Recent Trends in Clinical Diagnosis for Viral Disease Detection Based on Miniaturized Biosensors

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Naveen Kumar Singh, Himali Horo, and Vikky Rajulapati

Abstract

Rapid detection of a virus through its biomarkers over miniaturized device for disease diagnosis is in critical demand that requires optimal clinical outcomes for public health. Traditional in vitro testing for viral infectious diseases is time-consuming and requires well-equipped laboratories, skilled personnel, and bulky equipment. With the recent advancement in the multiplexed miniaturized diagnostic technologies, biosensor-based machineries can deliver point-of-care devices that match or outshine conventional standards concerning time, precision, and cost. Broadly classified, modern biosensors take advantage of nano and microfabrication technologies with diverse sensing strategies, such as mechanical, optical, and electrical transducers. This chapter reviewed the miniaturized biosensors for their point-of-care and point-of-need analytical performance in various diseases and their ground-level problems. Miniaturized biosensors for virus disease detection are complex analytical tools that combine interdisciplinary understandings based on biological chemistry, electrochemistry, materials science, and enzymology. This chapter discusses different types of biosensors for viruses and their biomarker detection for various diseases, their properties, the methods and techniques used for sensor fabrications, and their applications in different fields with some selected examples. We evaluate the advances of

N. K. Singh (✉)
Department of Electrical and Computer engineering, University of California, San Diego, CA, USA
e-mail: nsingh@eng.ucsd.edu

H. Horo
Center for the Environment, Indian Institute of Technology Guwahati, Guwahati, Assam, India

V. Rajulapati
School of Energy and Chemical Engineering, Ulsan National Institute of Sciences and Technology (UNIST), Ulsan, South Korea

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biosensors for virus diseases diagnostics and discuss the critical challenges that need to be overcome to miniaturize diagnostic biosensors in real-world settings. The future approach focuses on the advanced strategies to fulfill current unmet clinical needs.

Keywords

Clinical diagnosis · Miniaturized biosensors · Microbial detection · Virus

6.1 Introduction

Viruses are astonishing pathogens associated with severe morbidity and mortality throughout the world since human history. They are highly infectious in nature, and the absence of deep knowledge and an effective prevention system is the main reason behind their devastating health impact. In the present context, immigration, industrialization, and the gap in efficient point-of-care (PoC) detection systems potentially contribute to the possibility of commonness and outbreak of viral infections around the globe. The advancement of current science and availability of pathogen-specific therapy options for viral infection increased the need for point-of-need efficient diagnostic tests. Improved knowledge about surface chemistry and advanced nanomaterials led to the discovery of several novel methods for virus detection over miniaturized systems. But the outbreak of new viruses such as MERS coronavirus (MERS-CoV), novel strains of influenza viruses A and B, and SARS coronavirus 1 or 2 (SARS-CoV-1/2) brings new challenges to better diagnostic systems. Since symptoms may be similar in different viral infections and may range from a minor cold to severe respiratory disease, it requires a fast and accurate diagnosis. A rapid and reliable diagnostic test to identify the pathogenic virus in infected people is vital to control and eradicate the challenge caused by viral infection (Fig. 6.1). The viral testing is hampered by limited testing capacity, cost,

Fig. 6.1 Need of suitable viral diagnostic assay

and logistics of deployment, often leading to prioritized testing for specific high-risk groups. Real-time reverse transcriptase-polymerase chain reaction (RT PCR)-based assays are considered the gold standard for COVID-19 diagnosis. Several other methods such as loop-mediated isothermal amplification (LAMP), clustered regularly interspaced short palindromic repeats (CRISPER), RT-qPCR, duplex RT-qPCR, sequencing-based assay, enzyme-linked immunosorbent assay (ELISA), and lateral flow immune assay are employed for the detection of SARS CoV-2 (CDC 2020; La Marca et al. 2020). However, except lateral flow immunoassay (LFIA), all of these methods are difficult to be employed in point-of-care/point-of-need (PoC/PoN) applications and resource-constrained environments as they require skilled operators and expensive instruments (Tang et al. 2020). Most LFIA is based on the application of antibodies. However, they suffer from limitations such as false-positive results, poor stability, batch variation, and qualitative or semi-qualitative in nature (Ravi et al. 2020; Sidiq et al. 2020). Among the different types of available recognition probes for detecting antigens from a biological specimen, several other advanced bio-probes such as aptamer, affimer, and small fragment antibodies emerge as suitable probes compared to antibodies and possess several advantages. These advanced recognition probes are inexpensive, rapid scale-up, and can integrate with other technologies to strengthen their performance and application during this unprecedented condition (Acquah et al. 2021; Singh et al. 2018). Electrochemical spectroscopy, either in voltammetry or amperometry modes, has recently obtained huge attention in the field of biosensor due to its exceptional sensitive signal transduction ability and ability to integrate over miniaturized electronics systems (Barfidokht et al. 2019; Sun and Hall 2019; Venkatesh et al. 2018). The key rationales for using an electrochemical system as a biosensing platform are its quick reaction time, high sensitivity, and the possibility of developing small and low-cost integrated devices using advanced and existing technologies (Hsu et al. 2018; Saha et al. 2014).

