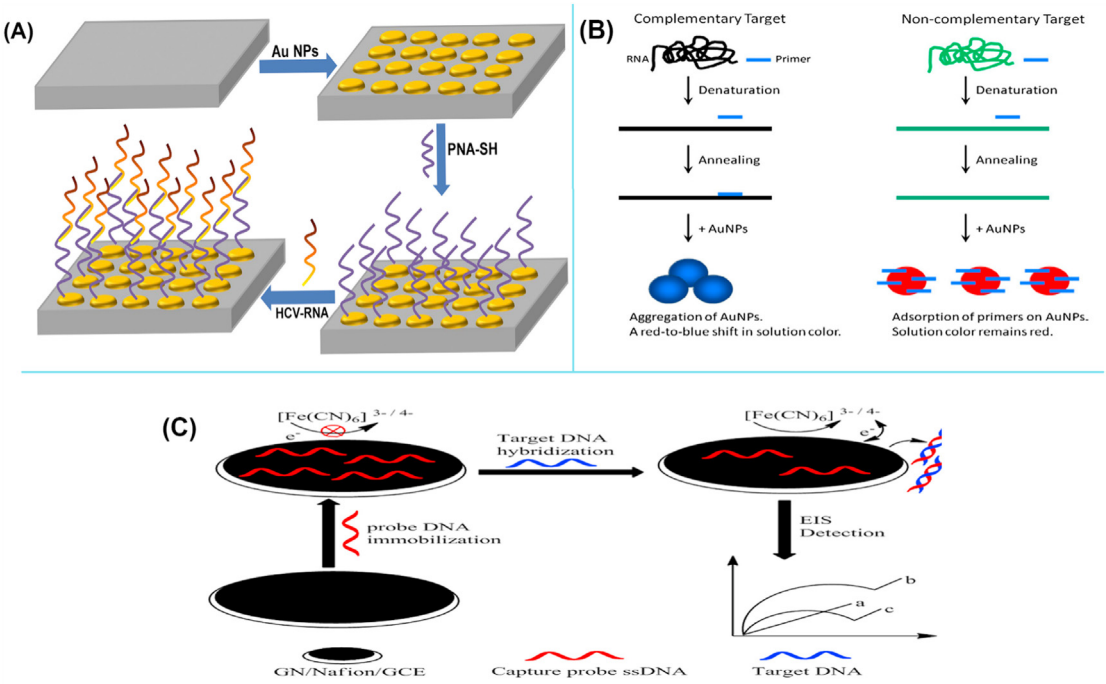

Fig. 4. (**A**): A schematic picture from the development of a voltammetric electrochemical HCV-RNA genosensor, first step: Fabrication of gold (Au) nanostructures modified indium tin oxide (ITO) substrate; Second step: immobilization of peptide nucleic acid (PNA-SH) onto Au nanodots modified ITO substrate; and Third step: probe binding with the complementary regions of HCV-RNA. (**B**): A schematic diagram of a label-free colorimetric SPR genosensor using gold nanoparticles for detection of unamplified 5′UTR of HCV RNA. In the presence of complementary target RNA and primer, the AuNPs were aggregated and the solution color changed from red to blue, while upon introduction of non-complementary target RNA, primers were adsorbed on AuNPs and the solution color remained red. (**C**): Schematic shows the structure of an impedimetric biosensor and detection of the target through the electrochemical impedance spectroscopy (EIS) strategy. The figures are obtained with permission from Refs. [140,152], and [176].

complementary region of HCV genome, the guanine oxidation signal was detected through SWV technique, which oxidation peak was seen at +1.05 V HCV1a RNA was detected in linear range 5 × 10^{-8} M to 1.0 × 10^{-9}M and limit of detection 40.6 nM by this cheap and disposable PGA-modified genosensor [142].

Despite recent advances in the production of antiviral therapeutics, there is no effective prophylactic vaccine for HCV infection due to the high rate of genetic variation of the HCV virus genome during replication in human host cells. HCV isolates are divided into six main genotypes (from 1 to 6) which subtype 3a is a more risky and dominant subtype globally [153]. Accordingly, the development of a reliable and sensitive HCV genotyping platform is very important to choose proper antiviral therapy and predicting of responses to treatment. Hence, many electrochemical genosensors were designed for HCV genotyping [153–155], and, in 2012, a commercial HCV genotyping electrochemical sensor with high sensitivity (97%) was enrolled in worldwide markets, called eSensor HCV Genotyping Test [156].

Publishes demonstrated that piezoelectric genosensor due to the real time monitoring of hybridization between the immobilized oligonucleotide probe and the complementary oligonucleotides of HCV genome in simple format are better than other types of NABSs. Also, it has been suggested that they are best choices for HCV RNA detection in real samples, from the economic point of view; however, only a few studies exist with HCV genome detection via these types of NABSs. An oligonucleotide probe modified piezoelectric quartz crystal was constructed for detection of RT-PCR amplification of HCV genome from sera of infected patients; the Amplicor commercial kit was used for production of HCV amplicons. For sensor designing, monolayer of cystamine was formed on the gold electrodes to generate the smooth and rough sensing surface and then immobilization of different biotinylated, avidin (streptavidin)-modified oligonucleotide probes for detection of HCV 1, HCV2A/C, HCV2B, and HCV3 was applied through the biotin/avidin

interaction. In this real-time monitoring QCM contribution, the total length 244 bases of HCV genome were diagnosed during only 10 min. Meanwhile, they pointed out that the reproducibility and performance of smooth crystals for HCV genome monitoring were better than rough sensors; this might be because of the less reproducible surface finish affecting also subsequently attached biolayers [143].

Also, for having the possibility of a high sensitive and label-free detection of HCV genome, Hwang et al. designed a nanomechanical microcantilever genosensor based on vibration modes for label-free detection of HCV helicase using RNA aptamer as receptor molecules. Production of detectable dynamic response on the microcantilever surface during the receptor-ligand binding event was the detection principle of their nanomechanical piezoelectric genosensor. Interaction between HCV helicase and RNA aptamer led to the detection of a low concentration of HCV genome (as much as 2.247×10^{-13} M) in the real samples. Furthermore, they suggested that using RNA aptamers as receptor molecules for the oscillating microcantilevers might be useful to increase the sensitivity of label-free detection [157].

It has been suggested that gold nanoparticles due to the showing of the unique phenomenon known as SPR are the best nanomaterials for use in the construction of colorimetric assays. Therefore, Sherif et al. designed the first rapid, simple, cheap, and label-free SPR genosensor using unmodified gold nanoparticles for detection of unamplified 5′UTR of HCV RNA obtained from infected sufferings. As depicted in Fig. 4B, the color changes from red to blue were detected in HCV positive samples within 1 min in the constructed colorimetric assay. The sensitivity, specificity, and detection limits of the colorimetric assay were 92%, 88.9%, and 50 copies/reaction, respectively. Detection of unamplified HCV RNA in clinical samples and elimination of the thermal cycling and detection instruments for cDNA synthesis are the main advantages of this SPR assay [140].

3.3. Human immunodeficiency virus (HIV)

Human immunodeficiency virus (HIV), a lentivirus, causes one of the great worldwide health challenges named acquired immunodeficiency syndrome (AIDS) [164]. The existed statistics demonstrated that around 36.9 million individuals of mankind were positive for HIV infection with a 0.8% prevalence rate at the end of 2017 [165]. Two types of HIV viruses including HIV-1 and HIV-2 are known as causative agents for AIDS, which have been classified according to their origin. Scientists suggested that HIV-1 was originated from chimpanzees, while HIV-2 originally transmitted from monkey species. However, HIV-1 is more viral and pandemic than HIV-2 and is responsible for most HIV infection cases [166]. The virus transforms from direct contact with body fluids and infects immune system cells which eventually led to immune system suppression of infected persons and susceptible them to opportunistic infections [167]. HIV infection is divided into three phases including acute, asymptomatic (window period), and AIDS phases [168]. Generally, antiretroviral therapy (ART) is a globally successful method to suppress HIV infection. But a remarkable ratio of HIV-infected individuals in low incoming and developing nations do not receive any effective ARTs because of the limited availability of rapid, sensitive, cheap diagnostic methods of HIV infection. For example, around 46% of HIV patients who needed ART worldwide did not receive medicine by the end of 2011 [169]. Therefore, HIV detection as soon as it enters the human body is a very important point to get rid of CD4 counting with flow cytometry and viral load measurement with real-time PRC gaps and also prevention of viral transmission during acute and window period phases of infection and increasing the efficiency of ART [164,166,170].

