Synthetic and Systems Biotechnology 10 (2025) 194–206

Contents lists available at ScienceDirect

Synthetic and Systems Biotechnology

journal homepage: www.keaipublishing.com/en/journals/synthetic-and-systems-biotechnology

Review Article

Biosensors for the detection of flaviviruses: A review

Ana-Belén Blázquez, Nereida Jiménez de Oya *

Departamento de Biotecnología, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC), Ctra. de La Coruña, km 7.5, 28040 Madrid, Spain

ARTICLE INFO

Keywords:
Flaviviruses
Biosensors
Point-of-care testing (POCT)
Diagnostic

ABSTRACT

Flaviviruses affect the lives of millions of people in endemic regions and also have the potential to impact non-endemic areas. Factors such as climate change, global warming, deforestation, and increased travel and trade are linked to the spread of flaviviruses into new habitats and host species. Given the absence of specific treatments and the limited availability of vaccines, it is imperative to understand the biology of flaviviruses and develop rapid and sensitive diagnostic tests. These measures are essential for preventing the transmission of these potentially life-threatening pathogens. Flavivirus infections are mainly diagnosed using conventional methods. However, these techniques present several drawbacks, including high expenses, time-consuming procedures, and the need for skilled professionals. The search for fast, easy-to-use, and affordable alternative techniques as a feasible solution for developing countries is leading to the search for new methods in the diagnosis of flaviviruses, such as biosensors.

This review provides a comprehensive overview of different biosensor detection strategies for flaviviruses and describes recent advances in diagnostic technologies. Finally, we explore their future prospects and potential applications in pathogen detection. This review serves as a valuable resource to understand advances in ongoing research into new biosensor-based diagnostic methods for flaviviruses.

1. Introduction

The genus *Flavivirus*, recently renamed *Orthoflavivirus*, is constituted by arthropod-borne positive-sense single-stranded RNA viruses belonging to the family *Flaviviridae* [1]. This genus comprises more than 70 different species, classified into 3 types according to the transmission vector: mosquito-borne, tick-borne, and unknown-vector-borne viruses. Flaviviruses include some of the most important human pathogens such as dengue virus (DENV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), Zika virus (ZIKV), West Nile virus (WNV), and tick-borne encephalitis virus (TBEV), causing a major global health concern [2]. Flavivirus infections display a wide variety of symptoms ranging from asymptomatic or mild fever to severe manifestations, which could be divided into two different categories: hemorrhagic and neurological complications [3]. The main hemorrhagic features of the disease can be liver failure, hemorrhagic syndromes, and vascular compromise, and may be fatal. Neurotropic flaviviruses can reach the brain and spinal cord and cause severe neurological syndromes such as meningitis, encephalitis, and acute flaccid paralysis [4]. On the other hand, ZIKV infection during pregnancy can be transmitted to the developing fetus, resulting in placental insufficiency, microcephaly, congenital malformations, and fetal demise. No specific anti-flaviviral treatments are currently available, and only a few vaccines have been approved for humans against JEV, DENV, YFV, and TBEV, for horses in the case of WNV and JEV [5,6] and for pigs in the case of JEV [7]. Therefore, knowledge of the biology of flaviviruses and the development of rapid and sensitive diagnostic tests is crucial to prevent the spread of these potentially life-threatening pathogens [4].

1.1. Virological features of flaviviruses

Flaviviruses are enveloped RNA viruses. Their genome is formed by a single-stranded positive RNA of approximately 11 kb in size that encodes a polyprotein within a single open reading frame (ORF), flanked by untranslated regions (UTRs) at both the 5' and 3' ends. The ORF is translated into a single polyprotein, which is processed by viral and cellular proteases to produce ten major viral proteins: three structural (C, prM/M, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [8] (Fig. 1).

Proteins are implicated in different steps of the replication cycle. The

Peer review under responsibility of KeAi Communications Co., Ltd.
* Corresponding author.
E-mail address: jdeoya@inia.csic.es (N. Jiménez de Oya).

https://doi.org/10.1016/j.synbio.2024.10.005
Received 27 July 2024; Received in revised form 26 September 2024; Accepted 21 October 2024
Available online 26 October 2024

C protein is involved in nucleocapsid formation, an essential step for viral assembly and replication [9]. The M protein is a transmembrane glycosylated protein resulting from the cleavage of the prM protein by a furin-like protease, leading to the formation of mature virions. The E protein is also a transmembrane glycosylated protein involved in different processes such as receptor binding, viral entry, and membrane fusion. This protein is considered the most immunogenic, and its glycosylation is crucial for efficient transmission and neuroinvasiveness.

The NS1 is involved in replication, immunomodulation, and pathogenesis. The NS2A participates in intracellular membrane rearrangements and virion assembly. The NS2B protein is the co-factor of the NS3 viral serine protease, allowing its activation and the consequent processing of the viral polyprotein. In addition, it interacts with the NS2A protein, playing a crucial role in viral replication and assembly [10]. The NS3 is a multifunctional protein, with a central role in infectivity, allowing the maturation of viral proteins through its protease activity, but it also presents helicase, nucleoside triphosphatase, and RNA triphosphatase activities involved in virus replication [11]. The NS4A is involved in membrane rearrangements, inhibition of IFN signaling, and is related to important processes such as autophagy or unfolded protein response. The NS4B participates in the formation of the viral replication complex. The NS5 is the most conserved protein among the different flaviviruses. Its methyl transferase enzymatic activity is necessary for the viral RNA capping and its RNA-dependent RNA polymerase (RdRp) activity for the replication of the virus genome [12].

Replication starts with viral entry in host cells by receptor-mediated endocytosis. Virions bind to receptor host endosomes in an acidic environment, allowing the fusion of the viral envelope with the endosomal host membrane. Then the viral genome is released into the cytosol [13]. Infectious virions emerged when immature viral particles assembled at the endoplasmic reticulum reach the Golgi complex for maturation. After this process, viral particles are released from the infected cell to the extracellular space by exocytosis (Fig. 2).

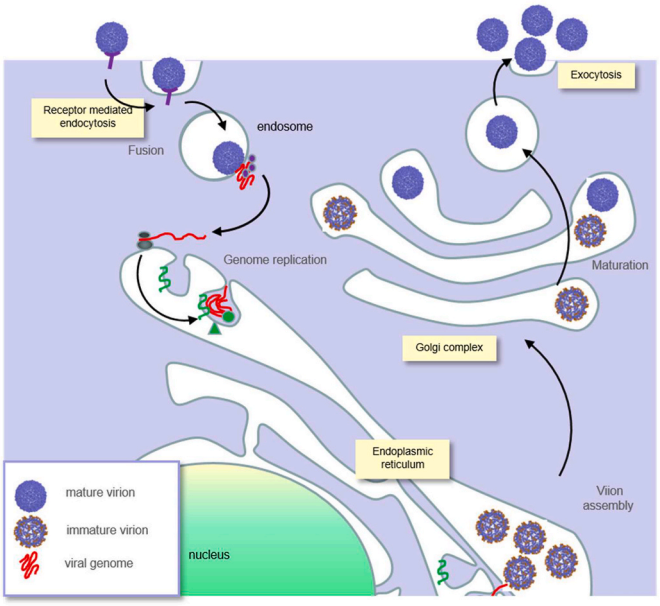

Fig. 2. Schematic view of flaviviruses infectious cycle. The major steps of infection, including receptor-mediated endocytosis, genome replication, immature virion in the endoplasmic reticulum, particle maturation, and mature virion release by exocytosis are schematized.

1.2. Geographic distribution and clinical manifestations

The worldwide geographic distribution of flaviviruses is well known. Even though these viruses are detected mainly in tropical and subtropical areas, factors such as climate change and global warming, deforestation, uncontrolled urbanization or traveling and trade are associated with flaviviruses colonizing new habitats and host species [14] thus contributing to the increase of flaviviral infections into previously non-endemic areas.

Dengue virus (DENV) causes more than 90 million cases and approximately 40000 deaths annually [15], being the most widespread arbovirus. According to the World Health Organization (WHO), DENV cases have been reported in over 80 territories in Africa, the Americas, Southeast Asia, the Western Pacific, and Eastern Mediterranean Regions during 2023. Particularly worrying is the fact that almost 80 % of these cases occurred in the Americas [16], where cyclic epidemics recurring every 3–5 years have been reported. Moreover, autochthonous dengue cases have also been described in the European region, since its mosquito vectors are increasing their presence northwards and westwards in Europe [17]. However, it is suspected that the number of cases is underestimated because most of the infections are usually asymptomatic.

DENV infections provoke a mild disease called dengue fever which displays a diverse array of symptoms, such as fever, headache, and myalgia, which frequently overlap with those of other febrile illnesses, posing a challenge for accurate differentiation without appropriate diagnostic methods. However, in some cases, DENV can trigger a more severe disease known as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), which presents potentially life-threatening symptoms like hemorrhage, thrombocytopenia, and vascular leakage [18].

Zika virus (ZIKV) was first identified in 1947 in Uganda, and it remained in the African continent until its detection in Southeast Asia in the 1980s, then in Micronesia and Oceania beginning in 2007, and finally, the virus reached the Americas in 2015 where it provoked an explosive outbreak, infecting hundreds of thousands of people, mainly affecting pregnant women and newborns [19]. Even though ZIKV disease is usually asymptomatic or presents mild symptoms, severe neurological manifestations such as Guillain-Barré syndrome (GBS) and microcephaly in newborns have been widely reported in the Americas [20].

West Nile virus (WNV) is currently considered one of the most important causative agents of human viral encephalitis worldwide [21]. The virus was first reported in the West Nile district of Uganda in 1937 [22]. In the following decades, WNV was considered a neglected

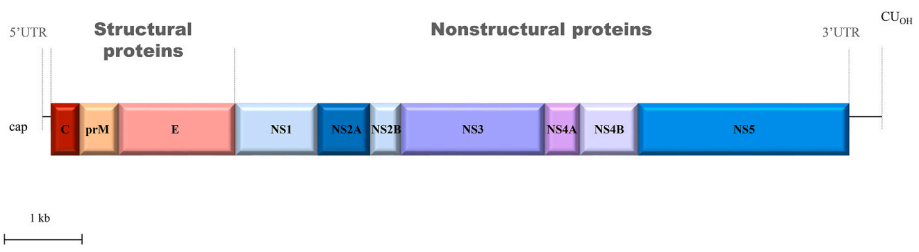

Fig. 1. Schematic view of the genomic organization of genus Orthoflavivirus. UTR: untranslated region; C: capsid or core protein; prM: pre-membrane protein; E: envelope protein; NS: non-structural proteins.

pathogen with infections sporadically reported in Africa, Israel, the Mediterranean Basin, Russia, and Australia [23]. It was not until 1999 that a WNV outbreak occurred in New York that spread explosively throughout the United States in the following years. Nowadays the virus is commonly found in Africa, Europe, the Middle East, North America, and West Asia. WNV is classified into several lineages that do not consistently correlate with its geographical distribution. Only lineages 1 and 2 have been involved in human outbreaks of WNV encephalitis, and both are now endemic in Europe. Infections are mainly asymptomatic or cause mild symptoms, but a small percentage of infected people (less than 1 %) develop severe neuroinvasive manifestations such as encephalitis and meningitis that can produce fatal consequences [9].