6.1.1 Viruses as Intracellular Parasites

Viruses are nanometer-sized entities that have the potential to cause severe threats to living cells. As they cannot grow, replicate, or produce their energy, they are not considered alive. However, they can infect a host cell by implanting their genetic materials, hijacking the cellular functions, and utilizing the cell's machinery and energy for replicating their genetic materials. In other words, an infected host cell synthesizes viral proteins instead of their standard metabolic products. The new progeny viruses that are generated attack other cells, and the process goes on (Knipe and Howley 2013). All types of viruses share a typical body structure consisting of a protein shell enclosing a nucleic acid genome. The nucleic acid can be a deoxyribonucleic acid (DNA) or a ribonucleic acid (RNA) and, in both cases, single- or double-stranded. The protein shell is called a capsid and is made of repeating units of a single protein or a few different proteins. In some cases, the capsid is surrounded by one or more lipid bilayer membranes studded with virus-coded glycoprotein on

its exterior surface. The capsid and this membrane are together called a viral envelope. These types of viruses are called enveloped viruses. The main goal of the capsid and the viral envelope is to safely carry the viral gene to the suitable host cell through the extra-cellular environment, retaining its infectious properties. After successfully transferring the viral gene, its next tasks are to attach the virus to the host cell, cross the plasma membrane, and then uncover the nucleic acid genome (Lucas 2010; Perlmutter and Hagan 2015). The diseases caused by viruses are vast and have the potential to cause severe risk to humans, animals, and plant life. In humans, some viruses may cause minor infections like the common cold, stomach flu, and measles, while some may cause severe threats like Ebola, rabies, HIV/AIDS, dengue, polio, hepatitis, and smallpox. HIV and hepatitis have killed millions of people over the years, while some evolving viruses have caused severe outbreaks. Not all viral infections are spread from person to person but can also be spread by the bites of infected insects and animals (Stollar 1993). The severity of infection depends upon the types of the virus and the immune system of the host.

6.1.2 Importance of Diagnosis

Viral infections and related diseases have become one of the major threats to mankind. A large number of highly infectious diseases and outbreaks are initiated by viruses, which have caused severe loss to life, society, and the economy (McKee and Stuckler 2020). Their minimal size and simple morphology, which is susceptible to mutation, challenge the development of wide-use and long-term viral detection systems. Their isolation and visualization are challenging compared to the other microorganisms, thus requiring advanced procedures and technologies (Draz and Shafiee 2018). Viral infections show diverse symptoms, including flu-like, gastrointestinal troubles, rashes, immunodeficiency, tissue and organ damage, etc. In several cases, a sign of viral infection might be confused with other infections such as bacterial, leading to an increased risk of improper prognosis and medication. Hence, a precise diagnosis of a viral infection is essential for a clinician to determine the proper clinical prognosis and effective treatment protocol (Qureshi and Niazi 2020). Moreover, with rapid and spontaneous mutation, viruses can infect host cells with different novel mechanisms, and if a new infection arises in the human population, it's highly required to detect and categorize the agent to prevent outbreaks and epidemics (Dangalle 2021; Parrish et al. 2008). Therefore, the development of a dynamic virus diagnostic system for rapid, precise, simple, and long-term detection is always of utmost importance (Draz and Shafiee 2018).

6.1.3 Virus Detection Approach, Past to Current

The nanoscale dimension of the virus enforces a great challenge in the development of a suitable point-of-care/need detection system for wide use. The first virus detection was performed in the early 1950s through an electron microscope. These

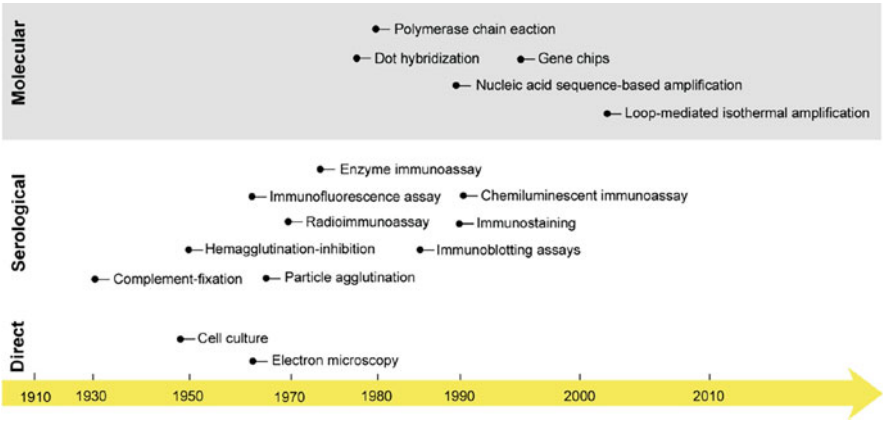

Fig. 6.2 A history and evaluation of virus detection techniques. The image is adapted from Draz and Shafiee (2018)

techniques formed the basis of all known classification and detection systems for a period of decades and remain the main tools for studying and investigating the biochemical and morphological properties of viruses. Although they are suitable for virus detection, their practical application has been restricted due to associated disadvantages such as cost, time, and safety. Two other major developments in the early 1980s bolstered the field of diagnostic virology: (1) the advent of a variety of immunoassays and (2) the introduction of polymerase chain reaction (PCR) (Fig. 6.2). It was accompanied by various successive serological and molecular detection techniques, which rapidly emerges as a mainstream laboratory-bound technique for clinical diagnosis of the virus. The serological immunoassay depends on the antibody and antigen-based detection of viral-specific antigen or corresponding humoral response generated against the specific virus, respectively. Tests that are commonly performed on serological specimens include complement fixation, neutralization, enzyme-linked or radiolabeled immunoassay, immunoprecipitation or immunochromatographic assay, and fluorescent or chemiluminescent immunoassay. Their principle of operation involves conjugating specific antibodies to a variety of signal reporting systems, such as chromo or fluorogenic substrate enzyme materials. The serological assay is a comparatively simple low-cost system and suitable for large-scale rapid testing but suffers from antibody cross-reactivity and high false-positive rate.

On the other hand, molecular techniques are more sensitive and specific (accurate), hence gaining interest in the field of virus detection. The discovery of new polymerases and the magnificent invention of polymerase chain reaction (PCR) have created a breakthrough for virus detection. Furthermore, a high degree of associated specificity in hybridization for DNA and simple modification capability led to many innovations in research and development of virus detection with genotyping and mutation prognostication. The molecular-based approach can be classified into amplification techniques (PCR, loop-mediated isothermal amplification, strand

displacement amplification, and sequencing) and nonamplification techniques (Southern or northern blotting, dot blot, and in situ hybridization). This system has revolutionized diagnostic testing in hospitals and in the community, enhancing the ability to control many viral infections. However, it is instrument dependent, expensive, and time-consuming and requires a highly skilled workforce.