In the past two recent decades, the integration of virology with nanotechnology and bio-sensing platforms has opened helpful avenues to the designing of new detection methods for the HIV. To date, scientists tested and designed both types of immunosensors and genosensors to detection of HIV in clinical and research studies, but genosensors are preferred more than immunosensors due to having high sensitivity and applicability in the early days upon infection especially window period which antibody against the virus is not detectable [166,171–176]. Table 4 is a list of various genosensors fabricated for HIV monitoring.

The electrochemical genosensor technology is one of the most studied and interesting NABSs devices for monitoring HIV genome sequences due to their inherent advantages mentioned in the previous sections of this review [177–179]. Forasmuch as, HIV-1 is responsible for most of the HIV infection around the world [180]; hence, most electrochemical constructions were designed for this type of virus [181–183]. Nevertheless, there is a few reports from HIV-2 genome monitoring by this type of genosensor [184]. Fig. 4C shows an impedimetric DNA biosensor that were fabricated by Qiaojuan and co-workers for HIV genome. In this report, the ssDNA probe was adsorbed on the graphene-Nafion modified on the surface of glassy carbon electrode through the $\pi-\pi^*$ stacking interactions. In the presence of HIV genome, the ssDNA probe hybridization with the target DNA formed a double-stranded DNA (dsDNA) helix, which formation of this helix induced releasing of ds-DNA from the biosensor surface. HIV DNA Hybridization of graphene-Nafion/GCE led to induction of changes in negative charge and conformational transition; which these changes were detected by electrochemical impedance spectroscopy (EIS) strategy. The fabricated biosensor was capable to detect the HIV genome in linear range 1×10^{-13} to 1×10^{-10} M and limit of detection 2.3×10^{-14}. Furthermore, it was point out that Nafion could stabilize graphene and also induce graphene dispersion [176].

In a report, an electrochemical biosensor consisting of exonuclease III-assisted target recycling and guanine nanowire amplification (GWA) techniques were utilized for HIV genome detection. The Hairpin probe 1 (HP1), Hairpin probe 2 (HP2), Exonuclease III (Exo III), c-myc, Hemin/G-quadruplex and gold electrode were utilized for the construction of the genosensor and improvement of its sensitivity and selectivity (Fig. 5). The presence of target HIV DNA caused structural conformations in the HP1 and also induced the Exo III cleavage activity for gaining a digestion product named help DNA towards double-strand DNA. The obtained help DNA hybridized with the G-quadruplex sequence-locked HP1, eventually, formation of G-quadruplex structure with help of K+ and Mg2+ triggered guanine nanowire amplification (GWA). The applicability of the developed genosensor was evaluated by doing recovery experiments in the serum of healthy individuals. In the standard addition test, 10^{-10} M, 10^{-9} M, and 25×10^{-9}M of HIV genome were added to the diluted human serum specimens. The developed biosensors showed good accuracy and potential for HIV genome detection in the clinical samples by exhibiting 99.5%, 97.8%, and 94.1% recoveries, respectively. The proposed bio-sensing strategy had good performance for diagnosis of target DNA at concentrations ranging from 10^{-11} M to 10^{-7} M and LOD 3.6×10^{-12}M, which was lower than reported genosensors [185].

Besides the electrochemical strategies, a potential microcantilever genosensor as a HIV detector was employed by Abdullah and coworkers to detection of HIV ssDNA and ssRNA at conserved regions. In this label-free manner, several HIV genome sequences at different lengths and concentrations were tested for a deeper understanding of DNA–DNA and DNA-RNA hybridization. The ssDNA probe hybridization with ssDNA complementary target formed a dsDNA that induced comprehensive stress on the microcantilever

Table 4
The various genosensing assays utilized for HIV genome detection.

Genosensor type	Method electrode	assay time	linear range	detection limit	Ref
Electrochemical	Voltammetric GR/AuNCs/GCE	-	10^{-16} -10^{-7}M	3×10^{-17} M	[186]
Piezoelectric	Voltammetric SPE	-	$5 \times 10^{-11} - 3 \times 10^{-10}$ M	5×10^{-11} M	[187]
SPR	Amperometric gold	2 h	10^{-10}-10^{-5}M	7.05×10^{-12} M	[188]
	Impedimetric platinum	3 min	1×10^{-9} M $- 1 \times 10^{-6}$ M	5×10^{-10} M	[189]
	Impedimetric GC	2.30 h	1×10^{-12} M $- 1 \times 10^{-9}$ M	3×10^{-13} M	[190]
	- CPE	30 min	1×10^{-7} M -1.2×10^{-6} M	4×10^{-9} M	[191]
	Microcantilever	-	25 min $10^{-6} - 2 \times 10^{-19}$M	400 ng/mL	[175]
	SERS gold	-	0-10^{-13}M	10^{-19}M	[192]

GR/AuNCs/GCE; GR-AuNCs modified glass carbon electrode, GC; Glassy carbon, CPE; carbon paste electrode, SERS; surface-enhanced Raman spectroscopy, SPE; Screen printed electrode.

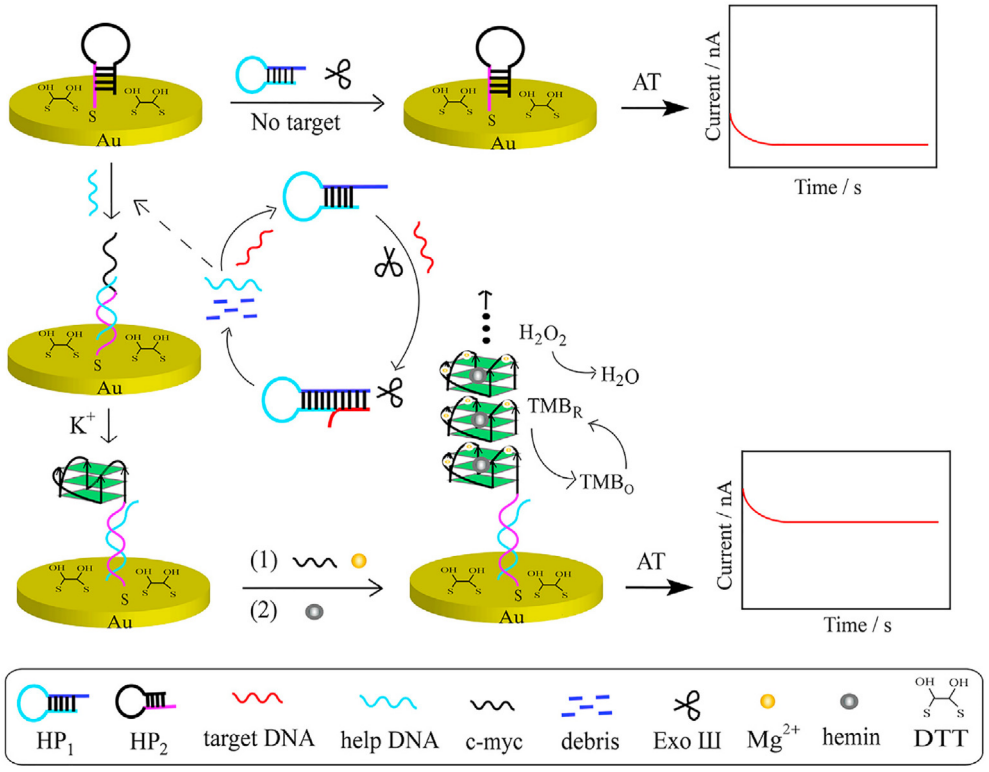

Fig. 5. Schematic exhibits the preparation process of the fabricated biosensor fabrication HIV diagnosis.

surface, while there was no deflection in active microcantilever and hybridization event when non-complementary sequences were introduced with ssDNA probe. It found that the variation in the microcantilever deflection upon the introduction of different concentrations of target DNA is the reason for the high sensitivity of this piezoelectric genosensor. The detection limit of 8.988×10^{-10} M was reported for the HIV genome by the fabricated microcantilever genosensor [175].