Japanese encephalitis virus (JEV) is the leading cause of viral encephalitis in Asia, causing around 60000 cases every year, and a 30 % mortality rate in those with encephalitis [24]. The disease is mainly developed during childhood, being endemic in 24 countries in Southeast Asia and Western Pacific regions. Outbreaks are unpredictably and spatially and temporally limited. Incidence in Asia has decreased, mainly attributed to vaccination [25].

Yellow fever virus (YFV) is found in tropical and subtropical areas of Africa and Central and South America. Even though most infected people have no symptoms or mild ones, a small percentage of patients can suffer severe complications in the liver and kidneys. They can develop jaundice and abdominal pain with vomiting or bleeding. There is a mortality rate of 50 % in those patients who enter the toxic phase [26].

Tick-borne encephalitis virus (TBEV) is the main causative agent of arboviral encephalitis in Europe. The virus is endemic in this continent and in regions of China and North Japan in Asia [27]. Neurological complications of the disease usually present as meningitis, meningoencephalitis, or meningoencephalomyelitis. Between 10,000 and 12,000 clinical cases of tick-borne encephalitis are reported annually, but the total number of clinical cases is believed to be underestimated [28].

1.3. Diagnostic methods for flavivirus identification

As previously mentioned, flaviviruses are globally distributed and produce dangerous life-threatening infections in tropical and subtropical areas. In this context, the search for rapid and efficient diagnostic methods represents a milestone in the control of flaviviral diseases.

Diagnosis of flavivirus infections is generally achieved by conventional methods, including molecular and serological assays (Table 1). In this sense, the most used molecular methods are reverse transcription polymerase chain reaction (RT-PCR) and real-time quantitative RT-PCR. The main disadvantage is their high cost, which makes them unaffordable in low-income countries. On the other hand, the window for virus detection using these techniques is relatively narrow, since they must be applied during the early stage of the disease [29].

Among serological methods, the most widely used are enzyme-linked immunosorbent assays (ELISAs), used either during the acute phase to detect early IgM antibodies or in the late phases of infection to detect IgG antibodies. However, it is essential to take into account that cross-reactivity is very common among flaviviruses, reducing the specificity of the diagnosis and producing false positive results [30].

In this sense, the plaque reduction neutralization test (PRNT) is considered the gold standard technique for the differential serological diagnosis of flavivirus [23]. PRNT is very specific and minimal cross-reactivity is observed between different flaviviruses. This method is used to detect neutralizing antibodies. However, the main drawback when working with infectious viruses is that most of them can only be handled within a biosafety level 3 (BSL-3) facility.

Another classical diagnostic method is cell culture for viral isolation. However, and as happened with PRNTs, these procedures may be conducted at designated research facilities placed in BSL-3 laboratories.

The main drawbacks of the application of classical diagnostic methods are the high economic and qualified personnel requirements, in addition to the fact that they are time-consuming methods and require expensive technical equipment and laboratory facilities. The search for fast, simple, and affordable alternative techniques as a feasible solution for developing countries is leading to the exploration of new methods for the diagnosis of flaviviruses, such as biosensors.

Table 1
Classical methods for the diagnosis of flaviviruses.

Molecular assays	Serological assays	Viral assays
Reverse transcription polymerase chain reaction (RT-PCR)	Enzyme-linked immunosorbent assay (ELISA)	Plaque reduction neutralization test (PRNT)
Real-time quantitative RT-PCR (qRT-PCR)		Cell culture for viral isolation

1.4. Biosensors

1.4.1. General principles

Biosensors are analytical tools that use chemical or biological mechanisms to identify particular target substances, principally including two primary elements: a receptor and a transducer [31]. The bioreceptor attaches to the target analyte, identified through physical or chemical interactions. The transducer converts this reaction into a measurable signal. Transducers produce a wide variety of signals, usually electrochemical, optical, acoustic, or calorimetric. This signal obtained from the transducer is usually amplified and analyzed by a detector (Fig. 3).

The first device considered a biosensor was developed by Leland C. Clark, Jr in 1956 who designed an electrode for oxygen detection [32]. In 1962, Clark and Lyons successfully developed an enzymatic electrode capable of converting glucose into a detectable signal, marking the beginning of biosensors as essential bioanalytical instruments [33].

Biosensors offer great potential such as outstanding performance, easy handling, high sensitivity and specificity, and the ability to provide a rapid response and perform analysis in real-time, thus allowing rapid intervention in case of health emergencies [34]. In addition to these features, their compact size and portability make these devices an ideal tool for point-of-care testing (POCT) in bioanalytical clinics. Nowadays there is a growing need for POCT for the rapid detection of infectious diseases, such as those caused by viruses. These devices play an essential role in preventing the spread of infectious diseases by enabling real-time testing and providing rapid, high-quality diagnosis [35].

The World Health Organization (WHO) has highlighted the relevance of developing POCT that meets the ASSURED criteria: Affordable, Sensitive, Specific, User-friendly, Robust and rapid, Equipment-free, and Deliverable. These criteria represent the essential attributes for an optimal POCT platform [36] (Fig. 4).

1.4.2. Characteristics of biosensors

Biosensors effectiveness is determined by some essential features: selectivity, reproducibility, stability, limit of detection, linear detection range, and response time [32].

Selectivity refers to the capability of the bioreceptor to identify a determined analyte within a sample that may contain a mixture of compounds. It is probably the most important characteristic of biosensors.

Reproducibility indicates the capacity of the biosensor to produce analogue responses when experimental conditions are replicated. This feature is mainly determined by the precision and accuracy of the transducer and the electronics of the biosensor.

The stability is attributed to the susceptibility of the biosensor system to external disturbances present in its surrounding environment, which induce fluctuations in the output signals of the biosensor during measurement. In applications involving long incubation periods or continuous monitoring, stability becomes a critical characteristic. Stability can

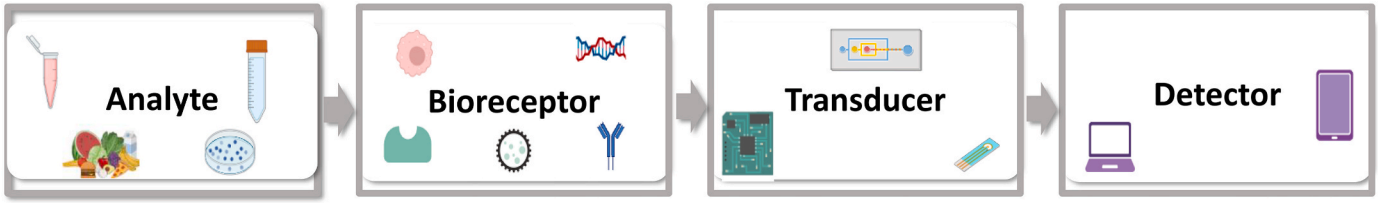

Fig. 3. Schematic design of a biosensor. The main elements of a biosensor are included: analyte, receptor, transducer, and detector.

Fig. 4. Representation of the assured criteria highlighted by the World Health Organization (WHO).

also be influenced by bioreceptor degradation over time or when temperature changes occur in transducers or detectors.

The limit of detection (LOD) of a biosensor is considered the minimum amount of analyte detected by the device. This parameter is directly related to sensitivity, which is determined by the correlation between the variation in the concentration of the analyte and the intensity of the signal monitored by the transducer. Ideally, biosensors would be able to produce a signal in response to even slight changes in the concentration of the target molecule [37]. Linearity is the characteristic that shows the accuracy of the measured response to follow a linear trend when various analyte concentrations are determined. This linear detection range is also associated with the sensitivity of the biosensor.

Response time is defined as the required time for the biosensor to produce a signal or response after interaction between the receptor and the target sample. It is generally taken as the time needed to achieve 95 % of the response [38].

1.4.3. Classification of biosensors

Different criteria are used in the classification of biosensors. The most frequent are shown in Table 2, according to the bioreceptor or transducer chosen. This choice depends mainly on the characteristics of the target analyte and the type of physical or chemical property to be measured [39].

1.4.3.1. Biosensors based on bioreceptors. As mentioned above, a bioreceptor is a biomolecule that uses a biochemical mechanism to identify an analyte. Its function is to capture the analyte of interest and attach it to the sensor for further study.

Table 2
Classification of biosensors.

Based on bioreceptors	Based on transducers
Enzyme-based biosensors	Electrochemical biosensors
Antibody-based biosensors	Optical biosensors
Nucleic acid-based biosensors	Thermal biosensors
Cell- and organelle-based biosensors	Gravimetric biosensors
Microbial-based biosensors	Magnetic biosensors

Bioreceptors can generally be classified into different categories including enzymes, antibodies, nucleic acids, cellular structures/cells, and other microorganisms. Enzymes and antibodies are the main types of bioreceptors used in biosensor applications (Fig. 5).

1.4.3.1.1. Enzyme-based biosensors. Enzyme-based biosensors stand out as one of the most advanced bioanalytical tools, due to the high catalytic activity and selectivity of enzymes to detect target analytes [38]. Thanks to the extensive development of enzyme-based receptors, a wide variety of biosensors can be generated based on enzyme specificity. Nevertheless, conventional enzyme-based biosensors often face challenges related to their sensitivity, selectivity, and stability. Consequently, different strategies are being explored to improve the performance of these biosensors, including the integration of nanoscale materials that improve physical and chemical properties [40].

1.4.3.1.2. Antibody-based biosensors. Antibody-based biosensors or immunosensors are one of the most important classes of affinity biosensors due to their specificity. These devices contain an embedded antibody as a ligand. A specific target analyte, the antigen, forms a stable immune complex with an antibody that acts as a capture agent based on the antibody-antigen interaction [41]. This interaction leads to the generation of a measurable signal provided by a transducer. Immunosensors have demonstrated remarkable selectivity and sensitivity due to precise antigen-antibody binding, making them highly suitable for various clinical applications, including pathogen detection [42].