6.1.4 Biosensor

A biosensor is an integrated device that can detect the presence or concentration of a specific chemical or biological analyte in a sample. It consists of a biorecognition element coupled to a transducer and an electronic processor. The biorecognition element can be any biomolecule like enzymes, aptamer, antibodies, cells, nucleic acids, tissues, molecularly imprinted polymers immunosystems, biomimetic, etc., that can specifically interact with the analyte and induce a biochemical activity. This activity is transformed into a decipherable signal by the transducer, followed by the processing of the transduced signal into visualized data by the signal processor (Aliofkhazraei and Ali 2014; Michelmore 2016). Biosensor finds wide application in the biomedical sector for the detection of numerous diseases. Detection of the biomarkers associated with different stages of a disease with high sensitivity and selectivity can help early detection and determine the infection rate and proper prognosis (Etzioni et al. 2003). Biosensors can be broadly classified based on the receptors and the transducing method used for the detection. Based on the receptors, they are classified by the types of biomolecules used. And based on the transducing method, they are classified as electrochemical, thermometric, piezoelectric, optical, and microbalance (QCM) biosensors (Atay et al. 2016). The electrochemical sensors are further classified into amperometric, potentiometric, voltammetric, and impedimetric (Srivastava et al. 2020).

6.1.5 Point-of-Care Biosensors

Point-of-care testing (POCT) or near-patient testing is one of the most effective approaches of a biosensor application. It is a diagnosis system that provides provision for rapid on-site testing and gives lab-quality results within minutes to a few hours. POCT kits are portable and easy to operate. They do not require specimen preparation, laboratory facilities, or trained personnel. POCT can be a very noteworthy approach to address an emergency, mass testing, remote and low resource healthcare sectors, patients requiring frequent health monitoring, and issues of the patients with chronic conditions and aged people. Since POCT ensures rapid diagnosis, it can enable proper disease monitoring and management and quick prognosis and can help a patient to start early treatment. POCT is designed as self-contained miniaturized kits with low detection limits, ultra-sensitivity, accuracy, specificity, and rapid and easy assaying methods. Various prototypes of POCTs have been fabricated, which include lab-on-chip, nanomaterial-based, labeled and label-free,

wearable, and wireless. Lab-on-chip mostly uses microfluidics to integrate multiple functions like sample preparation, reaction, separation, and detection into a few centimeters to millimeter-sized chip. Continuous flow of analyte through microchannels allows the fresh sample to encounter the sensing element embedded in the channels, thus increasing the sensitivity. Moreover, mass transfer is faster in the channels making the diagnosis faster.

Initially, microfluidic channels were fabricated using silica and glass by photolithographic technique. However, various types of polymeric materials such as poly (carbonates) (PC), poly(methyl methacrylate) (PMMA), poly(dimethylsiloxane) (PDMS), poly(ethyleneterephthalate glycol) (PETG), poly(vinyl chloride) (PVC), poly(styrene), poly(imide) (PI), cyclein olefin polymers (COP), and cyclein olefin copolymer (COC) have also been developed. PDMS is widely used in cell-based chips because of its high gas permittivity and optical transmissivity (Rodrigues et al. 2017; Tsao 2016). Paper-based microfluidics is also being in trend because of the large number of advantages it possesses. Paper is inexpensive, abundantly available, highly biocompatible, disposable, environment friendly, and easily functionalized for binding biological samples, it can be patterned by photolithography and easily stored, and most importantly, paper facilitates wicking of liquid, which induces transport without pump (Berthier et al. 2019; Xu et al. 2016).

Nanomaterial-based POCTs explore the exceptional properties of nanoparticles such as small size, high surface-to-volume ratio, electrical and thermal conductivity, magnetic, optical, and fluorescence. They are used as fluorescence quenchers, optical probes, biochemical labels, and biomolecule immobilizing platforms because of surface functional groups. The use of nanomaterials has been shown to enhance the working of sensors with various different mechanisms. For example, in electrochemical, they increase the electron transfer rates; in enzymatic, they reduce the distance between enzyme and electrode (Murphy 2006); in optical, the noble metal nanoparticles increase surface plasmon resonance (SPR) (Choi et al. 2020) and in colorimetric, the color change phenomena due to the state of nanoparticles aggregation can be beneficial (Aldewachi et al. 2018).

6.2 Virus Biomarkers and Associated Challenges with Virus Detection

National Institutes of Health (NIH) has defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" (Atkinson et al. 2001). Thus, biomarkers are a part of or are expressed in a biological system that can be analyzed for a normal and abnormal process occurring in the body. Biomarkers of viral infections have been expressed in various biological systems like plasma, sputum, urine, sweat, saliva, etc., and can be analyzed for monitoring the occurrence and severity of viral disease (Hwang et al. 2018).

6.2.1 Types of Virus Biomarkers

The viral biomarkers can be broadly classified into two classes: direct and indirect biomarkers. The former is extracted directly from a virus, which includes viral nucleic acid (DNA/RNA) and antigenic proteins, while the indirect biomarkers are expressed in the host cells in response to a viral infection such as an antibody.

6.2.1.1 Nucleic Acid Biomarkers

Viral nucleic acids (DNA/RNA) have been potential biomarkers for the detection, monitoring, and prognosis of specific viral infections. Advancement in nucleic acid detection techniques enabled wide use of the biomarkers in clinical implementation. PCR, microarray, and LAMP are the most generally practiced techniques for nucleic acid analysis (Shen et al. 2020). However, real-time polymerase chain reactions (qRT-PCR) are now being in trend for early, sensitive, and specific nucleic acids detection (Prabhakar and Lakhanpal 2020). A DNA/RNA biosensor consists of a single-stranded oligonucleotide immobilized on a transducer that can detect its complementary strand (biomarker) by surface hybridization. The transducer converts this hybridization event on the electrode surface to an analytical signal (Ozer et al. 2020).