An innovative label-free SPR bio-sensing strategy with maximum sensitivity and duplicability was proposed for online monitoring of HIV DNA using the hairpin capture probes for immobilization on the gold sensing chip through Au—S bond and generation of a double-stranded complex. The sensor worked based on entropy-driven strand displacement reactions (ESDRs) and double-layer DNA tetrahedrons (DDTs). The advanced SPR system could efficiently detect virus genome in a linear range from 10^{-12} M to 15×10^{-8} M and limit of detection 48×10^{-15}M during window period in absence of antibody. In comparison with other biological sensors, this simple and low-cost sensor could be done without any need for chemical complexes and enzymes for only 1 h [174].

3.4. Human papilloma virus

Human papillomavirus (HPV) has a circular, double-stranded DNA genome that encodes early (E), and late (L) genes, which among them, E6 and E7 are necessary for transformation. According to the risk of cancer, infections are classified into two high risk and low-risk groups [193]. High-risk subtypes of the HPVs are HPV-16 and HPV-18 that are responsible for carcinogenesis and cervical cancers [194]. Pap smear is a traditional method for the detection of cervical cancer. PAP test has a sensitivity ranging from 30 to 87% [195]. So high rate of false-negative, poor specificity, and sensitivity are these methods' limitations. However, by developing molecular methods, novel strategies based on the use of HPV DNA assays expanded for the detection and identification of the HPV types [196,197].

The Benefit of molecular methods is their high sensitivity for HPV detection. Nevertheless, the methods mentioned have drawbacks such as high cost, the requirement of the trained operator, and advanced equipment [193]. Real-time PCR Detection range is 12.8 DNA copies [198]. Therefore, Due to the disadvantages of the

existing methods, in recent years, DNA sensor platforms for HPV-DNA detection are expanding as a simple, rapid, economical, sensitive, and specific diagnostic method [199].

Avelino et al. developed a biosensor composed of polypyrrole (PPy) films and gold nanoparticles (AuNPs) for specific detection of HPV genotypes in cervical specimens [200]. The biosensor was fabricated by using flexible electrodes based on polyethylene terephthalate coated with indium tin oxide (PET/ITO electrodes). In this study, AuNP loaded polymer films were obtained by the electrosynthesis method, and then modified oligonucleotides were designed to recognize HPV families were chemically immobilized on the nanostructured platform. CV and EIS were used to measure the electrode modification and monitoring of molecular hybridization. They obtained a linear performance in a range of 2.247×10^{-10} M to 2.247×10^{-15} M, LOD of 199.983×10^{-14} M, and LOQ of 6.0669×10^{-12} M with a regression coefficient of 220.206×10^{-14}. Screening tests on cervical specimens showed high specificity, selectivity, and sensitivity for HPV families.

As shown in Fig. 6A, an electrochemical DNA sensors based on a sandwich-hybridization using pyrrolidinyl peptide nucleic acid probes was fabricated to detect high-risk human HPV DNA sequences [201]. They first immobilized a capture PNA probe on a gold-deposited SPCE. Second, to obtain a higher signal response, an anthraquinone-labeled signaling probe (AQ P2) was designed to bind to the target DNA sequence at upstream (ASU) or downstream (ASD) positions. Compare to the ASU sensor, the ASD sensor showed a higher signal response. This biosensor detected the target DNA selectively in a detection range of 5×10^{-10} -10^{-7} M, and the limits of detection of 150×10^{-12} and 153×10^{-12}M were acquired for HPV type 16 and 18 sequences respectively. Furthermore, to specific detection of HPV DNA in clinical samples, Huang et al. introduced an ultrasensitive electrochemical DNA biosensor platform based on graphene/Au nanorod/polythionine (G/Au NR/PT) by using electrochemical impedance spectroscopy and differential pulse voltammetry techniques [202] (Fig. 6B). In this biosensor, two auxiliary probes were used to long-range self-assemble DNA nanostructure, in which the target DNA triggered the connection of the DNA structure to the capture probe immobilized onto the electrode surface. $[Ru(phen)_3]^{2+}$ was utilized as a redox indicator and enhanced significantly electrochemical signals. The DNA biosensor displayed a detection range of 1.0×10^{-13} to 1.0×10^{-10} M and a detection limit value of 4.03×10^{-14} M.

In a research, a highly sensitive label-free electrochemical DNA hybridization biosensor was designed for the detection of specific DNA sequences of HPV [203]. In this genosensing platform, the thiol-modified single-stranded DNA probe (HS ssDNA) coated onto the sensing surface of a screen-printed gold electrode (SPGE). Hybridization among HS ssDNA with its target DNA (complementary DNA) in solution to form double-stranded DNA (dsDNA) onto the surface of SPGE was performed. Under optimal conditions, the detection limit value of this biosensor is lower than 4.494×10^{-15}M.Karimizefreh et al. introduced an impedimetric biosensor for the specific detection of HPV DNA type16based on glassy carbon-gold nanosheet (GC-GNS) electrodes [204]. They synthesized a glassy carbon electrode modified with gold nanosheets. A single-stranded oligonucleotide (ssDNA) acting as the DNA probe was placed on the surface of the GC GNS electrode. They have demonstrated that the use of gold nanosheet can increase the active area of the gold nanosheet modified electrode, furthermore, this sensor can distinguish between single base pair mismatches, complementary and non-complementary HPV ssDNA, and as a result improve the sensitivity of the biosensor. The suggested biosensor

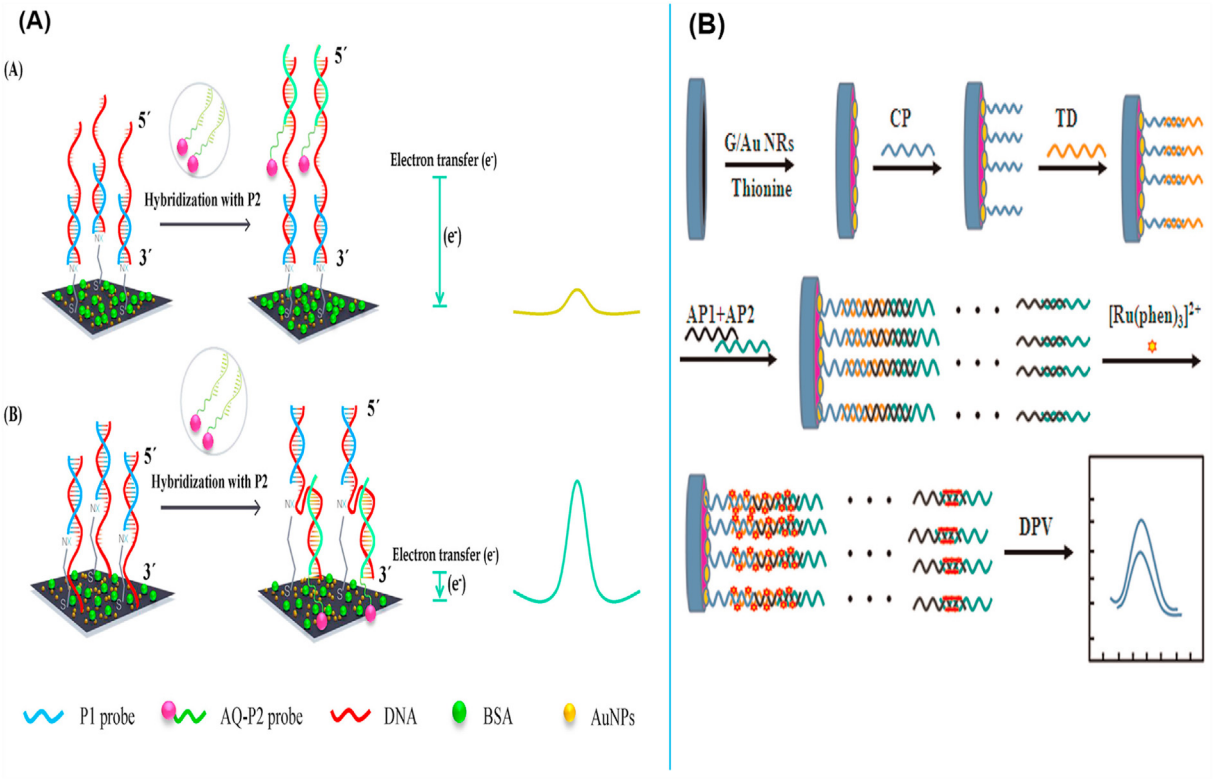

Fig. 6. (**A**): The fabricated electrochemical DNA sensors based on a sandwich-hybridization using pyrrolidinyl peptide nucleic acid probes to detect high-risk human HPV DNA sequences. (**B**): A schematic diagram of the constructed long-range self-assembled DNA electrochemical sensor for HPV detection. These figures are obtained with permission from ref [201,202].

had a good selectivity with a remarkable detection limit (15×10^{-14} M).