1.4.3.1.3. Nucleic acid-based biosensors. The most common biosensors that use nucleic acids consist of single-stranded DNA, which hybridizes with its complementary strand, exhibiting remarkable efficiency and specificity [43]. DNA sensors, also called genosensors, are an interesting tool to provide access to sequence-specific information. This capability can be widely used across numerous fields, particularly in clinical, environmental, and food analysis [44].

Other commonly used nucleic acid biosensors have been generated using aptamers or microRNA [45]. An aptamer is a short single-stranded nucleic acid, whether ssDNA or RNA, that binds to a specific target molecule [46]. Due to their synthetic and chemical simplicity unlike antibodies, aptamer-based biosensors or aptasensors offer improved stability and functionality for detecting environmental contaminants [47] or for biomedical applications [48] among others.

1.4.3.1.4. Cell- and organelle-based biosensors. Biorecognition in cell-based biosensors relies on the whole cell or on a particular cellular component or organelle that is competent for specific binding to certain species [39]. Cell-based biosensors integrate living cells with sensors or transducers to detect cellular physiological parameters, thus acting as a

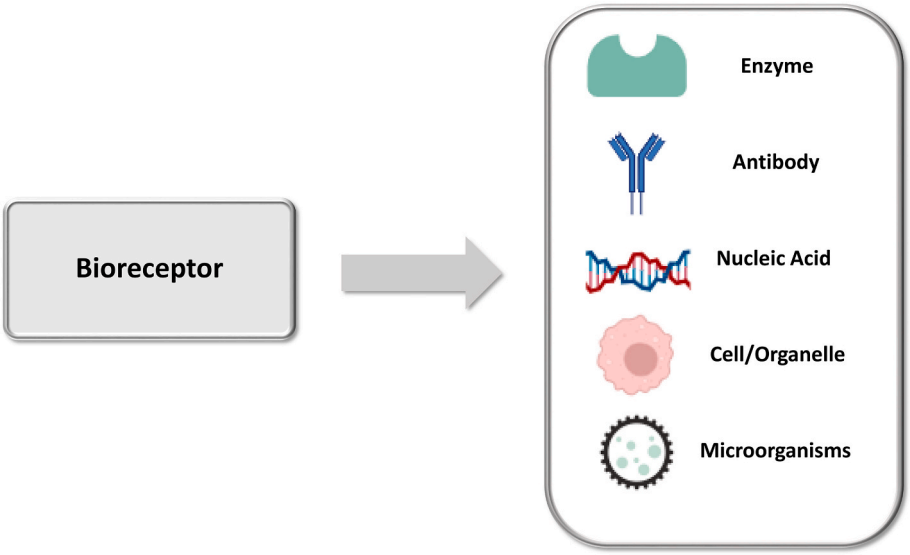

Fig. 5. Classification of biosensors according to the type of bioreceptor.

connection between biology and electronics. These biosensors present evident advantages, such as prolonged non-invasive recording, fast response times, and label-free experimentation [49]. In this sense, they are being used in a vast variety of applications that include the detection of biologically active signaling molecules, antimicrobial strategies, or cancer therapy, among others [50]. However, despite these advantages, there are still some obstacles, such as regeneration and storage lifespan, cell population heterogeneity, significant interference, and high costs, which need to be resolved before larger-scale implementation of cell-based biosensors [51].

1.4.3.1.5. Microbial-based biosensors. A microbial-based biosensor is an analytical tool produced by combining immobilized viable or non-viable microorganisms with a physical transducer to produce a measurable signal proportional to the concentration of the analyte [52]. The immobilization of microorganisms on transducers plays an essential role in microbial biosensors, so there is a huge variety of methods for this immobilization such as adsorption, encapsulation, covalent binding, etc. [53].

Although metabolites produced by microorganisms are generally non-specific, achieving highly selective microbial biosensors is potentially feasible by excluding unwanted metabolic pathways and inducing relevant ones. This can be accomplished by adjusting the microorganisms to suitable substrates of interest. Additionally, recent advances in molecular biology have introduced a novel approach to creating genetically modified microorganisms, offering a new way to improve the selectivity and sensitivity of microbial biosensors [54].

1.4.3.2. Biosensors based on transducers. As mentioned above, the transducer essentially works as an interpreter, detecting the interaction of various biochemical reactions and converting it into another signal ready to be analyzed by the detector. Depending on the mechanism by which the transducers perform the conversion, the signal generated by the interaction between bioreceptor and analyte can be different and, as shown in Fig. 6, biosensors can be classified according to the transduction methods they employ.

1.4.3.2.1. Electrochemical biosensors. Transducers depending on electrochemical detection mechanisms are the most commonly used in the development of biosensors.

Electrochemical biosensors rely on the interactions between the biorecognition element that is included on its surface and the binding molecule present in the analyte. These interactions induce changes in electrochemical properties, which subsequently translate into a detectable electrical signal. Electrochemical biosensors can be classified into

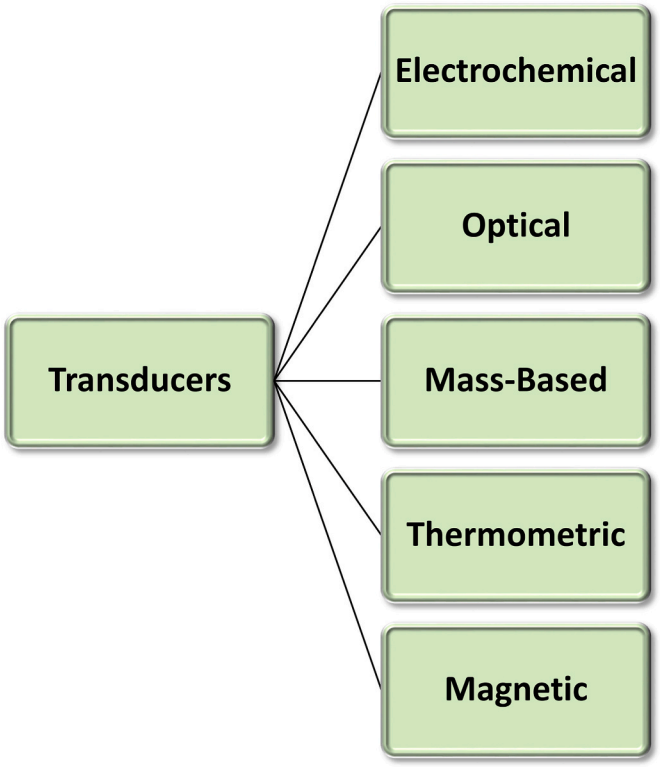

Fig. 6. Classification of biosensors according to the type of transducer.

amperometric, potentiometric, impedimetric, conductometric, voltammetric, polarographic, capacitive, or piezoelectric, depending on the detection principle and application [55]. On the other hand, label-free biosensors constitute a category of electrochemical biosensors in which the quantification of the target analytes is based on the techniques described above but no other signal labels are required. The inclusion of a tag can modify the specific binding of the analyte, leading to potential systematic errors in the measurement. Direct detection eliminates the labeling steps, reducing the time and cost of analysis [56].

Electrochemical methods offer significant advantages, including high sensitivity, rapid signal generation and detection, miniaturization,

and affordability [57]. Another good feature is that these devices have the possibility of being coupled with other biosensing techniques for enhanced detection.

All these characteristics make electrochemical biosensors a good platform to be used in a wide spectrum of applications ranging from monitoring water [57], biomedical diagnostics [58], food analysis [59], or pathogen detection [60,61].

1.4.3.2.2. Optical biosensors. Optical biosensor detection relies on the interaction between optical technologies with a biorecognition element. They have received considerable attention in recent decades as powerful detection and analysis tools with broad applications, as they present important advantages compared to other well-established biosensor technologies, such as noise reduction and immunity to electromagnetic interference [62]. Optical biosensing can be classified into two main categories: label-free and label-based. As previously mentioned in electrochemical biosensors, in label-free detection the signal originates directly from the interaction between the analyzed sample and the transducer, while label-based detection employs a tag [63].

Based on the detection principle, these devices can be classified as those that measure luminescence, fluorescence, color changes, absorbance, reflectance, or fluorescence emissions that occur in the ultraviolet (UV), visible, or near-infrared (NIR) spectral ranges [64]. Table 3 summarizes the most commonly used optical techniques in terms of their detection mechanism.

These biosensors have demonstrated valuable efficacy in the detection of biological analytes and have shown notable advances in their use in biomedicine [65,66], food safety [67,68], pathogen detection [69], and the biotechnology industry [70,71].

1.4.3.2.3. Thermometric biosensors. Thermometric biosensors, also known as calorimetric, quantify heat changes in a sample and its environment. These biosensors are created by immobilizing the bioreceptor in a temperature sensor, which detects and measures the energetic alterations, such as heat exchange, produced in the analyte [72]. The technique is available for the analysis of any reaction that generates a measurable amount of heat. In this sense, the wide usefulness of calorimetric biosensors is based on the fact that all biochemical reactions are associated with a change in heat, either generating or absorbing heat. Consequently, a single calorimetric transducer can serve as a versatile platform to quantify multiple biomarkers [73]. Therefore calorimetric biosensors are used in a wide range of applications, such as food processing and safety [73], pathogen detection [74], clinical monitoring [75,76], or environmental determinations [77].

1.4.3.2.4. Mass-based biosensors. Mass-based biosensors, also called gravimetric, react to a small variation in the mass of the binding analyte generating a detectable signal [78]. The most commonly used gravimetric transducers are thin piezoelectric quartz crystals that resonate at a particular frequency in response to both the applied current and the mass of the detected material [38]. These piezoelectric biosensors stand out as optimal tools, as they facilitate rapid, label-free, real-time detection of analytes without requiring specific reagents or complex sample manipulations. Acoustic biosensors are a type of piezoelectric devices that use the acoustic waves generated by these materials to identify the target analyte through induced changes in the features of the acoustic wave [79].

Table 3
Optical biosensors.

Detection mechanism
Fluorescence
Phosphorescence
Reflection
UV/Vis/IR absorbance
Förster Resonant Energy Transfer (FRET)
Interferometry
Surface Plasmon Resonance (SPR)

Mass-based biosensors are important in the development of miniaturized, portable devices for pesticide detection [80], virus detection [81], food processing technologies [82], or medical diagnosis [83], among others.