Based upon the type of nucleic acid they hold, viruses are classified as DNA and RNA viruses. The DNA virus consists of double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA). Replication in DNA viruses occurs by DNA-dependent DNA polymerase. Large DNA viruses (>10 kb) comprise dsDNA, while small DNA viruses comprise circular, ssDNA, or dsDNA (Sanjuán et al. 2016). The pathogens under this type are African swine fever virus (ASFV), varicella-zoster virus (VZV) causing chickenpox and shingles, variola virus (VARV) causing smallpox, herpes simplex virus 1 (HSV-1), and hepatitis B virus (HBV) (Babkin and Babkina 2015; Bauer et al. 2013; Chen et al. 2020; Liang 2009; Vizoso Pinto et al. 2011).

RNA virus generally consists of single-stranded RNA (ssRNA) or sometimes double-stranded RNA (dsRNA). The ssRNA can be categorized into positive sense (ssRNA (+)) and negative sense (ssRNA (−)) RNA. An ssRNA (+) genetic material can execute both as a genome and messenger RNA (mRNA). Thus, they can be directly translated into protein by the host cell ribosomes. During replication, the ssRNA (+) encodes genes for an RNA-dependent RNA polymerase (RdRp), which catalyzes the synthesis of negative-sense antigenome that acts as a template for the formation of new ssRNA (+) (Payne 2020). This virus covers over one-third of all virus genera and includes several pathogens such as human immunodeficiency virus (HIV), coronaviruses (CoV), West Nile virus (WNV), Zika virus (ZIKV), hepatitis A virus (HAV), hepatitis C virus (HCV), hepatitis E virus (HEV), dengue virus (DENV), Japanese encephalitis virus (JEV), human rhinoviruses (HRVs), and chikungunya virus (CHIKV) (Nagy and Pogany 2012). The genetic material in ssRNA (−) acts as a complementary strand for mRNA and needs to be converted to ssRNA (+) before translation using RNA polymerase. The replication in ssRNA (−) occurs by synthesis of positive-sense antigenome as the template by RdRp. All

the virus under this type consists of a lipid envelope enclosing the nucleocapsid. Some of the pathogens that come under this type are influenza A causing Spanish flu, swine flu, bird flu, Asian flu, Hong Kong flu, influenza B and influenza C viruses, measles morbillivirus (MeV), Ebola virus (EV), Marburg virus (MARV), hepatitis delta virus/hepatitis D (HDV), human parainfluenza viruses (HPIVs), Nipah virus (NiV), mumps virus (MuV), and human respiratory syncytial virus (RSV).

The nucleic acid detection approaches possess several advantages, such as excellent sensitivity, specificity, and beneficial for virus that cannot be cell-cultured. However, some major challenges that are associated along with the detection procedures of nucleic acid biomarkers (Leland and Ginocchio 2007; Zhong et al. 2007) can be listed as follows:

(a) DNA can get modified or degraded by nucleases and other substances.
(b) RNA isolation requires high precautions as it is very unstable and susceptible to chemical and enzymatic hydrolysis. The presence of 2′-hydroxyl group on the pentose ring and the ubiquitous presence of RNase enzyme makes it liable to chemical and enzymatic degradation.
(c) RNase inhibitors like diethyl pyrocarbonate (DEPC) used to protect RNA from degradation are highly toxic and carcinogenic, which has to be handled with high precautionary measures.
(d) Any mutations in the DNA/RNA sequence may be missed by the particular primers and probes employed in the detection procedures.
(e) Detection of RNA in diagnosing some viruses like Zika is possible only after a few days of onset of the symptoms.
(f) Technical expertise and expensive instrumentations are required mainly for low-volume analytes.

6.2.1.2 Protein Biomarkers

A virus contains a large number of proteins in its body structure, comprising of structural and nonstructural proteins. For example, the SARS-CoV-2 virus is made of four structural and 29 nonstructural proteins. Detection of these viral proteins can deliver information about the existence of a particular viral infection in a body. Protein biomarkers have always been favored over nucleic acid biomarkers as the nucleic acid biomarkers encompass tedious steps of isolation, purification, and processing stage, which is time-consuming and expensive, while protein biomarkers are easy to isolate and require simple sample preparation steps. Moreover, an extensive range of analytical instrumentation is available that can identify and quantify proteins (Kaur et al. 2020).

The antigenic viral surface glycoproteins are a significant component of an enveloped human pathogenic virus. They play a pivotal role in viral infectivity and immune evasion. Glycoproteins are formed by glycosylation (covalent attachment of carbohydrate to protein backbone), which is a post-translational modification process. Direct detection of these glycoproteins or indirect detection of the developed antibodies in the host cell is an evolving discipline in virus diagnosis. Lectins or monoclonal antibodies mostly do the glycoprotein recognition. However,

various other biochemical processes have been established to investigate glycoproteins (Banerjee and Mukhopadhyay 2016).