Besides the discussed genosensing approaches, Bartolome et al. synthesized carbon nano onions (CNOs) modified Glassy carbon electrodes as a biosensing platform for the detection of oncogenic human papillomaviruses [205]. These GCE/CNO electrodes were used for the amperometric detection of oncogene sequences of HPV DNA by immobilizing short biotinylated or thiolated DNA probes. They showed that this biosensor had a better sensitivity and a low limit of detection (54×10^{-11} M).

3.5. Influenza viruses

Influenza is a viral infectious disease that has threatened human health and life as well as caused numerous medical issues and considerable financial burdens. The influenza viruses are negative-stranded enveloped RNA viruses that belong to the Orthomyxoviridae family andare divided into A, B, and C types [206].

In recent years, significant advances have been achieved in laboratory diagnostics for the detection of influenza virus infection. Virus isolation via cell culturing, shell vial culturing, Serological assay, and polymerase chain reaction (RT-PCR), are the methods currently used for the laboratory diagnosis of influenza viruses [207,208]. Routinely real-time PCR technique has been developed for the specific detection of influenza viruses and also have been for typing and subtyping. However, it has its limitations: relatively long detection time (~2 h), expensive instruments, sample handling, post-PCR analysis, and requirement of well-trained technicians [208,209]. Nevertheless, rapid and accurate detection of influenza viruses to prevent the occurrence of serious influenza outbreaks is instantly necessary. Recent advances in biosensors for virus detection have led to the development of simple, rapid, and sensitive biosensors for the detection of influenza viruses, including Electrochemical DNA-Biosensor and impedance biosensors [206,210–212].

Tam et al. presented a direct and label-free detection method for the type A influenza virus by using carbon multi-walled nanotubes (MWCNTs) [213]. They have been used DNA-functionalized MWCNTs to determine the concentration of the target DNA sequence on the surface of sensors. The results show that this biosensor can reduce the response time required for influenza virus type A to 4 min. they also obtained detection limit values as low as 5×10^{-10}M of the target DNA samples.

Additionally, DNA based label-free electrochemical biosensor with a duel amplification strategy was developed for the detection of the avian influenza A virus [214]. The isothermal exponential amplification reaction (EXPAR) coupled with hybridization chain reaction (HCR) of DNAzymes nanowires has been developed for the ultra-sensitive detection of low-abundance DNA of Avian influenza A(H7N9) in the clinical samples (Fig. 7A). This method has low cost, high efficiency, and rapid amplification detection under isothermal conditions. They demonstrated that the detection limit of this system were 9.4×10^{-15}M.

On the other study, an electrochemical biosensor by using DNA tetrahedral nanostructural probe was immobilized onto the gold surface was developed to detect the hemagglutinin (HA) gene of influenza A (H7N9) virus from clinical throat-swab samples by amperometric measurements by Dong et al. [215]. This biosensor can specifically detect the target DNA of influenza A virus (H7N9) from other types of influenza A viruses (H1N1 and H3N2) and even could detect single-stranded mismatch oligonucleotides. They showed that through the combined use of the DNA tetrahedral structure as a probe and avidin HRP as the signal amplifier the detection limit of the biosensor could reach 10^{-13} M.

3.6. Arboviruses

Arboviruses are known as viruses that are transmitted by blood-feeding arthropods [216]. Arthropod-borne viruses (arboviruses) are a unique, diverse, and fascinating group of RNA viruses that belong to different families, including Bunyaviridae, Flaviviridae, and Togaviridae, which have the same characteristic of being transmitted to humans or other hosts by arthropods [217]. Some of the most well-known arboviruses include the zika virus, dengue virus, and chikungunya virus. This group of viruses is associated with worldwide outbreaks and a serious public health problem [7]. Dengue virus (DENV) and zika virus (ZIKV) are enveloped positive-sense single-stranded RNA viruses, members of the Flaviviridae family, genus Flavivirus. Chikungunya virus (CHIKV) is a single-stranded RNA virus, belonging to the Togaviridae family and genus Alphavirus [218]. Similarities in some clinical manifestations of the disease caused by the CHIKV to the DENV and the ZIKV, make the diagnosis of CHIKV difficult, especially in endemic areas with DENV and ZIKV circulation. Conventional diagnostic assays that are exploited for Zika, DENV-1–4, and CHIKV detection are RT-PCR and IgM capture ELISA and as well as MAC-ELISA. However, the RT-PCR detection method is highly sensitive and specific for ZIKV, considered the gold standard test for ZIKV [219–221]. However, the RT-PCR method is cumbersome, requires centralized laboratory facilities, skilled individuals to operate, and as well as MAC-ELISA has reduced sensitivity due to false-negative results associated with cross-reaction caused by arboviruses [222–224].

By identification of ZIKV genome in only one drop of sample, an impedimetric electrochemical DNA biosensor, using disposable, three-contact electrodes, has shown great selectivity with a $25.0 \pm 1.7 \times 10^{-9}$ M detection limit. Being a label-free genosensor, allows EIS to easily measure electrical changes of the electrode surface, caused by hybridization of capture probes and target sequences [89]. The detection of ZIKV RNA has been also successfully conducted by using fluorescence signals from semiconductor quantum dot (Qdot) nanocrystals, mediated by localized surface plasmon resonance (LSPR) from four different plasmonic nanoparticles (NPs). The NP-Qdot conjugates to DNA loop sequence of molecular beacon (MB) probes, which hybridization of them with target RNA, makes alterations in optical transduction in NP-Qdot reporter fluorophore (Fig. 7B) [225]. Moreover, genosensors are capable of differentiating the ZIKV genome from its homologous arboviruses, DENV and CHIKV. To achieve this goal, the target genome binds to capture probe and signal probe, from the 3′ and 5′-ends, then, signal probe is recognized by specific antibodies and signals are measured by both electrochemical and calorimetric formats [91].

Detection of DENV has been experimented by several silicon nanowires (SiNW)-based genosensors including the one, accomplished by G Zhang et al. In this sensing method, SiNW is functionalized with the attachment of peptide nucleic acid (PNA) on its surface and hybridization of the complementary target sequence of DENV, which is acquired by RT-PCR, to PNA makes alterations in SiNW resistance, measured by electric sensing technique with LOD of 10^{-14} M [226]. Piezoelectric biosensors, also called quartz crystal microbalance (QCM), are considered sensitive and specific in identifying the DENV genome as well as other sensing methods. To be more detailed, first, the target DNA sequence, obtained from the DENV genome, hybridize into two different probes from 3′ and 5' ends in a sandwich format, second, this complex binds to another probe via by excess target, that acts as a bridge in this layered probe complex. Ultimately the frequency changes (Δf), amplified by probes for enhancing detection sensitivity, are being measured by QCM system with LOD of 2 pfu/mL [227]. Another procedure, in which DENV DNA is detected with high sensitivity and selectivity, is