1.4.3.2.5. Magnetic biosensors. A magnetic biosensor is a device able to transform a magnetic field into an electrical signal. In recent years, these biosensors have been increasingly used in the development of biosensors thanks to the special characteristics of magnetic materials. The general procedure for biological detection using a magnetic biosensor involves initially immobilizing the probe on the sensor surface and subsequently allowing the sample, which contains magnetic labels, to flow across the surface of the sensor [84]. Magnetic nanoparticles (MNPs) have recently emerged as suitable labels for the development of this technology, enabling the detection and identification of a huge variety of physical, chemical, and biological agents [85].

Magnetic biosensors are widely applied to monitor biological interactions and rapid detection of analytes as POCT, mainly in drug discovery [86], virus detection [87], biomedical applications [88,89], or food analysis [90].

1.5. Biosensors for flavivirus detection

As mentioned above, the diagnosis of flavivirus infections is usually performed by traditional methods, mainly serology and molecular assays. However, these techniques have a series of disadvantages, such as the high economic burden that makes diagnostic tests unaffordable in low-income countries, where the impact of flaviviruses is usually important. Other drawbacks are the need for qualified personnel and the fact that they are time-consuming methods. In recent years, these obstacles are being overcome thanks to the development of biosensors as new, fast, and sensitive methods in the diagnosis of flaviviruses (Fig. 7).

Biosensors for diagnosing flavivirus offer notable advantages over conventional methods, particularly their ability to produce easy handling portable devices. Most techniques allow for direct analysis of samples without requiring any pre-treatment since the most commonly used samples include serum, saliva, and other body fluids from patients. These samples facilitate easy handling and rapid results. Sometimes, biosensor samples require prior processing, typically following the same procedures used in conventional diagnostic methods, such as nucleic acid extraction or similar techniques [91]. However, it is essential to consider that biosensors also have some drawbacks such as potential stability issues with the components or alteration in pathogen detection due to mutant viruses (Table 4).

1.5.1. Biosensors for the detection of dengue virus

DENV is a serious global public health concern affecting more than 90 million cases and approximately 40,000 deaths per year. Currently, many researchers have explored biosensors as a novel alternative technology to detect the virus or the presence of antibodies. This approach offers several advantages, including sensitivity, cost-effectiveness, easy production, rapid results with quantitative analysis, and the possibility of developing POCT devices [100].

A large majority of researchers have developed electrochemical biosensors for the diagnosis of DENV, mainly based on the electrochemical impedance spectroscopy (EIS) technique [101]. In this sense, a

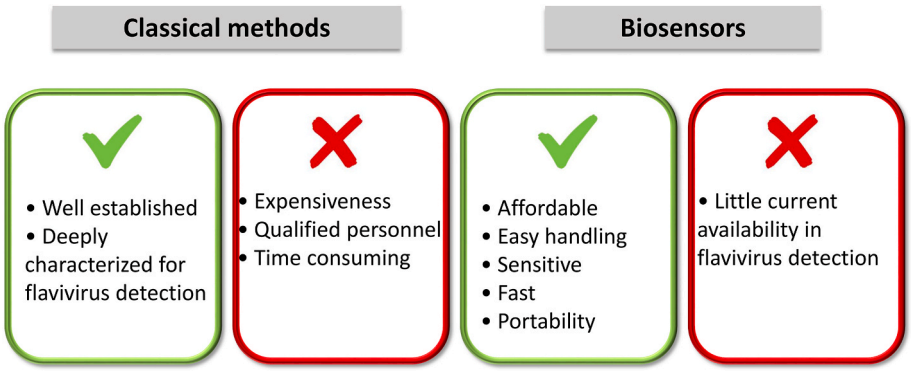

Fig. 7. Advantages and disadvantages of classical methods versus biosensors applied in the detection of flaviviruses.

Table 4
Advantages, disadvantages and detected targets presented according to the principal flavivirus diagnostic approaches.

Methods		Advantages	Disadvantages	Detected targets	Ref.
Classical methods	RT-PCR	Sensitivity	Expensiveness Time-consuming Only applicable in early stages of infection	Flavivirus RNA molecular detection	[29]
	qRT-PCR	Sensitivity Quantitative results	Expensiveness Qualified personel required Only for early stages of infection	Flavivirus RNA molecular detection	[29]
	ELISA	Detection in early and late stages of infection	Cross-reactivity Low specificity	Flavivirus antibody detection (IgM/IgG)	[30]
	PRNT	Gold-standard method for flaviviruses	BSL-3 facilities Qualified personel required Only for early stages of infection	Flavivirus neutralizing antibodies detection	[23]
	Cell culture	Viral isolation capability	BSL-3 facilities Qualified personel required Only for early stages of infection	Flavivirus isolation	[23]
Biosensors (based on bioreceptors)	Enzyme	Affordable Easy handling Specificity	Stability challenges	DENV	[56]
	Antibody	High specificity Sensitivity Portable	Proper immobilization of antibodies	DENV ZIKV JEV WNV	[92] [93] [94] [95]
	Nucleic acid	Affordable Specificity	Limitations in detection of mutated viruses Nucleic acid extraction required Low sensitivity	DENV ZIKV JEV WNV	[96] [97, 98] [99]

huge number of different electrodes have been designed, the most common being those made of graphene and gold. Graphene electrodes have been used recently due to their characteristics of improved sensitivity, thus achieving low detection limits. A graphite-based DNA biosensor was developed specifically to identify the DENV-3 serotype [102]. Additionally, another method was introduced to detect the dengue virus, capable of discriminating between the different serotypes, using an electrochemical method based on graphene polymer [103].

Gold electrodes are also widely used in DENV biosensors. Luna et al. [104] immobilized the lectin concanavalin A on the gold electrode. This approach was also employed by Oliveira et al. with sera from infected patients who developed dengue fever (DF) or dengue hemorrhagic fever (DHF) [105]. In this case, variations in charge transfer resistance were utilized to differentiate the sensor responses for the sera examined (from patients with DF or DHF), thereby aiding in the discrimination of the stages or severity of the disease. Researchers have also used other different lectins immobilized on gold electrodes, such as Cramoll, identified from *Cratylia mollis* seeds [106,107], or Bauhinia monandra lectin (BmoLL) [96] for the detection of DENV-1, DENV-2, and DENV-3 serotypes.

Most electrochemical biosensors for the detection of DENV have been developed targeting the non-structural proteins (NS) of the virus as a bioreception element. Different studies indicate that NS1 antigen is abundant in the serum of patients during the early stages of DENV infection [108,109], making it a potential marker for acute dengue virus infection. Immunosensors targeting this protein have been produced with different electrodes. In this sense, Parkash et al. developed an electrochemical immunosensor modified with the streptavidin/biotin system on screen-printed carbon electrodes (SPCEs) for the detection of the NS1 antigen. The biosensor was tested in patient serum samples [110]. NS1 detection system was also developed by Junior et al. [111], using a DNA aptamer, and other immunosensors based on screen-printed electrodes were developed by different authors [112–114]. Cecchetto et al. also developed different capacitive electrochemical methods for the detection of NS1 in human samples [92, 115]. Similar approaches have been used with anti-DENV2 IgG or other antibodies immobilized on nanoporous alumina electrodes [116,117].

The use of DENV DNA probes has also been widely exploited as bioreception elements in the development of electrochemical biosensors. In this regard, Shingai et al. created a biosensor where the DNA was immobilized on the surface of a ZnO/Pt–Pd nanocomposites electrode [118]. Different DNA probes were also assessed by many other authors [119–121].

More recently, CRISPR-based detection approaches have been

developed as a sensitive method to reveal the presence of DENV in different samples, such as blood and saliva [122], or RNA samples [123,124].

Although electrochemical biosensors are the most commonly used in the detection of DENV, there is also a wide variety of approaches that use optical biosensors. As happened with electrochemical biosensors, different procedures have been developed. In this sense, viral RNA has been evaluated by Chen et al. [125] with gold nanoparticles coupled to quartz crystals. Other authors have performed different RNA biosensors [126–129].

Among optical biosensors, the use of antibodies in the development of immunosensors is a technique also exploited for the detection of DENV. Different immunosensors based on surface plasmon resonance (SPR) have been designed for DENV IgM antibody detection [130–132] or the identification of dengue NS1 antigens [133]. Atias et al. developed a diagnostic tool based on a chemiluminescent optical fiber immunosensor (OFIS), for the detection of anti-DENV immunoglobulin M (IgM) in human serum samples [134].

Mass-based biosensors have also been described for the detection of DENV. In this case, the most commonly used are piezoelectric devices such as immunosensors that detect viral E or NS1 proteins [135–137], or nucleic acid biosensing [125].

Different approaches have been used by authors to determine and compare the sensitivity and recognition capabilities of biosensors, thus confirming the detection of this flavivirus. These methods include techniques such as ELISA, the use of previously titrated viruses or commercially available protein standards, among others.

Despite great efforts to develop DENV biosensors, only a few have been commercialized. Commercially available devices are ViroTrack Dengue Acute, capable of detecting dengue NS1 antigen, an important biomarker of early DENV infection [138], and Bioline™ DENGUE DUO, which detect both DENV NS1 and anti-DENV specific IgM/IgG antibodies [139].

As previously mentioned, the main characteristics of DENV biosensors should be portability, low cost, and easy handling to make them ideal detection systems for POCT and field applications. Likewise, the ability to distinguish between different serotypes and the potential for early detection of infection make biosensors for DENV a highly effective tool in pathogen diagnosis.

1.5.2. Biosensors for the detection of Zika virus

ZIKV is a relatively recent virus identified in the mid-20th century. Hence, studies carried out on the development of biosensors for its detection are scarce. The virus can cause serious diseases such as fetal microcephaly or Guillain-Barré syndrome. Since most infections occur in developing countries, there is an urgent need for affordable and effective biosensors capable of rapidly and accurately identifying ZIKV in epidemic areas [19]. In the search for electrochemical biosensors, different platforms have been used, such as an immunosensor based on ZnO nanostructures immobilized with ZIKV-NS1 antibody [140] or the immobilization of protein E with the development of quantum dots in combination with screen-printed carbon electrodes [141]. Using electrochemical impedance spectroscopy and square wave voltammetry, a biosensor capable of discriminating ZIKV antibodies in blood and saliva from DENV virus-specific antibodies was also assessed [142]. A graphene-enabled biosensor was created to detect ZIKV with a specific NS1 monoclonal antibody [97]. Likewise, the electrochemical modification of pencil carbon graphite electrodes [143], or the detection of genomic RNA using a new platform based on graphite electrodes have been used [91]. Other relevant electrochemical techniques are the development of impedance electrical sensing assay on paper microchips [144] or the immobilization of surface imprinted polymers for sensitive and specific detection of ZIKV [145].