A viral envelope is composed of three types of glycoproteins: membrane protein (M), envelope protein (E), and spike protein (S). The S protein is a type of large class I fusion protein that plays a key role in binding and penetrating the host cell. They bind to the angiotensin-converting enzyme 2 (ACE2) receptor present on the host cells and facilitate virion transfer. The M and E proteins are primarily responsible for forming the virus assembly (Shajahan et al. 2020). Some of the glycoproteins associated with the particular types of viruses are spike (S) glycoprotein in SARS-CoV-2, hemagglutinin and neuraminidase in influenza virus, gp120, gp160, and gp41 in HIV, spike Gp1-Gp2 in EV, nonstructural glycoprotein NS1 in DENV, and G-1 and G-2 glycoprotein of HSV-1. Some of the techniques used for the detection of the viral protein are ELISA, chromatographic techniques, radiolabeling, and fluorescence-based assays (García-Cañas et al. 2007). However, some of the major challenges are associated with the detection of viral proteins (Feng et al. 2020; Leland and Ginocchio 2007). Some have been listed below:

(a) Detection of trace amounts of viral protein is a challenge, as they cannot be amplified like nucleic acids.
(b) The nonexistence of antibodies against each protein of a virus limits the development of the protein detection process.
(c) Because of the complex structure and high molecular weight of the glycoproteins, their separation and purification are difficult.

6.2.1.3 Serological (Antibody) Biomarkers

Antibodies, also known as immunoglobulins, are the Y-shaped proteins formed as a body's defense mechanism against infection by specialized white blood cells called B lymphocytes. An antibody binds to a specific antigen protein and inhibits its action in various ways, such as neutralization, opsonization, and complement activation. Antibodies produced for a particular virus differ from the antibody produced for another. Thus, the detection of antibodies produced in response to a specific viral antigen can be an effective approach in diagnosing the occurrence of a particular viral infection.

An antibody test analyzes the level of a specific antibody in the sample. Serum samples are generally used for the detection of developed antibodies. However, saliva, sputum, nasal swab, and dried blood spots have also been used depending upon the type of infection.

Five major classes of antibodies are produced in a body in response to viral infections. They are as follows:

(a) Immunoglobulin A (IgA), found in high concentration in the mucosal secretions, serum, salivary glands, lacrimal glands, intestinal fluids, and colostrum.
(b) Immunoglobulin G (IgG), the most common antibody found in blood and tissue fluids and the only antibody that can cross the placenta.

(c) Immunoglobulin M (IgM), the first antibody produced in response to an infection, found in blood and lymph fluid.
(d) Immunoglobulin E (IgE), produced in the very low level in the serum; however, the level increases in the presence of allergens.
(e) Immunoglobulin D (IgD), mainly found on the B cell surface, acting as a receptor for antigen.

The IgG, IgM, and IgA antibodies are primarily responsible for neutralizing the infectivity of a viral infection. IgG antibodies are very important and show high specificity to their respective antigens. Therefore, IgG detection is in common practice for virus diagnosis. Some of the conventional methods used for the detection are ELISA, immunofluorescence assay, hemagglutination inhibition assay, neutralization assay, and western blot (Corrales-Aguilar et al. 2016). IgG-based diagnostics have been used to detect some glycoproteins such as spike (S) glycoprotein of SARS-CoV-2 and G-1 and G-2 glycoprotein of herpes simplex virus and have shown high sensitivity and specificity.

Some of the major challenges associated with the antibody biomarkers are listed below:

(a) Occurrence of cross-reactivity between antigens if the antibody against a specific antigen has a competing high affinity toward another antigen. This might occur when two antigens have similar epitopes.
(b) False-negative results can occur if the amount of antibodies in the test specimen is below the detection limit of the assay or insufficient antibodies have been produced at that stage of infection.

6.3 Sensor against Viral Diseases

6.3.1 COVID-19 or Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2)

There are several biomarkers that can be significant for the detection of SARS-CoV2. These are ssRNA, antigen, antibody, blood, urine, infection, blood gas index, hemagglutination level, and cytokine levels. In the clinically used approaches, several biosensors have been established for the recognition of COVID-19 (Jalandra et al., 2020). This biosensing system can be used as a body wearable, smart band, plasmonic photothermal sensor, optical sensor, and nano- and cell-based sensors. Seo et al. (2020) reported a biosensor identifying SARS-CoV2 from clinical samples based on the field-effect transistor (FET) approach. The sensor was fabricated with graphene sheets on a coated transistor, followed by an antibody precisely against the SARS-CoV2. The manufactured sensor performs current clinical samples from COVID-19 patients with better results as antigen protein. The samples are collected from COVID-19 patients' nasal swab specimens. This biosensor has been detecting the SARS-CoV2 protein from clinical samples up to the level of 1 fg/mL