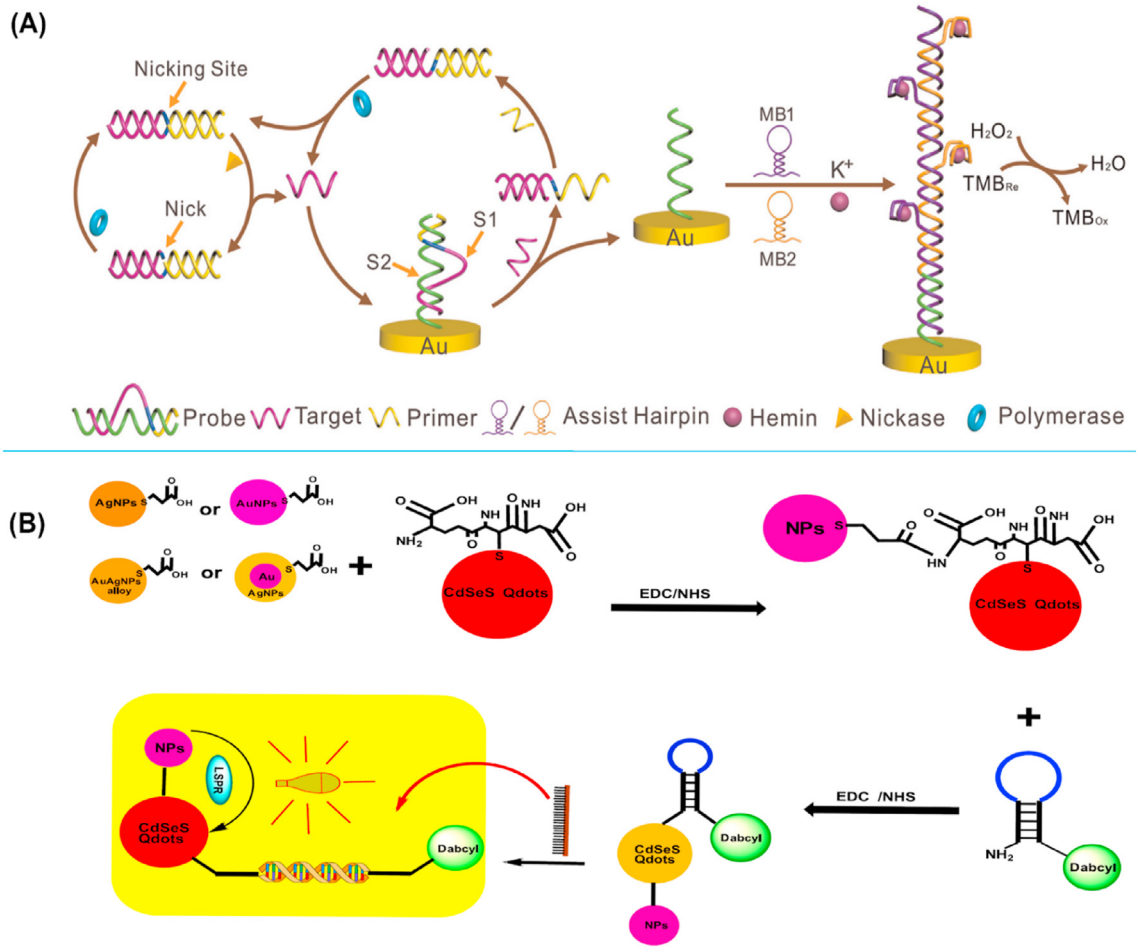

Fig. 7. (**A**); Schematic picture of dual amplification strategy based on isothermal exponential amplification reaction (EXPAR) coupled with hybridization chain reaction (HCR) of DNAzymes nanowires for electrochemical genosensor technique. (**B**); Conjugation of plasmonic NPs with L-glutathione-capped CdSeS alloyed Qdots makes NP-Qdots hybrids that further conjugate to MB. Finally, an LSRP-mediated fluorescent signal is detected as a result of the hybridization of target RNA and MB. The figures are obtained from ref [214,225].

a label-free impedimetric sensor based on nanoporous alumina membrane (Fig. 8). Both sides of the alumina membrane are coated by thin platinum electrodes and DNA probes are linked into the pores of the alumina membrane. The impedance changes, induced by binding of target DNA and probes, are measured by impedance spectroscopy and show a 2.7×10^{-12} M limit of detection [228]. Other DENV and ZIKV-specific genosensors are shown in Table 5. Unless two other arboviruses, chikungunya virus detection has been little investigated by genosensors, however, the studies whose summaries are displayed in Table 5, have been demonstrated to be efficient and reliable in detecting the virus.

3.7. Other viruses

Aside from the viruses discussed earlier, genosensors have been utilized to detect other viruses like adeno and herpes virus and recently discovered viruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that will be reviewed in this section.

3.7.1. Coronaviruses

Coronaviruses in general have been detected by different genosensors as well as other respiratory viruses. However, since some of the species of this family are related to severe diseases including middle eastern respiratory syndrome (MERS) and SARS-CoV-2, the necessity of a sensitive, selective, and rapid detecting method based on biosensor is more important [239,240]. With so many deaths and hospitalized cases around the world, the coronavirus disease (COVID-19) caused by SARS-CoV-2 is known to be the third widespread pandemic of coronaviruses in the last 2 decades. Despite several false-positive and false-negative cases reported in laboratory diagnosis, RT-PCR has been regularly used for SARS-CoV-2 detection. Thus, biosensors especially the ones that detect the viral genome can compensate for the deficiencies of conventional methods. To develop an efficient genosensor, the plasmonic photothermal (PPT) effect combined with LSRP sensing transduction, have made a double-function biosensor in which the hybridization of the target sequence with complementary DNA receptor-functionalized gold nanoislands (AuNIs), can produce two different wavelengths that contribute to high sensitivity and reliability of this approach [241]. The combination of a lateral flow biosensor (LFB) with a multiplex reverse transcription loop-mediated isothermal amplification (mRT-LAMP)had also led to the development of an assay for SARS-CoV-2 detection with an LOD of 12 copies of detection target per reaction (Fig. 9). By utilizing two LAMP primer sets, two different sequences of the virus are amplified at the same time then labeled to be detected. The interpretation of the results accomplished by LFB becomes appears on the LFB test line with three lines that two of them are indicators of 2 different SARS-CoV-2 genes and one for control [242]. To provide

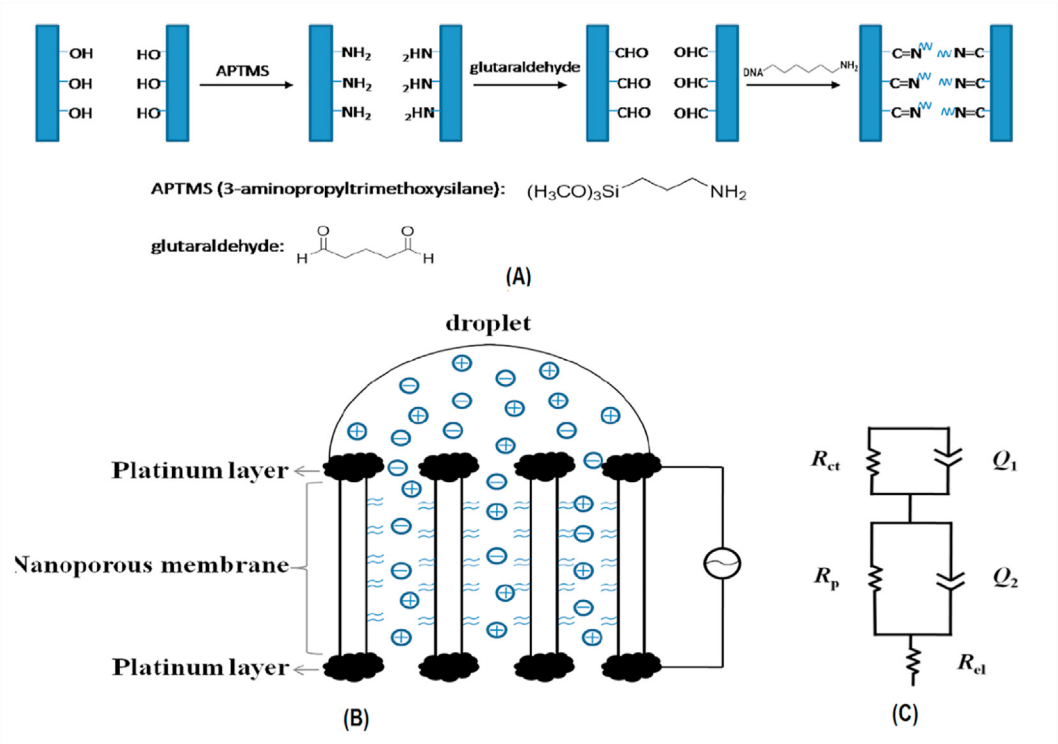

Fig. 8. (**A**) A picture of DNA immobilization procedure; (**B**) Schematic representation of nanoporous alumina membrane for impedimetric biosensing of DENV DNA; (**C**) The equivalent circuit model of the impedimetric experimental results. The figure is obtained from ref [228].

Table 5
Summary of the various genosensing studies regrading arbovirus virus.