Label-free biosensors have also been described, such as an impedimetric electrochemical DNA genosensor [146] or an E protein-based immunosensor [93].

Among optical biosensors, some authors have used colorimetry for the development of different platforms showing high specificity in the detection of ZIKV [93]. Another work described the development of localized surface plasmon resonance technology to detect the NS1 protein in an immunofluorescence biosensor [147].

Moreover, a mass-based biosensor has been described for the detection of ZIKV using susceptometry measurement techniques [148].

As mentioned in the case of DENV biosensors, various strategies have been employed by researchers to assess and compare the sensitivity and recognition capabilities of devices, thereby confirming the detection of this flavivirus [91,140].

However, further research is needed to achieve rapid and accurate identification using biosensing technologies in the case of ZIKV [149].

1.5.3. Biosensors for the detection of West Nile virus

The research currently being carried out in the development of biosensors for the detection of WNV is very limited. As happened with other flaviviruses, assays are mainly aimed at the development of electrochemical biosensors. In this sense, Park et al. applied an alternating current electrothermal flow technology to provide a rapid biosensor platform based on WNV DNA aptamers exhibiting high selectivity [150]. Other genosensors using DNA have also been described [99].

On the other hand, different assays based on surface-enhanced Raman scattering (SERS) technology have been reported for WNV detection. An immunoassay for the detection of DNA of the pathogen was described using Au nanoparticles [151]. These techniques enable rapid and sensitive detection of WNV, thus contributing to the diagnosis and control of the virus.

Label-free biosensors for WNV detection have also been described based on capacitive techniques [152] or using a paper-based microfluidic analytical device with integrated microwire Au electrodes [153]. These biosensors can detect complementary DNA fragments or viral particles in a rapid and low-cost way, making them suitable for POCT devices.

In the case of optical biosensing, research was carried out to develop a fiber optic immunosensor for the detection of anti-WNV IgG antibodies in serum [95].

1.5.4. Biosensors for the detection of Japanese encephalitis virus

JEV outbreaks predominantly affect rural regions. Therefore, it is not feasible to establish complex laboratory facilities and deploy trained technicians for its diagnosis. Efforts in advances in diagnostic techniques aim to create faster, cost-effective, and more sensitive methods to detect JEV [154]. These innovations, including nanotechnology, are being integrated into biosensors to enhance their sensitivity, thereby facilitating highly effective detection mechanisms.

Electrochemical biosensors have been reported for the detection of JEV. A device consisting of carbon nanoparticles modified SPCEs was assessed using cyclic voltammetry (CV) and EIS to detect JEV antigens in serum samples [155]. Related procedures were based on gold [98] or silver [94] nanoparticles modified SPCE. Other electrochemical strategies have been employed such as gold-coated magnetic beads [156], graphene derivatives [157,158], or surface-enhanced Raman spectroscopy-based biosensors [159].

Label-free-based techniques have also been described. In this regard, two electrochemical immunosensors based on anti-JEV IgG antibodies immobilized on different polyaniline microelectrodes have been reported for the detection of JEV antigens [160,161]. Another reported label-free biosensor was based on the immobilization of JEV-specific serum antibodies on a silanized surface of an interdigitated sensor [162].

Regarding optical biosensors, Liang et al. produced a fluorescent sensor based on virus-molecular imprinted polymers anchored on the surface of silica [163], while He et al. designed a fluorescent sensor based on virus-imprinted polymers [164]. The fluorescence intensity was enhanced in the first work by the fluorescence resonance energy

transfer (FRET) technique. Likewise, other fluorescence molecularly imprinted sensors based on different frameworks were described [165,166], showing remarkable selectivity and sensitivity in detecting JEV.

1.5.5. Biosensors for the detection of yellow fever and tick-borne encephalitis viruses

Ongoing research into the development of biosensors for the detection of YFV and TBEV is rather limited. Only a few works describe the development of biosensors used in the diagnosis of YFV in human serum or plasma samples [167,168], and no devices have been developed for the specific detection of TBEV. However, biosensors have been developed for the diagnosis of multiple flaviviruses, including these two pathogens.

1.5.6. Biosensors for multiple flavivirus detection

As previously mentioned, cross-reactivity between flaviviruses is frequent, particularly in regions with viral co-circulation. The widespread distribution of mosquitoes acting as vectors promotes the coexistence of flaviviral infections in overlapping regions. Most flaviviruses exhibit significant structural similarities, triggering a cross-reactive immune response that can result in false positives in conventional serological tests, especially in secondary infections. To aid in virus recognition, biosensors capable of distinguishing between them have been devised.

The most common devices are those designed for the concurrent detection of ZIKV and DENV, using different approaches for their development, such as the application of DNA-nanotechnology-based detection biosensors, the development of electrochemical devices with different working electrodes for each virus, or the use of CRISPR technology, among others [169–171].

The identification of DENV and YF has been conducted with a multiplexed pathogen detection platform using multi-colored silver nanoplates [172]. Another complex biosensor has been designed to differentiate DENV, ZIKV, and YFV infections. Atomic force microscopy analyses validated the electrode surface modification and unveiled varied topography throughout the biorecognition process. CV and EIS were used for the characterization of the biosensor [173].

For the diagnosis of TBEV, a bi-parametric serological microarray was developed to detect TBEV and WNV. The detection system was based on the specific sequential detection of antibodies [174]. Detection of TBEV, ZIKV, YFV, and JEV, as well as other related arboviruses, has been described using a multiplex recombinase polymerase amplification-based nucleic acid detection platform. The optimal conditions enable fluorescence detection of nucleic acids with high velocity, specificity, and sensitivity. Furthermore, a low-cost, easy-to-handle POCT device was engineered for visualization [175].

A commercially available test is the SD Biosensor STANDARD Q Arbo Panel I (Z/D/C/Y). The test consists of a chromatographic immunoassay for the detection of ZIKV, DENV, and YFV in human serum, plasma, or whole blood (https://www.sdbiosensor.com/product/product_view?product_no=219).

2. Conclusions

Flaviviruses (genus *Orthoflavivirus)* are arboviruses (arthropod-borne viruses) transmitted mainly by mosquitoes or ticks. This genus includes multiple well-known human, animal, and zoonotic pathogens. The spectrum of symptoms induced by flavivirus infections ranges from asymptomatic or mild fever to severe manifestations, mostly hemorrhagic or neurological complications, which can ultimately lead to death. Due to various factors, such as the globalization of travel and trade, climate change, alterations in land use, and changes in vector behavior, several flaviviruses are emerging as significant global health concerns, expanding their presence to new habitats not previously colonized [176]. There are currently no specific antiviral treatments for flaviviruses, and only a limited number of vaccines have been approved for human use against some of them. Hence, understanding the biology of flaviviruses and developing rapid and sensitive diagnostic tests is essential to prevent the spread of these potentially life-threatening pathogens.

Flavivirus infections are usually diagnosed by conventional methods, predominantly serology and molecular assays. However, these techniques have several drawbacks, including high costs, making diagnostic tests unaffordable in low-income countries where the impact of flavivirus is significant. Moreover, these methods require qualified personnel and are time-consuming. It is worth mentioning that cross-reactivity between flaviviruses is frequent, especially in areas where multiple viruses circulate simultaneously. The wide distribution of mosquitoes, which act as vectors, facilitates the co-occurrence of flaviviral infections in overlapping geographical areas. Many flaviviruses share notable antigenic similarities, leading to a cross-reactive immune response that can produce false positives in serological tests. To address this challenge, the development of biosensors has been overcoming these obstacles in recent years, showing new, rapid, and sensitive approaches for the diagnosis of flaviviruses. In this sense, biosensors offer a wide range of advantages such as exceptional sensitivity and specificity easy handling, low cost, and the ability to provide rapid responses and perform real-time analyses [101]. All these features facilitate rapid intervention in the event of health emergencies such as pandemic situations.

As previously mentioned, most techniques enable the direct analysis of samples without the need for pre-treatment, as the commonly utilized samples—such as serum, saliva, and other bodily fluids—allow for straightforward handling and quick results. However, in some cases, biosensor samples may require prior processing, usually employing methods similar to those used in conventional diagnostics, such as nucleic acid extraction and other related techniques.

Furthermore, the possibility of designing compact-sized portable devices renders biosensors ideal for point-of-care testing (POCT) in bioanalytical clinics [154]. Currently, there is a growing demand for POCT to swiftly detect infectious diseases, including those caused by viruses. These devices are crucial to slowing the spread of infectious diseases by enabling real-time testing and providing rapid, high-quality diagnoses, as flavivirus outbreaks occur mainly in rural areas, making it unfeasible to have specialized laboratories and skilled workers to carry out diagnoses. Hence, biosensors are the most notable advance in the detection of these life-threatening pathogens. In this sense, many researchers have developed several types of equipment classified according to the technology used by their design. In the case of flaviviruses, most of the devices developed use electrochemical transducer technology, combined with a huge variety of bioreceptors, thus achieving a significant number of devices with different specificity and sensitivity for the rapid and efficient diagnosis of the aforementioned virus.

Since DENV is the most significant life-threatening flavivirus, causing approximately 40,000 deaths each year, biosensing technologies are primarily focused on the early detection of this pathogen. Some studies have shown that the dengue virus nonstructural 1 (NS1) antigen is present in the serum of patients during the early stages of infection, indicating that NS1 may serve as an effective marker for acute dengue virus infection. In this context, biosensors designed to detect DENV NS1 could provide a reliable means of identifying early acute dengue infections, thereby potentially improving disease, as no specific treatments are available for dengue or any other flavivirus. The existing treatment options are only supportive and focused on mitigating complications and reducing the severity of symptoms.

On the other hand, biosensors in flavivirus research are enabling effective discrimination between related strains, or even, in the case of DENV, between serotypes. Of particular importance is to highlight that reinfections with various serotypes of this virus can exacerbate the disease, potentially leading to fatal outcomes due to antibody-dependent enhancement (ADE) [177]. In this context, recent advancements in biosensor technology for flavivirus are focused on achieving accurate infection diagnosis. Notably, new devices are being developed that

enable multiplex analysis of various flaviviruses, including those designed for the simultaneous diagnosis of DENV and YF [172], as well as DENV, ZIKV, and YFV [173].

3. Future perspectives

Since there are no specific treatments and only a few available vaccines for human life-threatening flaviviruses, swift and early diagnosis is crucial to implement timely health interventions, minimizing the risk of health complications and preventing further virus transmission. In this review, a comprehensive overview of the advances in the field of biosensors for the detection of these pathogens has been described. These innovative technologies offer simplicity, user-friendliness, and cost-effectiveness, and have substantial potential to replace conventional, lengthy, and time-consuming diagnostic methods.