concentration using buffer and 100 fg/mL from the biological sample fluid. Furthermore, the sensor detected the virus-related culture in clinical samples with approximately 2.42×10^2 copies/mL, as Seo et al. (2020) reported. A recently reported biosensor was manufactured with the help of gold nanoparticles and SnO_2/F electrodes (fluorine doped with tin oxide), immobilized with monoclonal antibodies of COVID-19. The sensor showed high sensitivity between 1 fM and 1 μM for recognition of COVID-19Ag (antigen). The fabricated sensor effectively senses COVID-19Ag in buffer up to 10 fM, 90 fM with eCovSens, and 120 fM for spiked samples from saliva (Mahari et al., 2020). This biosensor device detected COVID-19Ag within 10–30 s from patient saliva samples. Djaileb et al. (2020) reported a surface plasmon resonance sensor identifying antibodies of nucleocapsid against SARS-CoV2 from human serum. The sensor could respond with 10 μg/mL anti-r nucleocapsid and yield of 221 RU. Qiu et al. (2021) designed a biosensor with the coupled characteristics of plasmonic, such as merging the photothermal plasmonic effect and surface resonance plasmonic. The detection of changes in clinical samples is an additional and encouraging approach for COVID-19 diagnosis. The dual-functional biosensor showed a high response toward SARS-CoV2 samples with the lowest recognition of 0.22 pM and detected a precise target from a gene pool. A recent study of SARS-CoV2 RapidPlex has been reported, which targets the multiplex of biomarkers such as C-reactive protein of saliva samples and serum, anti-spike protein of IgM and IgG, and nucleocapsid protein (NP) (Torrente-Rodríguez et al., 2020). RapidPlex showed the samples' S/B relation range between 10.5 and 12.4 in serum and 2.81 NP, 3.24 S1-IgG, 1.62 S1-IgM, and 1.76 CRP in saliva; the NP concentration range of 0.1 to 0.8 μg/mL for serum and 0.5 to 2.0 ng/mL for saliva in COVID-19 clinical patient samples; IgG in the range of 20–40 μg/mL in serum and 0.2–0.5 μg/mL in saliva and IgM in the range of 20–50 and 0.6–5.0 μg/mL in COVID-19 patient serum and saliva, respectively; and a CRP range of 10–20 and 0.1–0.5 μg/mL in COVID-19 patient serum and saliva, respectively. The positive samples showed higher signals than negative samples, which significantly proved the accurate evaluation of the COVID-19 biomarkers in biofluids using biosensors. Recently, a biosensor label-free miniaturized smartphone-supported signal detection for COVID-19 diagnosis has been reported (Chandra, 2020). This development of an electrochemical immunosensor-based approach will be a potential for the point-of-care device for the detection of SARS-CoV2. The commercially viable and clinical practice nano-bioengineered approaches for COVID-19 diagnosis are minute details on RT-PCR immunodiagnostic assays. Tripathi and Agrawal (2020) reported the electrochemical label-free detection of DNA hybridization, a possible method for identifying COVID-19. The high contagious (rate of infectivty) nature of SARS CoV-2 virus imposes serious challenges and restrictions on the healthcare workers at diagnostic centers. It brings up the need for a suitable user-friendly and pragmatic sensor for detection of COVID-19 at home with minimal settings. With a particular focus, researchers have developed a method to employ a glucometer for detection of SARS CoV-2 virus from human saliva at very low cost, i.e., ~ 3 USD. The assay was based on antisense (complementary DNA) displacement assay from aptamer on binding of target antigen (SARS CoV-2 spike or nucleocapsid protein). To translate

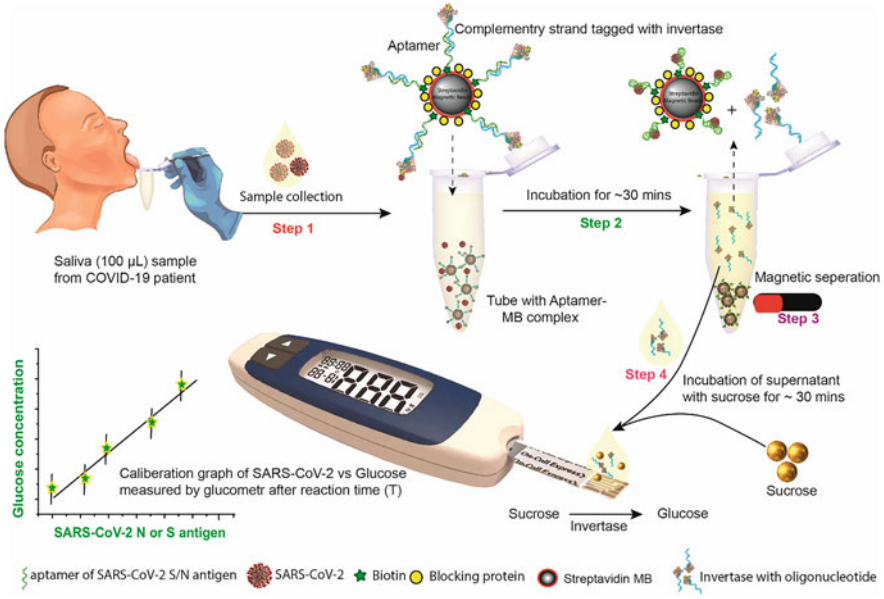

Fig. 6.3 Assay scheme. Biotin-aptamers (anti-S or anti-N protein) are annealed to the complementary invertase–oligonucleotide and pre-assembled on streptavidin-coated magnetic beads (MBs). Next, samples containing SARS-CoV2 virus, or viral (S/N) protein, are incubated with this pre-assembled complex (Steps 1 and 2). The binding of the virus or viral protein to the aptamer triggers a conformational switch releasing the invertase–oligonucleotide into solution (Step 3). The virus-bound aptamer–MB complex is separated using a magnet, and the supernatant containing invertase–oligonucleotide is collected (Step 4). The invertase–oligonucleotide solution is then incubated with sucrose, which is converted to glucose and measured by a commercially available glucometer

COVID-19 viral antigen binding into glucose production, they have exploited the native catalytic properties of invertase and engineered a novel aptamer-based competitive assay. Under the assay, anti-S (or anti-N) protein aptamers conjugated to the enzyme invertase through a small oligonucleotide (15–25 base pairs antisense) that is complementary to a portion of the aptamer sequence. The biotinylated aptamer-oligo-invertase complex is pre-assembled on magnetic beads, and in the presence of respective target anti-N or -S aptamer undergoes conformation change. Hence, complementary strand along with invertase enzyme was displaced from MB, thus creating an antigen-sensitive switch for signal production. The incubation of released invertase enzyme with sucrose for unit time converts it into glucose, thus providing necessary amplification. The formed glucose is readout with a glucometer; the amount of glucose formed is directly proportional to the viral antigen (Fig. 6.3). The core advantage of this approach relies on distributed devices that were already ubiquitous in the market today, rather than developing custom hardware or expensive instruments.

6.3.2 Recent Challenges with SARS CoV-2 Diagnosis

A kind of new mutation in the genetic material in SARS CoV-2 was reported in reference to the genetic sequence of Wuhan-Hu 1 or USA-WA1/2020. The mutation in RNA viruses was up to million times higher than their host, as it supported their survival by enhancing their variability and evolvability. The probable reason for high mutation in the viruses is faster replication rate through faster polymerase, and high-rate kinetics polymerases make more mistakes (Duffy 2018). The list of SARS CoV-2 variants with their respective mutations is given in Table 6.1**.** These mutants have high transmission and virulence and lessen the effort of the social health measure. The SARS CoV-2 delta variant is a major concern due to its higher transmission rate, pathogenicity, and ability to evade the immune system of the vaccinated person.