Virus	Genosensor type	Method	Electrode	Assay time	Linear rang	Detection limit	Ref
ZIKV	Electrochemical	Impedimetric	Gold	50 min	1.0×10^{-12} _ 1.0×10^{-6}M	82×10^{-14}M	[229]
ZIKV	Electrochemical	Voltammetric	1.SPAuE 2.SPCE/Au	_	1.1×10^{-15}_6×10^{-13} M 2.5×10^{-15}_10^{-11}M	0.2–33 $\times$ 10^{-15}M	[230]
ZIKV	Optical	Fluorescence	MOF	_	0–6×10^{-7} M	23×10^{-11}M	[231]
DENV	Electrochemical	Voltammetric	Alumina	45 min	10^{-12}-10^{-6} M	9.55×10^{-12} M	[232]
DENV	Electrochemical	Voltammetric	ZnO/Pt–Pd	_	1×10^{-6}_100×10^{-6} M	4.3×10^{-5} M	[97]
DENV	Optical	Calorimetric	PSiNs	90 min	1.0×10^{-16}-1.0×10^{-10} M	2×10^{-19} M	[233]
DENV	Electrical	Electrical	Silicon	_	10^{-14}-10^{-5} M	2.0×10^{-15}M	[234]
DENV	Electrical	Electrical	SiNW	_	1.0×10^{-11}-1.0×10^{-7} M	1.63×10^{-12} M	[235]
DENV	Electrical	Electrical	SiNW	_	1.0×10^{-9}-1.0×10^{-13} M	1.985×10^{-14} M	[236]
CHIKV	Electrochemical	Voltammetric	Gold	_	10^{-10} -10^{-4} M	3.4×10^{-9} M	[237]
CHIKV	Electrochemical	Voltammetric	Gold	_	10^{-10} -10^{-4} M	1.0×10^{-9} M	[238]

SPAuE; screen-printed gold electrode, SPCE/Au; screen-printed carbon electrode decorated with gold nanostructure, MOF; metal-organic framework, ZnO/Pt–Pd; Zinc oxide/platinum-palladium, PSiNs; porous silica nanospheres, SiNW; silicon nanowire.

more feasibility in result interpretation, an AuNPs-based colorimetric biosensor with the visual naked-eye response is fabricated to identify SARS-CoV-2 nucleocapsid protein gene from extracted RNA sample and shows LOD of 4×10^{-16} M. In this system, shown in picture 15, when antisense oligonucleotide (ASO)-capped AuNPs hybridize with target RNA, they become agglomerated and change their surface plasmon resonance. After adding RNase H to this solution, target RNAs will be removed from AuNPs which results in visible precipitation from the solution [243]. In COVID-19 pandemic, some evidence supporting the possibility of transmitting SARS-CoV-2 through food products. So, monitoring and assessment food products for hazardous agents are important to progress in healthcare. Thus, advances in the producing mobile sensor devices to screening food products for fecal–oral transmission of viruses offer a significant outlook in-home healthcare [244]. Other approaches for genome detection of SARS-CoV-2 are summarized in Table 6.

3.7.2. Herpes viruses

From transient erythematous vesicles to various cutaneous manifestations including malignancies, all eight members of the Herpesviridae family are capable of developing an asymptomatic infection in humans. What makes different species of this family to be important from a diagnostic point of view, is the characteristic of the latent phase in the virus infection cycle, especially in immunocompromised individuals [251]. Here we refer to some of the studies that have employed genosensors for the detection of herpes viruses.

One of the efficient electrochemical genosensors for herpes virus detection is developed by immobilization of oligonucleotides on graphite electrodes which hybridization of the target sequence with complementary probes, making voltammetric changes measured by voltammogram with LOD of approximately 14.77×10^{-12} M. Moreover, this DNA biosensor can distinguish between herpes simplex virus I (HSV I) and Herpes simplex virus II (HSV II),

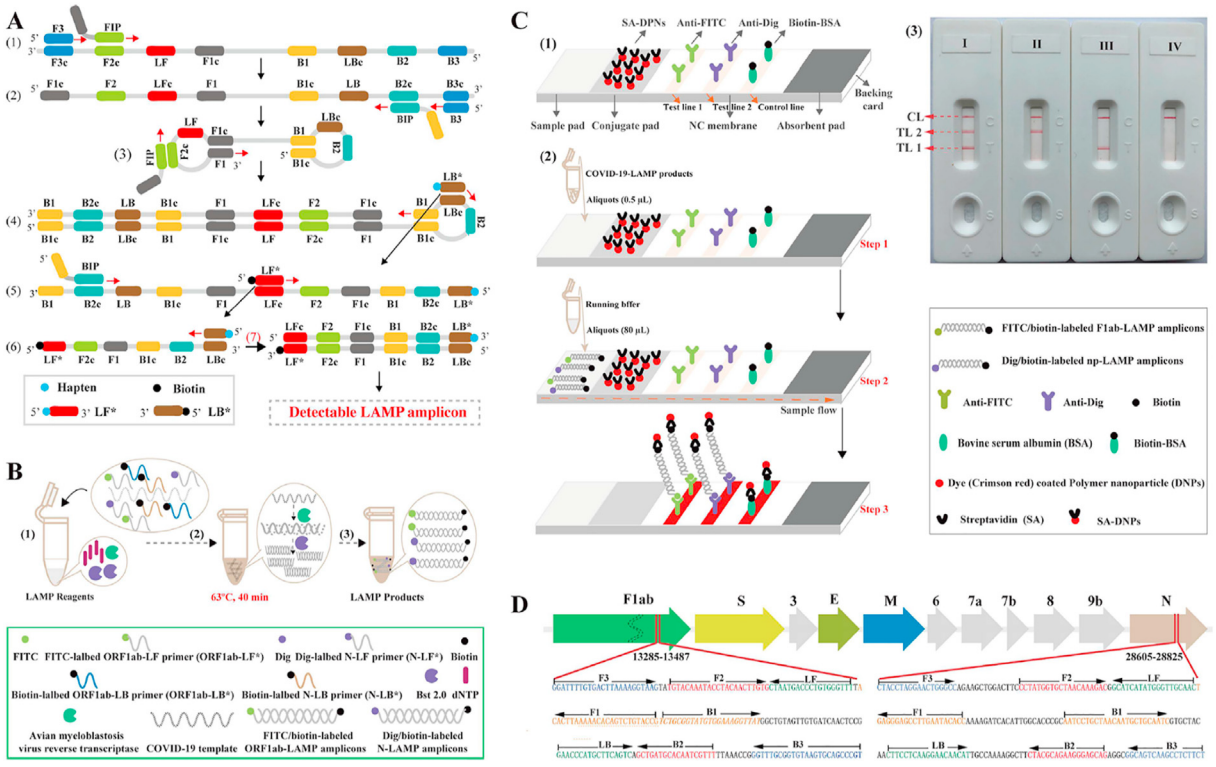

Fig. 9. The combination of a lateral flow biosensor (LFB) with a multiplex reverse transcription loop-mediated isothermal amplification (mRT-LAMP) biosensor for detection of SARS-CoV-2 RNA. **A**) Is a schematic picture of LAMP assay with LF* and LB*. Top row is an outline of LAMP with LF* and LB*, while bottom row, schematically represents the new forward/backward loop primers (LF*/LB*). LF* was labeled with a hapten and LB* was labeled with biotin. **B**) Mechanistic representation of the SARS-CoV-2 RNA detection by RT-LAMP-LFB technique; this assay steps are amplification mixtures preparation (1), RT-LAMP reaction (2), and detectable SARS-CoV-2 RT-LAMP products (3). **C**) The principle of LFB for visualization of SARS-CoV-2 RT-LAMP products; the details of LFB (1), the principle of LFB for SARS-CoV-2 RT-LAMP products (2), Interpretation of the SARS-CoV-2 RT-LAMP results (3). **D**) Primer design of SARS-CoV-2 mRT-MCDA-LFB assay; up row shows SARS-CoV-2 genome organization and bottom row indicates nucleotide sequence and location of ORF1ab and N gene used to primer designing. The figure is obtained with permission from ref [242].

Table 6
Summary of genosensors of SARS-CoV-2 detection.