The main advantage of biosensors is that they can be used in POCT. As previously mentioned, the ideal POCT device should meet the ASSURED criteria proposed by the WHO. However, significant drawbacks still need to be resolved. In this sense, there are currently just a few biosensors available for flavivirus detection and the majority of them are still non-portable devices. Therefore, there is a need to develop innovative biosensors with appropriate technology for cost-effective production to be used as POCT platforms. On the other hand, the production of biosensors that can specifically differentiate between flaviviruses without exhibiting cross-reactivity between viruses sharing similar genomes and antigenic structures must be crucial. This issue could restrict the applicability of biosensors, making it essential to carry out important ongoing research in this field. This represents one of the most significant challenges currently faced in the field of biosensors for flavivirus. As previously mentioned, DENV is the most widely spread arbovirus and poses a considerable threat to human health, particularly in low-income regions with greater healthcare needs. Furthermore, reinfections with this virus can lead to an exacerbation of the disease due to the phenomenon of antibody-dependent enhancement (ADE). Thus, it is crucial to develop rapid, cost-effective, and portable diagnostic methods for prompt detection and response in health emergencies. Likewise, other highly important flaviviruses, such as WNV, are re-emerging due to circumstances such as climate change and global warming. This pathogen, regarded as one of the leading causes of encephalitis globally, is spreading to regions where it was previously undetected, thereby presenting substantial risks to human health.

The development of biosensors for flaviviruses is increasingly centered on multiplexed platforms that can detect multiple viruses from the same family simultaneously, which is essential in areas where several flaviviruses co-circulate. These multiplexed systems often utilize microfluidics and advanced nanomaterials to enhance sensitivity and specificity while ensuring portability and user-friendliness. Research is also investigating wearable biosensors and smartphone-integrated devices for real-time monitoring and surveillance of flavivirus infections in endemic regions. The aim is to develop cost-effective, user-friendly devices that can be implemented in remote or resource-limited settings, offering vital tools for public health monitoring and outbreak management.

Therefore, further research is necessary in the future to ensure consistent production and performance of biosensors, and the adoption of rapid readout methods, such as this smartphone technology, which would advance the biosensor industry and transform POCT for the diagnosis of flavivirus.

Funding statement

This work was supported by the Spanish Ministry of Science and Innovation AEI under grant PID2020-119195RJ-I00 (to NJO).

CRediT authorship contribution statement

Ana-Belén Blázquez: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Nereida Jiménez de Oya:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Investigation, Funding acquisition, Data curation, Conceptualization.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

Not applicable.

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.

References

[1] Postler TS, et al. Renaming of the genus Flavivirus to Orthoflavivirus and extension of binomial species names within the family Flaviviridae. Arch Virol 2023;168(9):224.
[2] Blazquez AB, et al. Stress responses in flavivirus-infected cells: activation of unfolded protein response and autophagy. Front Microbiol 2014;5:266.
[3] van Leur SW, et al. Pathogenesis and virulence of flavivirus infections. Virulence 2021;12(1):2814–38.
[4] Pierson TC, Diamond MS. The continued threat of emerging flaviviruses. Nat Microbiol 2020;5(6):796–812.
[5] Dutta SK, Langenburg T. A perspective on current flavivirus vaccine development: a brief review. Viruses 2023;15(4).
[6] Goto H. Efficacy of Japanese encephalitis vaccine in horses. Equine Vet J 1976;8(3):126–7.
[7] Nah JJ, et al. The present and future of veterinary vaccines for Japanese encephalitis in Korea. Clin Exp Vaccine Res 2015;4(2):130–6.
[8] Simmonds P, et al. ICTV virus taxonomy profile: Flaviviridae. J Gen Virol 2017;98(1):2–3.
[9] Saiz JC, et al. Pathogenicity and virulence of West Nile virus revisited eight decades after its first isolation. Virulence 2021;12(1):1145–73.
[10] Latanova A, Starodubova E, Karpov V. Flaviviridae nonstructural proteins: the role in molecular mechanisms of triggering inflammation. Viruses 2022;14(8).
[11] Brinton MA. Replication cycle and molecular biology of the West Nile virus. Viruses 2013;6(1):13–53.
[12] Blazquez AB, Saiz JC. Potential for protein kinase pharmacological regulation in Flaviviridae infections. Int J Mol Sci 2020;21(24).
[13] Martin-Acebes MA, Saiz JC. West Nile virus: a re-emerging pathogen revisited. World J Virol 2012;1(2):51–70.
[14] Chan KR, et al. Serological cross-reactivity among common flaviviruses. Front Cell Infect Microbiol 2022;12:975398.
[15] Kading RC, Brault AC, Beckham JD. Global perspectives on arbovirus outbreaks: a 2020 snapshot. Trav Med Infect Dis 2020;5(3).
[16] www.who.int/emergencies/disease-outbreak-news/item/2023-DON498.
[17] www.ecdc.europa.eu.
[18] Wang WH, et al. Dengue hemorrhagic fever - a systemic literature review of current perspectives on pathogenesis, prevention and control. J Microbiol Immunol Infect 2020;53(6):963–78.
[19] Saiz JC, et al. Zika virus: the latest newcomer. Front Microbiol 2016;7:496.
[20] Blazquez AB, Saiz JC. Neurological manifestations of Zika virus infection. World J Virol 2016;5(4):135–43.
[21] Bai F, et al. Current understanding of West Nile virus clinical manifestations, immune responses, neuroinvasion, and immunotherapeutic implications. Pathogens 2019;8(4).
[22] Smithburn KC, H TP, Burke AW, Paul JH. A neurotropic virus isolated from the blood of a native of Uganda. Am J Trop Med Hyg 1940;20:471–92.
[23] Maeda A, Maeda J. Review of diagnostic plaque reduction neutralization tests for flavivirus infection. Vet J 2013;195(1):33–40.
[24] www.who.int/news-room/fact-sheets/detail/japanese-encephalitis.
[25] www.ecdc.europa.eu/en/japanese-encephalitis/facts.
[26] www.who.int/news-room/fact-sheets/detail/yellow-fever.
[27] www.who.int/health-topics/tick-borne-encephalitis#tab=tab_1.
[28] Kvam KA, et al. Outcome and sequelae of infectious encephalitis. J Clin Neurol 2024;20(1):23–36.