Moreover, the structural and functional mutation in SARS CoV-2 RNA or protein imposes a serious threat on an effort to curb the challenges caused by SARS CoV-2 infection. The presence of new SARS CoV-2 variants in patent samples can possibly impact the performance of diagnostic tests; for example, several FDA-approved diagnostic tests such as Linea, Taq Path, and X-pert showed slightly reduced sensitivity against SARS CoV-2 variants B.1.1.7. (Health 2021). Some other potential challenges become apparent by different SARS CoV-2 variants such as the following:

Table 6.1 List of SARS CoV-2 variants and respective mutation

WHO label	Pango lineages	Additional amino acid changes monitored[a]	Earliest documented samples	Date of designation
Alpha	B.1.1.7	+Spike:484 K +Spike:452R	United Kingdom, Sep-2020	18-Dec-2020
Beta	B.1.351 B.1.351.2 B.1.351.3	+Spike:L18F	South Africa, May-2020	18-Dec-2020
Gamma	P.1 P.1.1 P.1.2 P.1.4 P.1.6 P.1.7	+spike:681H	Brazil, Nov-2020	11-Jan-2021
Delta	B.1.617.2 AY.1 AY.2 AY.3 AY.3.1	+Spike:417 N	India, Oct-2020	VOI: 4-Apr-2021 VOC: 11-May-2021

[a]*VOI* variants of interest (need to be monitored and characterized repeatedly), *VOC* variants of concern (need to be monitored and characterized by central agencies). Table adapted from WHO SARS CoV-2 declaration

1. As mentioned above, mutation in the genetic material of virus, especially primer binding region, can escape the detection by molecular diagnostic tests such as RT-PCR or LAMP. Hence, multiple target-based assay or multiplex assays are needed to minimize the chance of error.
2. Mutation in epitope (antibody binding region) or aptatope (aptamer binding region) can reduce the susceptibility and sensitivity of therapeutic agents, for example, aptamer and monoclonal antibody.
3. Having the ability to evade polyclonal antibody, an immunized person with natural infection or vaccine of SARS CoV-2 generates a polyclonal response that recognizes the receptor-binding domain (RBD) or other parts of the spike protein. Hence, any mutation in spike protein can evade the immunity of a person or any diagnostic assay based on the polyclonal antibody.

6.3.3 Dengue

Dengue is the fastest spreading viral disease by mosquitos. A nonstructural 1 (NS1) protein is a particular and sense biomarker for the detection of dengue. The detection methods of IgM- and NS1-based diagnostic tests for dengue are most widely used in many countries. The chemically modified peptide approach was used to design an electrochemical sensor for the diagnosis of dengue virus protein NS1. Young et al. (2000) reported the clinical test on arrest antigen in an infected patient by ELISA. This approach reveals a better understanding and significant detection of protein NS1 in the serum from dengue virus-infected specimens. The sensitivity of detection was approximately 4 ng/mL. ELISA targets NS1 protein, using the antigen in the bloodstream in clinical samples of infected patients at a critical stage of dengue. Infected patients with dengue fever contained NS1 protein level in their serum with 0.04–2.00 μg/mL in the initial stage of infection and 0.01–2.00 μg/mL in the later stage (Alcon et al., 2002). Cui et al. (2020) reported voltammetric electrochemical activities of synthetic dengue virus RNAs detected by indium tin oxide sensing electrode, and the limit of detection was shown to be 2 Amol. Cecchetto et al. (2020) reported serological point-of-care of free-label electrochemical capacitive identification for dengue virus infection. The modified approach employed a ferrocene-flagged peptide surface that contained anti-NS1 as the receptor. The assay capacitive had a limit of 1.36%, with an interval confidence of 99.99% (Cecchetto et al. 2020).

6.3.4 Encephalitis

An amperometric biosensor was constructed by sandwich gold label immunoassay for detecting the forest spring encephalitis antibodies' concentration maintained between 10^{-7} and 10^{-2} mg/mL with a sensor limit of recognition of 10^{-7} mg/mL (Brainina et al. 2003). A label-free amperometric immunosensor specifically detects Japanese B encephalitis (JBE) in the range of 1.1×10^{-8} to 1.9×10^{-6} lg pfu/mL. The correlation data coefficient was found as 0.995. A biosensor was

established for the detection of JBE using the label-free Fe^{2+}/Fe^{3+} target of immunoassay, and the detection limit of the device was 6×10^{-9} lg pfu/mL (Yuan et al., 2005). Similarly, a potentiometric biosensor was designed for JBE as an immunoassay approach, and the device limit was 6×10^{-9} lg pfu/mL (Jinchi et al., 2004). The light potentiometric biosensor was designed for the detection of Venezuelan equine encephalitis by using sandwich enzyme-label immunoassay, and the detection limit of the sensor was 30 ng/mL (Weaver et al., 2004). An extremely sensitive detection of JBE and avian influenza virus (AIV) by a field-effect transistor-functionalized graphene sensor has been reported. An antigen–antibody interaction assay was observed in both cases, and the current signal in the sensor analyzed it. These sensors showed the detection range of 1 fM to 1 μM for both cases. The detection limit of 1 fM for JBE and 10 fM for AIV was seen (Roberts et al., 2020). Lai et al. (2017) reported another way of detection of JBE by a carbon nanoparticle-based electrochemical biosensor. Immobilization of JBE antibody was done through carboxylic group linkage with the nanoparticles' amide group. The electrochemical biosensor showed a linear way observation range of data of 1–20 ng/mL with a lower recognition limit of 0.36 ng/mL, and detection sensitivity was 0.024 ng/mL for JBE analysis obtained in 10 min.