Genosensor type	Method	Electrode	Assay time	Linear rang	Detection limit	Ref
Electrochemical	EIS	Carbon	_	10^{-17}-10^{-12} M	200 copies/mL	[245]
	Voltammetry	Gold	5 min	585.4 × 10^3_5.854 × 10^{10}copies/mL	6.9 × 10 ^{3}copies/mL	[246]
	Voltammetry	Carbone	>2 h	1–1 × 10^{12} copies/mL	10^3 copies/mL	[247]
	Voltammetry	PANI nanowire	_	10^{-14}- 10^{-9} M	3.5 × 10^{-15} M	[248]
Electrochemiluminescence	EIS	Gold	_	10^{-15} _ 10^{-10}M	2.67 × 10 $^{-15}$M	[249]
Optomagnetic	Volumetric	MNP	100 min	10 $^{-16}$ -10 $^{-14}$M	4 × 10 $^{-16}$M	[250]

PANI; electropolymerized polyaniline, MNP; magnetic nanoparticle.

which are responsible for facial and genital sores respectively [252]. Similar to other members of the Herpesviridae family, cytomegalovirus has double-stranded DNA and is related to several diseases particularly to immunosuppressed patients like organ transplant recipients [251]. By utilizing two different methods for the preparation of gold electrode, a direct capacitive DNA biosensor demonstrates to be efficient when CMV DNA specifically hybridize to oligonucleotides that are immobilized on the surface of electrodes followed by displacement of water and ions from electrode surface which results in capacitance alteration with LOD of 10^2 molecules/mL [253]. This was one of the first DNA biosensors applied for herpesvirus detection, however, subsequent researches are greater in complexity and specificity. Table 7 shows DNA biosensors for HSV, CMV, Epstein - Barr virus (EBV), human herpesvirus 5 (HHV-5) and Kaposi's sarcoma-associated herpesvirus (KSHV).

3.7.3. *Adenovirus*

Adenoviruses are double-stranded DNA viruses that are transmitted via either the respiratory tract or the fecal-oral route. Human adenoviruses are capable of establishing severe disease in children, the elderly, and immunocompromised patients, however, they are mostly asymptomatic in healthy individuals [270]. Despite the prosperity of most genosensors in virus detection, eSensor respiratory viral panel (eSensor RVP) shows some cross-reactivity in the detection of different adenovirus serotypes when compared to real-time PCR. eSensor RVP is a commercial electrochemical biosensor with gold electrodes using voltammetry for the detection of the genome of respiratory viruses such as influenza and rhinoviruses [271]. While commercial biosensors might not be highly selective and sensitive in the diagnosis of some virus serotypes, the ones developed in laboratories indicate more success compared to conventional methods like PCR. Table 8 summarizes some of the genosensors, applied for adenovirus and also

Table 7
Summary of herpes viruses' DNA biosensor.

Virus	Genosensor type	Method	Electrode	Assay time	Linear rang	Detection limit	Ref
HSV I	Piezoelectric	Acoustic	QCM	3 min	5.2×10^{-11}_1.3×10^{-7} M	10^{-18} M	[254]
HSV I	Piezoelectric	Acoustic	Gold	6 min	_	5.2×10^{-12} M	[255]
HSV I	Capacitive	Impedimetric	Aluminum	30 s	_	21×10^{-17} M	[256]
CMV	Electrochemical	Voltammetric	SPE	_	1×10^{-10}M	6×10^{-16}M	[257]
CMV	Electrochemical	Amperometric	SPE	_	3×10^{-8}_ 2×10^{-7} M	3×10^{-11} M	[258]
CMV	Optical	Fluorometric	GO	_	_	4.15×10^{5}IFU/ml	[259]
CMV	Optical	SPR	Gold	<1 h	_	24_108 $\times 10^{-15}$M	[260]
CMV	Piezoelectric	PCR	QCM	_	0_500 $\times 10^{-9}$ M	25×10^{-9} M	[261]
CMV	Piezoelectric	SDA	Gold	>3 h	_	_	[262]
EBV	Electrochemical	Voltammetric	Graphite	_	3.78_756 $\times 10^{-6}$ M	17.32×10^{-9} M	[263]
EBV	Electrochemical	Microfluidic	Gold	10 min	1.0×10^{3}_1.0×10^{-1} copies/mL	1.1×10^{3} copies/mL	[264]
EBV	Electrochemical	EIS	Gold	_	1×10^{-15}_1×10^{-9} M	38×10^{-17} M	[265]
EBV	LF	LF	Gold	_	_	_	[266]
EBV	Electrochemical	Signal amplification	QCM-D	_	11.235×10^{-11} _2.247×10^{-9} M	112.35×10^{-12}M	[267]
HHV-5	Electrochemical	Voltammetric	Zn−Ag	5 s	113_10^{3} and 3×10^{5}_10^{6}copies/mL	97 copies/mL	[268]
KSHV	LF	SERS	Gold	20 min	0_100 $\times 10^{-12}$ M	0.043×10^{-12} M	[269]

SPE; screen-printed carbon electrode, IFU; immunofluorescence focus unit, SPR; surface plasmon resonance, GO; graphene oxide, SDA; strand displacement amplification, EIS; electrochemical impedance spectroscopy, LF; lateral flow, QCM-D; quartz crystal microbalance with a dissipation monitoring, Zn−Ag; zinc-silver, SERS; surface-enhanced Raman scattering.

picornaviruses detection. In addition to before mentioned viruses, other viruses are applied for genome detection by biosensors. In Table 9 we have displayed the information about related researches for paramyxovirus, rhabdoviruses, and reoviruses diagnosis.

4. Simultaneous multi-target detection of viruses

Besides the mentioned mono-target detection genosensor platforms that were constructed for the determination of one viral infection, some scientists are focused to design biosensors with capability to simultaneously diagnose different viruses from clinical specimens at the same time. Because this strategy provides multiplex virus genome detection systems with the lowest price and time-wasting which is an important health issue for all nations especially developing ones [286,288–290]. A multi-target SPR genosensor was combined with gene chip technology by Lei Shi et al. for simultaneous detection of nine respiratory viruses' genome including influenza A and B, H1N1, parainfluenza virus 1–3 (PIV-1, 2, 3), respiratory syncytial virus (RSV), adenovirus (ADV), and severe acute respiratory syndrome coronavirus (SARS) from clinical swaps within 30 min. In this strategy, specific oligonucleotides of the mentioned respiratory viruses' genome have been immobilized in a chip. In the present SPR sensor, PCR primers were labeled with biotin and also streptavidin used for further amplifying the signal to increase the sensitivity of the sensor. According to this report, the complementary probes are specifically hybridized with the genome of the target virus without any nonspecific and cross-hybridization between the viruses' genome and probes. This type of multi-target genosensor is enabling to concurrent identifying of respiratory viruses with high sensitivity, specificity, and reproducibility at the same time with LOD: 5, 1, 1, 2.5, 3.5, 3, 0.5, 2, and 3×10^{-9} M for Influ A, Influ B, PIV1, PIV2, PIV3, RSV, ADV, SARA, H1N1, respectively [291].

In 2019, for the first time, a multifunctional molecularly imprinted sensor for simultaneous detection of hepatitis viruses including HAV and HBV was provided. As simultaneously detection of HAV and HBV viruses is very difficult because of their similarity of the outer structure therefore some modifications such as hydrophilic monomers and metal chelation are used to reduce the cross-hybridization and non-specific binding as well as to increase the specificity of the biosensor. The acceptable selectivity and sensitivity were achieved for the detection of the two viruses with the limits of detection of 3.4×10^{-12} M and 5.3×10^{-12} M for HAV and HBV, respectively, less than 20 min [289]. Additionally, an innovative electrochemical genosensor array with high sensitivity was fabricated for real-time multiplexed monitoring of three of the most common high-risk HPV types (including 16, 18 and 45) DNA sequences from the clinical samples using a co-immobilization strategy of a thiolated probe and bipodal alkanethiol for the modification of the gold electrodes and sandwich assay with a horseradish peroxidase (HRP) labeled reporter probe for the diagnosis of the three HPV types. The proposed electrochemical genosensor array detected HPV16, 18, and 45 DNA with limits of detection of 220×10^{-12} M, 170×10^{-12} M and 110×10^{-12} M, respectively at the same time. Also, there was insignificant cross-reactivity and non-specific binding between all target viruses and HRP-labeled probes [288].

On the other study, a simple and very cheap label-free

Table 8
Information about some of the genosensors applied for adenovirus and picornaviruses detection.