[29] Ayers M, et al. A single tube RT-PCR assay for the detection of mosquito-borne flaviviruses. J Virol Methods 2006;135(2):235–9.
[30] Kuno G. Serodiagnosis of flaviviral infections and vaccinations in humans. Adv Virus Res 2003;61:3–65.
[31] Brahmkhatri V, et al. Recent progress in detection of chemical and biological toxins in Water using plasmonic nanosensors. Trends in Environmental Analytical Chemistry 2021;30:e00117.
[32] Bhalla N, et al. Introduction to biosensors. Essays Biochem 2016;60(1):1–8.
[33] Clark Jr LC, Lyons C. Electrode systems for continuous monitoring in cardiovascular surgery. Ann N Y Acad Sci 1962;102:29–45.
[34] Saylan Y, et al. An alternative medical diagnosis method: biosensors for virus detection. Biosensors 2019;9(2).
[35] Pashchenko O, et al. A comparison of optical, electrochemical, magnetic, and colorimetric point-of-care biosensors for infectious disease diagnosis. ACS Infect Dis 2018;4(8):1162–78.
[36] Jain S, et al. Internet of medical things (IoMT)-integrated biosensors for point-of-care testing of infectious diseases. Biosens Bioelectron 2021;179:113074.
[37] Phumlani T, Poslet Morgan S, Zikhona N-T. Biosensors: design, development and applications. In: Sadia A, Akhtar MS, Hyung-Shik S, editors. Nanopores. Rijeka: IntechOpen; 2021. Ch. 3.
[38] Naresh V, Lee N. A review on biosensors and recent development of nanostructured materials-enabled biosensors. Sensors 2021;21(4).
[39] Velusamy V, et al. An overview of foodborne pathogen detection: in the perspective of biosensors. Biotechnol Adv 2010;28(2):232–54.
[40] Kucherenko IS, et al. Advances in nanomaterial application in enzyme-based electrochemical biosensors: a review. Nanoscale Adv 2019;1(12):4560–77.
[41] Mollarasouli F, Kurbanoglu S, Ozkan SA. The role of electrochemical immunosensors in clinical analysis. Biosensors 2019;9(3).
[42] Byrne B, et al. Antibody-based sensors: principles, problems and potential for detection of pathogens and associated toxins. Sensors 2009;9(6):4407–45.
[43] Du Y, Dong S. Nucleic acid biosensors: recent advances and perspectives. Anal Chem 2017;89(1):189–215.
[44] El-Safty SA, Shenashen MA. Nanoscale dynamic chemical, biological sensor material designs for control monitoring and early detection of advanced diseases. Mater Today Bio 2020;5:100044.
[45] Cecilia C, et al. Immunosensors. In: Toonika R, editor. Biosensors. Rijeka: IntechOpen; 2015. Ch. 6.
[46] Sypabekova M, et al. Selection, characterization, and application of DNA aptamers for detection of Mycobacterium tuberculosis secreted protein MPT64. Tuberculosis 2017;104:70–8.
[47] McConnell EM, Nguyen J, Li Y. Aptamer-based biosensors for environmental monitoring. Front Chem 2020;8:434.
[48] Zhou H, Li Y, Wu W. Aptamers: promising reagents in biomedicine application. Adv Biol (Weinh) 2024;8(6):e2300584.
[49] Liu Q, et al. Cell-based biosensors and their application in biomedicine. Chem Rev 2014;114(12):6423–61.
[50] Gheorghiu M. A short review on cell-based biosensing: challenges and breakthroughs in biomedical analysis. J Biomed Res 2020;35(4):255–63.
[51] Gupta N, et al. Cell-based biosensors: recent trends, challenges and future perspectives. Biosens Bioelectron 2019;141:111435.
[52] Su L, et al. Microbial biosensors: a review. Biosens Bioelectron 2011;26(5):1788–99.
[53] D'Souza SF. Microbial biosensors. Biosens Bioelectron 2001;16(6):337–53.
[54] Liu C, et al. Engineering whole-cell microbial biosensors: design principles and applications in monitoring and treatment of heavy metals and organic pollutants. Biotechnol Adv 2022;60:108019.
[55] Stukovnik Z, Bren U. Recent developments in electrochemical-impedimetric biosensors for virus detection. Int J Mol Sci 2022;23(24).
[56] Khristunova E, et al. Label-free electrochemical biosensors for the determination of flaviviruses: dengue, zika, and Japanese encephalitis. Sensors 2020;20(16).
[57] Hui Y, et al. Recent advancements in electrochemical biosensors for monitoring the water quality. Biosensors 2022;12(7).
[58] Blair EO, Corrigan DK. A review of microfabricated electrochemical biosensors for DNA detection. Biosens Bioelectron 2019;134:57–67.
[59] Mishra GK, et al. Food safety analysis using electrochemical biosensors. Foods 2018;7(9).
[60] Chen Y, et al. Amperometric DNA biosensor for Mycobacterium tuberculosis detection using flower-like carbon nanotubes-polyaniline nanohybrid and enzyme-assisted signal amplification strategy. Biosens Bioelectron 2018;119:215–20.
[61] Layqah LA, Eissa S. An electrochemical immunosensor for the corona virus associated with the Middle East respiratory syndrome using an array of gold nanoparticle-modified carbon electrodes. Mikrochim Acta 2019;186(4):224.
[62] Chen Y, et al. Optical biosensors based on refractometric sensing schemes: a review. Biosens Bioelectron 2019;144:111693.
[63] Damborsky P, Svitel J, Katrlik J. Optical biosensors. Essays Biochem 2016;60(1):91–100.
[64] Tatiana Duque M, et al. New insights on optical biosensors: techniques, construction and application. In: Toonika R, editor. State of the art in biosensors. Rijeka: IntechOpen; 2013. Ch. 5.
[65] Nejati-Koshki K, et al. Biomarkers and optical based biosensors in cardiac disease detection: early and accurate diagnosis. Anal Methods 2023;15(41):5441–58.
[66] Gharatape A, Yari Khosroushahi A. Optical biomarker-based biosensors for cancer/infectious disease medical diagnoses. Appl Immunohistochem Mol Morphol 2019;27(4):278–86.
[67] Zhou X, et al. Smartphone-based pH responsive 3-channel colorimetric biosensor for non-enzymatic multi-antibiotic residues. Food Chem 2023;429:136953.
[68] Rathi BS, Kumar PS, Vo DN. Critical review on hazardous pollutants in water environment: occurrence, monitoring, fate, removal technologies and risk assessment. Sci Total Environ 2021;797:149134.
[69] Yoo SM, Lee SY. Optical biosensors for the detection of pathogenic microorganisms. Trends Biotechnol 2016;34(1):7–25.
[70] Chen C, Wang J. Optical biosensors: an exhaustive and comprehensive review. Analyst 2020;145(5):1605–28.
[71] Bleher O, et al. Development of a new parallelized, optical biosensor platform for label-free detection of autoimmunity-related antibodies. Anal Bioanal Chem 2014;406(14):3305–14.
[72] Danielsson B. Calorimetric biosensors. J Biotechnol 1990;15(3):187–200.
[73] Gaddes D, Reeves WB, Tadigadapa S. Calorimetric biosensing system for quantification of urinary creatinine. ACS Sens 2017;2(6):796–802.
[74] van Grinsven B, et al. Label-free detection of Escherichia coli based on thermal transport through surface imprinted polymers. ACS Sens 2016;1(9):1140–7.
[75] Raghavan V, et al. An enzyme thermistor-based assay for total and free cholesterol. Clin Chim Acta 1999;289(1–2):145–58.
[76] Harborn U, et al. Evaluation of a miniaturized thermal biosensor for the determination of glucose in whole blood. Clin Chim Acta 1997;267(2):225–37.
[77] Satoh I. Use of immobilized alkaline phosphatase as an analytical tool for flow-injection biosensing of zinc(II) and cobalt(II) ions. Ann N Y Acad Sci 1992;672:240–4.
[78] Cali K, Tuccori E, Persaud KC. Gravimetric biosensors. Methods Enzymol 2020;642:435–68.
[79] Nair MP, Teo AJT, Li KHH. Acoustic biosensors and microfluidic devices in the decennium: principles and applications. Micromachines 2021;13(1).
[80] Marrazza G. Piezoelectric biosensors for organophosphate and carbamate pesticides: a review. Biosensors 2014;4(3):301–17.
[81] Narita F, et al. A review of piezoelectric and magnetostrictive biosensor materials for detection of COVID-19 and other viruses. Adv Mater 2021;33(1):e2005448.
[82] Thakur MS, Ragavan KV. Biosensors in food processing. J Food Sci Technol 2013;50(4):625–41.
[83] Akgonullu S, Ozgur E, Denizli A. Quartz crystal microbalance-based aptasensors for medical diagnosis. Micromachines 2022;13(9).
[84] Ren C, et al. Biomarkers detection with magnetoresistance-based sensors. Biosens Bioelectron 2020;165:112340.
[85] Nabaei V, Chandrawati R, Heidari H. Magnetic biosensors: modelling and simulation. Biosens Bioelectron 2018;103:69–86.
[86] Baselt DR, et al. A biosensor based on magnetoresistance technology. Biosens Bioelectron 1998;13(7–8):731–9.
[87] GhaderiShekhiAbadi P, et al. Magnetic biosensors for identification of SARS-CoV-2, Influenza, HIV, and Ebola viruses: a review. Nanotechnology 2023;34(27).
[88] Lin G, Makarov D, Schmidt OG. Magnetic sensing platform technologies for biomedical applications. Lab Chip 2017;17(11):1884–912.
[89] Llandro J, et al. Magnetic biosensor technologies for medical applications: a review. Med Biol Eng Comput 2010;48(10):977–98.
[90] Garkani Nejad F, et al. Magnetic nanomaterials based electrochemical (bio) sensors for food analysis. Talanta 2021;228:122075.
[91] Moço ACR, et al. Electrochemical detection of zika virus in biological samples: a step for diagnosis point-of-care. Electroanalysis 2019;31(8):1580–7.
[92] Cecchetto J, et al. Serological point-of-care and label-free capacitive diagnosis of dengue virus infection. Biosens Bioelectron 2020;151:111972.
[93] Kaushik A, et al. A sensitive electrochemical immunosensor for label-free detection of Zika-virus protein. Sci Rep 2018;8(1):9700.
[94] Chin SF, et al. Electrochemical determination of Japanese encephalitis virus antigen using silver nanoparticles modified screen-printed carbon electrode. Nano Biomedicine and Engineering 2019;11(4):333–9.
[95] Herrmann S, et al. Chemiluminescent optical fiber immunosensor for the detection of anti-West Nile virus IgG. Talanta 2005;66(1):6–14.
[96] Andrade CA, et al. Diagnosis of dengue infection using a modified gold electrode with hybrid organic-inorganic nanocomposite and Bauhinia monandra lectin. J Colloid Interface Sci 2011;362(2):517–23.
[97] Afsahi S, et al. Novel graphene-based biosensor for early detection of Zika virus infection. Biosens Bioelectron 2018;100:85–8.
[98] Geng X, et al. Sensitive impedimetric immunoassay of Japanese encephalitis virus based on enzyme biocatalyzed precipitation on a gold nanoparticle-modified screen-printed carbon electrode. Anal Sci 2016;32(10):1105–9.
[99] Cosnier S, et al. Electroenzymatic polypyrrole-intercalator sensor for the determination of West Nile virus cDNA. Anal Chem 2006;78(19):7054–7.
[100] Parkash O, Shueb RH. Diagnosis of dengue infection using conventional and biosensor based techniques. Viruses 2015;7(10):5410–27.
[101] Cordeiro TAR, et al. Electrochemical biosensors for neglected tropical diseases: a review. Talanta 2021;234:122617.
[102] Khan MZH, et al. Ultrasensitive detection of pathogenic viruses with electrochemical biosensor: state of the art. Biosens Bioelectron 2020;166:112431.
[103] Navakul K, et al. A novel method for dengue virus detection and antibody screening using a graphene-polymer based electrochemical biosensor. Nanomedicine 2017;13(2):549–57.
[104] Luna DM, et al. Biosensor based on lectin and lipid membranes for detection of serum glycoproteins in infected patients with dengue. Chem Phys Lipids 2014;180:7–14.
[105] Oliveira MD, Correia MT, Diniz FB. Concanavalin A and polyvinyl butyral use as a potential dengue electrochemical biosensor. Biosens Bioelectron 2009;25(4):728–32.

[106] Avelino KYPS, et al. Biosensor based on hybrid nanocomposite and CramoLL lectin for detection of dengue glycoproteins in real samples. Synth Met 2014;194:102–8.
[107] Oliveira MDL, et al. Detection of dengue virus serotypes on the surface of gold electrode based on Cratylia mollis lectin affinity. Sensor Actuator B Chem 2011;155(2):789–95.
[108] Xu H, et al. Serotype 1-specific monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: implications for early diagnosis and serotyping of dengue virus infections. J Clin Microbiol 2006;44(8):2872–8.
[109] Dussart P, et al. Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. Clin Vaccine Immunol 2006;13(11):1185–9.
[110] Parkash O, Yean CY, Shueb RH. Screen printed carbon electrode based electrochemical immunosensor for the detection of dengue NS1 antigen. Diagnostics 2014;4(4):165–80.
[111] Bachour Junior B, et al. Electrochemical aptasensor for NS1 detection: towards a fast dengue biosensor. Talanta 2021;233:122527.
[112] Siqueira Silva M, et al. Rational selection of hidden epitopes for a molecularly imprinted electrochemical sensor in the recognition of heat-denatured dengue NS1 protein. Biosens Bioelectron 2021;191:113419.
[113] Silva MM, et al. A thiophene-modified screen printed electrode for detection of dengue virus NS1 protein. Talanta 2014;128:505–10.
[114] Dias AC, et al. A sensor tip based on carbon nanotube-ink printed electrode for the dengue virus NS1 protein. Biosens Bioelectron 2013;44:216–21.
[115] Cecchetto J, et al. The capacitive sensing of NS1 Flavivirus biomarker. Biosens Bioelectron 2017;87:949–56.
[116] Peh AE, Li SF. Dengue virus detection using impedance measured across nanoporous alumina membrane. Biosens Bioelectron 2013;42:391–6.
[117] Nguyen BT, et al. Electrochemical impedance spectroscopy characterization of nanoporous alumina dengue virus biosensor. Bioelectrochemistry 2012;88:15–21.
[118] Singhal C, Pundir CS, Narang J. A genosensor for detection of consensus DNA sequence of Dengue virus using ZnO/Pt-Pd nanocomposites. Biosens Bioelectron 2017;97:75–82.
[119] Dutta Chowdhury A, et al. Femtomolar detection of dengue virus DNA with serotype identification ability. Anal Chem 2018;90(21):12464–74.
[120] Oliveira N, et al. A sensitive and selective label-free electrochemical DNA biosensor for the detection of specific dengue virus serotype 3 sequences. Sensors 2015;15(7):15562–77.
[121] Souza E, et al. Label-free electrochemical detection of the specific oligonucleotide sequence of dengue virus type 1 on pencil graphite electrodes. Sensors 2011;11(6):5616–29.
[122] Raza S, et al. Innovations in dengue virus detection: an overview of conventional and electrochemical biosensor approaches. Biotechnol Appl Biochem 2024;71(3):481–500.
[123] Lee Y, et al. Fabrication of ultrasensitive electrochemical biosensor for dengue fever viral RNA Based on CRISPR/Cpf1 reaction. Sensor Actuator B Chem 2021;326:128677.
[124] Wang J, et al. A sensitive electrochemical method for rapid detection of dengue virus by CRISPR/Cas13a-assisted catalytic hairpin assembly. Anal Chim Acta 2021;1187:339131.
[125] Chen SH, et al. A method of layer-by-layer gold nanoparticle hybridization in a quartz crystal microbalance DNA sensing system used to detect dengue virus. Nanotechnology 2009;20(21):215501.
[126] Cam Duyen VT, et al. A novel colorimetric biosensor for rapid detection of dengue virus upon acid-induced aggregation of colloidal gold. Anal Methods 2023;15(32):3991–9.
[127] Jamaluddin ND, et al. G-quadruplex microspheres-based optical RNA biosensor for arthropod-borne virus pathogen detection: a proof-of-concept with dengue serotype 2. Int J Biol Macromol 2022;199:1–9.
[128] Yrad FM, Castanares JM, Alocilja EC. Visual detection of dengue-1 RNA using gold nanoparticle-based lateral flow biosensor. Diagnostics 2019;9(3).
[129] Baeumner AJ, et al. Biosensor for dengue virus detection: sensitive, rapid, and serotype specific. Anal Chem 2002;74(6):1442–8.
[130] Jahanshahi P, Sekaran SD, Adikan FR. Optical and analytical investigations on dengue virus rapid diagnostic test for IgM antibody detection. Med Biol Eng Comput 2015;53(8):679–87.
[131] Jahanshahi P, et al. Rapid immunoglobulin M-based dengue diagnostic test using surface plasmon resonance biosensor. Sci Rep 2014;4:3851.
[132] Kumbhat S, et al. Surface plasmon resonance based immunosensor for serological diagnosis of dengue virus infection. J Pharm Biomed Anal 2010;52(2):255–9.
[133] Farooq S, et al. Optimizing and quantifying gold nanospheres based on LSPR label-free biosensor for dengue diagnosis. Polymers 2022;14(8).
[134] Atias D, et al. Chemiluminescent optical fiber immunosensor for the detection of IgM antibody to dengue virus in humans. Sensor Actuator B Chem 2009;140(1):206–15.
[135] Pirich CL, et al. Piezoelectric immunochip coated with thin films of bacterial cellulose nanocrystals for dengue detection. Biosens Bioelectron 2017;92:47–53.
[136] Tai DF, et al. Artificial receptors in serologic tests for the early diagnosis of dengue virus infection. Clin Chem 2006;52(8):1486–91.
[137] Wu TZ, et al. Piezoelectric immunochip for the detection of dengue fever in viremia phase. Biosens Bioelectron 2005;21(5):689–95.
[138] cordis.europa.eu/project/id/811953.
[139] www.globalpointofcare.abbott/ar/es/product-details/bioline-dengue-duo-ns1-ag-ab-combo.html.
[140] Faria AM, Mazon T. Early diagnosis of Zika infection using a ZnO nanostructures-based rapid electrochemical biosensor. Talanta 2019;203:153–60.
[141] Ribeiro JFF, et al. Sensitive Zika biomarker detection assisted by quantum dot-modified electrochemical immunosensing platform. Colloids Surf B Biointerfaces 2023;221:112984.
[142] Cabral-Miranda G, et al. Biosensor-based selective detection of Zika virus specific antibodies in infected individuals. Biosens Bioelectron 2018;113:101–7.
[143] da Fonseca Alves R, et al. Novel electrochemical genosensor for Zika virus based on a poly-(3-amino-4-hydroxybenzoic acid)-modified pencil carbon graphite electrode. Sensor Actuator B Chem 2019;296:126681.
[144] Draz MS, et al. Nanoparticle-enhanced electrical detection of Zika virus on paper microchips. Nanoscale 2018;10(25):11841–9.
[145] Tancharoen C, et al. Electrochemical biosensor based on surface imprinting for zika virus detection in serum. ACS Sens 2019;4(1):69–75.
[146] Faria HAM, Zucolotto V. Label-free electrochemical DNA biosensor for zika virus identification. Biosens Bioelectron 2019;131:149–55.
[147] Takemura K, et al. A localized surface plasmon resonance-amplified immunofluorescence biosensor for ultrasensitive and rapid detection of nonstructural protein 1 of Zika virus. PLoS One 2019;14(1):e0211517.
[148] Tian B, et al. Attomolar Zika virus oligonucleotide detection based on loop-mediated isothermal amplification and AC susceptometry. Biosens Bioelectron 2016;86:420–5.
[149] Nicolini AM, McCracken KE, Yoon JY. Future developments in biosensors for field-ready Zika virus diagnostics. J Biol Eng 2017;11:7.
[150] Park H, et al. Fast-response electrochemical biosensor based on a truncated aptamer and MXene heterolayer for West Nile virus detection in human serum. Bioelectrochemistry 2023;154:108540.
[151] Harpster MH, et al. SERS detection of indirect viral DNA capture using colloidal gold and methylene blue as a Raman label. Biosens Bioelectron 2009;25(4):674–81.
[152] Wang L, et al. A sensitive DNA capacitive biosensor using interdigitated electrodes. Biosens Bioelectron 2017;87:646–53.
[153] Channon RB, et al. Development of an electrochemical paper-based analytical device for trace detection of virus particles. Anal Chem 2018;90(12):7777–83.
[154] Roberts A, Gandhi S. Japanese encephalitis virus: a review on emerging diagnostic techniques. Front Biosci (Landmark Ed) 2020;25(10):1875–93.
[155] Lai HC, et al. Carbon nanoparticles based electrochemical biosensor strip for detection of Japanese encephalitis virus. J Nanomater 2017;2017(1):3615707.
[156] Li F, et al. Facile fabrication of magnetic gold electrode for magnetic beads-based electrochemical immunoassay: application to the diagnosis of Japanese encephalitis virus. Biosens Bioelectron 2011;26(10):4253–6.
[157] Roberts A, et al. Electroactive reduced graphene oxide for highly sensitive detection of secretory non-structural 1 protein: a potential diagnostic biomarker for Japanese encephalitis virus. Biosens Bioelectron 2022;198:113837.
[158] Roberts A, et al. Graphene functionalized field-effect transistors for ultrasensitive detection of Japanese encephalitis and Avian influenza virus. Sci Rep 2020;10(1):14546.
[159] Tripathi MN, et al. SERS based rapid and ultrasensitive detection of Japanese Encephalitis Virus. Antivir Res 2022;205:105382.
[160] Hien HT, et al. Enhancement of biosensing performance using a polyaniline/multiwalled carbon nanotubes nanocomposite. J Mater Sci 2017;52(3):1694–703.
[161] Tuan CV, et al. Polyaniline nanowires-based electrochemical immunosensor for label free detection of Japanese encephalitis virus. Anal Lett 2013;46(8):1229–40.
[162] Huy TQ, et al. A novel biosensor based on serum antibody immobilization for rapid detection of viral antigens. Talanta 2011;86:271–7.
[163] Liang C, et al. A virus-MIPs fluorescent sensor based on FRET for highly sensitive detection of JEV. Talanta 2016;160:360–6.
[164] He K, et al. Highly selective recognition and fluorescent detection of JEV via virus-imprinted magnetic silicon microspheres. Sensor Actuator B Chem 2016;233:607–14.
[165] Yang J, et al. A novel fluorescence molecularly imprinted sensor for Japanese encephalitis virus detection based on metal organic frameworks and passivation-enhanced selectivity. Talanta 2020;212:120744.
[166] Luo L, et al. Fast and sensitive detection of Japanese encephalitis virus based on a magnetic molecular imprinted polymer-resonance light scattering sensor. Talanta 2019;202:21–6.
[167] Liv L, Ozerdem Z. First DFT-supported point of care and novel electrochemical biosensing: determination of yellow fever NS1 antibody in human plasma. Int J Biol Macromol 2024;269(Pt 2):132169.
[168] Ofosu-Appiah LH, et al. An evaluation of the diagnostic performance characteristics of the Yellow Fever IgM immunochromatographic rapid diagnostic test kit from SD Biosensor in Ghana. PLoS One 2022;17(1):e0262312.
[169] Park G, et al. Recent developments in DNA-nanotechnology-powered biosensors for zika/dengue virus molecular diagnostics. Nanomaterials 2023;13(2).
[170] Sampaio I, et al. Electrochemical detection of Zika and Dengue infections using a single chip. Biosens Bioelectron 2022;216:114630.
[171] Gootenberg JS, et al. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science 2018;360(6387):439–44.
[172] Yen CW, et al. Multicolored silver nanoparticles for multiplexed disease diagnostics: distinguishing dengue, yellow fever, and Ebola viruses. Lab Chip 2015;15(7):1638–41.
[173] Simao EP, et al. Nanostructured impedimetric lectin-based biosensor for arboviruses detection. Talanta 2020;208:120338.
[174] Tagliabue G, et al. A label-free immunoassay for Flavivirus detection by the Reflective Phantom Interface technology. Biochem Biophys Res Commun 2017;492(4):558–64.

[175] Ma Y, et al. FEN1-aided recombinase polymerase amplification (FARPA) for one-pot and multiplex detection of nucleic acids with an ultra-high specificity and sensitivity. Biosens Bioelectron 2023;237:115456.
[176] Daep CA, Munoz-Jordan JL, Eugenin EA. Flaviviruses, an expanding threat in public health: focus on dengue, West Nile, and Japanese encephalitis virus. J Neurovirol 2014;20(6):539–60.
[177] Martin-Acebes MA, Saiz JC, Jimenez de Oya N. Antibody-dependent enhancement and zika: real threat or phantom menace? Front Cell Infect Microbiol 2018;8:44.