Virus		Genosensor type	Method	Electrode	Assay time	Linear rang	Detection limit	Ref
Adenovirus	Adenovirus	Electrochemical	Amperometric	Carbone	3_5 h	_	_	[272]
		Electrochemical	Conductometric	Ag/AgCl	_	0.4_1.0 μM/tDNA	_	[273]
		Optical	Fluorescent	Silver	_	_	1×10^{-9} M	[274]
Picornaviruses	HAV	Electrochemical	Voltammetry	Gold	Few minutes	_	13.693×10^{-9}M	[275]
	HAV	Optical	Ratiometric	Silver	_	_	0.5×10^{-9} M	[276]
	HAV	Optical	FRET	QD	30 min	2×10^{-10}-5×10^{-8}M	1.3×10^{-11}M	[277]
	HAV	Optical	Chemiluminescent	Silica Fiber	_	11.23×10^{-6}_2.247 × 10-5 M	11.23×10^{-6}M	[278]
	Coxsackievirus	Electrochemical	EIS	GO	_	0.01_20 $\times 10^{-6}$M	2.5×10^{-9} M	[279]
	Enterovirus	LF	LF	Gold	<60 s	_	1×10^{-7} M	[280]

Ag/AgCl, silver chloride; tDNA, target DNA; HAV, hepatitis A virus; FRET, fluorescence resonance energy transfer; QD, quantum dot; EIS, electrochemical impedance spectroscopy; GO, graphene oxide; LF, lateral flow.

Table 9
The list of genosensors for applied for paramyxovirus, rhabdoviruses, and reoviruses diagnosis.

Virus		Genosensor type	Method	Electrode	Assay time	Linear rang	Detection limit	Ref
Paramyxovirus	SV40	Optical	Fluorescence	GO	_	40.0_260 × 10^{-9} M	14.3 × 10^{-9}M	[281]
	SV40	Optical	Fluorescence	MB	_	1_600 × 10^{-9} M	0.4 × 10^{-9} M	[282]
	RSV	Electrochemical	Voltammetric	Gold	_	_	13.6 × 10^{-18} mol	[283]
	NDV	Electrochemical	Potentiometric	Nitrocellulose	45_60 min	_	0.30 × 10^{-15} mol	[284]
Rhabdoviruses	Rabies	Optical	1.fluorescence 2.colorimetric	_	_	1.0.1_6 × 10^{-9}M 2.0.5_60 × 10^{-9} M	1.15 × 10^{-12}M 2.60 × 10^{-12} M	[285]
	Lyssaviruses	Optical	Fluorescent	_	_	_	_	[286]
Reovirus	Ibaraki virus	Magnetic	FRET	Magnetic	18 min	_	1.9 × 10^{-12} M	[287]

SV40, Simian virus 40; GO, graphene oxide; MB, molecular beacon; RSV, respiratory syncytial virus; NDV, Newcastle disease virus; FRET, fluorescent resonance energy transfer.

electrochemical multiplexed genosensor by using the six gold working electrodes, a gold auxiliary electrode, and a printed Ag/AgCl reference electrode to simultaneously detect the specific oligonucleotide sequences of HIV-1 and HIV-2 genome. The voltammetric system could detect the DNA of HIV-1 and HIV-2 with good sensitivity and specificity at the detection limits of 0.1 × 10^{-9} M without the obvious cross-interference under the optimized conditions. Meanwhile, single-base mutation oligonucleotides, random oligonucleotides, and complementary target genome easily discriminated from each other. This research demonstrates that using different hairpin-DNA probes can be helpful to design the sensitive label-free electrochemical genosensor for simultaneous diagnosis of DNA sequences for HIV-1 and HIV-2 as well as various viral pathogens [184].

Although cross-hybridization and interference are the main concerns of genosensors application for simultaneous detection of viruses at the same time. But the cross-interference assays that have been conducted for the multiplexed genosensing systems showed that there is no report of being obvious cross-interference and non-specific binding between the genome of the tested pathogens and used specific probes in all fabricated and reported genosensors [201,286,288–290]; however further investigations especially proteomics research are needed for more confirmations.

5. Commercial genosensors

As previously mentioned, low cost, high sensitivity and Low limit of detection of biomolecules have resulted in the development of biosensors. Although, only small types of biosensors are commercially available, Innovations in this technology have enabled many biosensors to be commercialized. At-home testing kits give rapid results, Self-testing is safe and do not require you to send samples to a laboratory for analysis. Commercial biosensors are available for viral infections specifically detecting the viral antigens, Nucleic acid, and antibodies in biological specimens [292]. The commercially available gene sensors and their characteristics are shown in Table 10.

6. Conclusion and future perspectives

Among all infectious agents, viruses, in general, are considered to be the most life-threatening pathogens not only for humans but for another alive creature including bacteria. The virus's unique non cellular structure makes it resistant to antimicrobial therapies and calls for specific antiviral medicines which target the specific components of the virus, with precise diagnosis as a prerequisite for antiviral treatment. Another challenging issue in defeating these vicious foes is their ability to trick the immune system and generate an anergy phase in which immune cells cannot respond to antigens. This condition impedes antibody-based detection of the virus because the immune cells do not recognize them as pathogens and thus the production of specific antibodies will not take place as the window period of HIV. In the case of hepatitis B window period, nor HBV-specific antibody nor it's a surface antigen (HBsAg) is detectable in patient's serum [294]. Moreover, some viruses like the Herpesviridae family have a latent phase in the host cells in which they only transcribe some of their vital genes and hide from the immune system.

Among molecular conventional methods, PCR an example of a precise one, has been vastly utilized in the recent pandemic of COVID-19. With regards to its approximate accuracy in SARS-CoV-2 genome detection, the number of false positivity or false negativity necessitates an alternative method to prevent prevailing errors. Noted obstacles in rapid and accurate detection of viruses and their infectious phase, leads to the development of biosensor that does not possess the deficiencies of conventional detecting methods and is even capable of discriminating virus serotypes that have cross-reactivity. While detection of antibody, antigen, or whole virus

Table 10
Commercially available clinical genosensors and their characteristics.

Company	Model	Sensitivity	Specificity	Sample	**Virus**	Reference
Quidel, USA	Solana Influenza A + B Assay	Influenza A: 97.2% (95% CI 95.0%–98.4%) Influenza B: 100.0% (95% CI 95.4%–100%)	Influenza A: 96.7% (95% CI 95.4%–97.7%) Influenza B: 98.9% (95% CI 98.2%–99.4%)	nasal and nasopharyngeal swabs	Influenza A&B	[292]
	Lyra Influenza A + B Assay	A: 100% B: 98.4%	A: 98.7% B: 95.5%	nasal swabs	Influenza A (HIN1,H3N2,H7N9) &B	
	Solana SARS-CoV-2 Assay	Nasal: 97.2% Nasopharyngeal: 100%	Nasal: 100% Nasopharyngeal: 96.9%	Nasal swabs and nasopharyngeal swabs	SARS-CoV-2	
	Lyra RSV + hMPV Assay	RSV 96% hMPV 95%	RSV 98% hMPV 98.9%	Nasal swabs And nasopharyngeal swab	Respiratory syncytial virus (RSV) and Human metapneumovirus (hMPV)	
Alere, USA	Alere i Influenza A & B assay	A: 55.2–100% B: 45.2–100%	A: 62.5–100% B: 53.6–100%	Nasal swabs And nasopharyngeal swab	Influenza A & B	[293]

might undergo inaccuracy due to delayed immune response or latency of virus life cycle, sensing techniques that are designed to detect virus genome can specifically be applied for different stages of infection by targeting particular genes. Despite conventional methods, genosensors can be operated in a selective, specific, time-saving, and effortless manner even by inexpert individuals and the difficulties in early stages of manufacturing different genosensors including costly instruments and materials, suitable DNA probe availability in label-based sensors, and other complications in small nucleic acid detection like sensitivity and specificity, have been resolved over time in respect of researcher's efforts.

From various types of fabricated genosensors, the majority of them have been used electrochemical sensing techniques and the most utilized electrodes have been the gold ones. Sensing methods especially, optical ones by which the results can be observed with naked-eye are more feasible, besides, label-free genosensors have provided a less-laborious detecting procedure. The limit of detection represented by different studies has been mostly comparable or lower to the PCR method indicating that genosensors are promising for future laboratory diagnostic approaches and can be a potential alternate for current methods. In this review we attempt to bring up the application of genosensors for virus detection, however many viruses remained unassessed because they were less of a consideration in terms of epidemiology or severity of the diseases they cause. Unlike plenty of researches accomplished about virus detection by genosensor, this advanced device needs more investigation to be proved for routine application.

Compliance with ethical standards

The authors declare that they have no competing interests.

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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