6.3.5 Hepatitis

Chronic hepatitis increases the risk of developing hepatocellular cancer, chronic hepatitis, and liver cirrhosis. Hepatitis B virus diagnosis kit was designed by Uzun et al. (2009) using surface plasmon resonance-based assay. The assay exhibited a detection limit of 208.2 mIU/mL and showed 0.015 mIU/mL association constant (K_A) and 66.0 mL/mIU dissociation constant (K_D). Seroprotection showed levels of 10 mIU/mL reported earlier in the case of HB surface antibody. The electrochemical biosensor for the HBV and TT virus detection by DNA amplified from polymerase chain reaction with clinical samples was reported. The biosensor was immobilized with 21–24 single-stranded oligonucleotides as a probe for HBV and TTV sequences and paste carbon electrode (Meric 2002). The detection of the hepatitis A virus by PCR using ssDNA as the probe was designed and tested. The fabrication of the electrochemical biosensor, HAV cDNA synthesis, which is complementary to ssDNA using the gold as the electrode, was tested. This device showed a limitation of signal cut edge to 0.65 pM for the ssDNA and 6.94 fg/μL for viral cDNA (Manzano et al., 2018). Another design approach established hybridization of DNA on a piezoelectric sensor for the detection of HBV. This is known as HBV DNA biosensor, which is more reliable and more sensitive. HBV DNA probe was crippled with gold electrodes with a frequency up to the range of 9 MHz. The quartz crystal in the piezoelectric sensor forms the adhesion cross-linking of glutaraldehyde and polyethyleneimine. The probe frequency shifts showed the significant linearization relation of hybridization with HBV DNA. The amount of HBV DNA showed better results between 0.02 and 0.14 μg/mL (Zhou et al., 2002). The identification of the hepatitis E virus using the pulse-electrochemical approach was reported. The

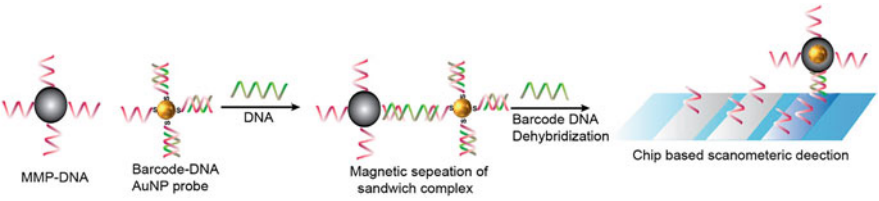

Fig. 6.4 Barcode-based detection of hepatitis B virus (HBV) (Wang et al. 2003)

sensor was fabricated using the combination of graphene quantum dots and nanowires of polyaniline embedded with gold. The device linearity was a concentration range of 10^2–10^7 copies/mL and HEV concentration in between 1 fg/mL and 100 pg/mL with a low-signal limit point of 0.8 fg/mL reported earlier (Chowdhury et al., 2019). A recent report on the design of biosensors with an electrochemical approach with nucleic acid combined with a new indicator of quercetin electroactive has been reported. It detects both ssDNA and dsDNA with the signal limit of 83 pM with a standard deviation of 4.6%. This study reports the fabrication of a biosensor as a successful technique for the detection of the hepatitis C virus with short sequences (Alipour et al., 2020).

A biobarcode amplification strategy was adapted by Wang et al. (2003) for detection of HBV DNA. This gold nanoparticle (AuNP) biobarcode scanometric assay was based on gold nanoparticle-enhanced reduction of silver ions into metallic silver, which is emerged as a visible black spot. In detail, it employed two different sets of DNA-functionalized AuNP and magnetic particle to capture and detect the HBV signature DNA sequence, respectively. The AuNPs modified with ssDNA specific to a target strand (barcode DNA) provide amplification and detection, while the second set with magnetic particle (MP) with ssDNA is specific to the target HBV strand. The presence of released complementary strand supports the aggregation of AuNP/DNA/MP conjugate. After washing, barcode DNA was substituted from nanoparticles and hybridized with a capturing DNA probe immobilized over the chip. Later silver staining reagents were used to amplify the detection signal (Fig. 6.4).

6.3.6 Human Immune Deficiency Virus (HIV)

Acquired immune deficiency syndrome is a severe transmissible immune disease caused by HIV. There have been many novel approaches of nanomaterial-based biosensors for HIV diagnosis. HIV infection response to CD4+ cells, CD4+ T lymphocytes, p24 core protein, HIV gene, p17 peptide, virus-like particles, and HIV-related enzymes and a viral duplicate within the host cell have been studied (Farzin et al., 2020). The new sandwich HIV p24 immunosensor based on chronoamperometry was developed. The electrochemical signal showed the concentration of p24, ranging from 0.01 ng/mL to 100 ng/mL, with a detection limit of 0.008 ng/

mL (Zheng et al., 2012), which was more sensitive than ELISA (1 ng/mL) and enhanced the reversibility and conductivity of the electrode. Shafiee et al. (2014) reported a nanostructured optical photonic crystal biosensor for HIV-1 viral load measurement with concentrations ranging from 10^4 to 10^8 copies/mL. It has been a rapid and sensitive optical detection method for biomolecules, cells, and viruses by monitoring the dielectric permittivity changes at the interface of a transducer substrate and a liquid media. An electrical sensing mechanism was also developed to detect captured HIV-1 on magnetic beads conjugated with anti-gp120 antibodies through impedance spectroscopy of viral lysate samples. Gray et al. (2018) reported the dual-channel surface acoustic wave biosensor, a pilot clinical sample study to diagnose HIV. This biosensor is a small lab prototype, portable, functionalized with ink-jet printing and dual-channel biochips, miniaturized, and requiring 6 μL of plasma. It can detect anti-p24 or anti-gp41 antibodies, with sensitivities of 100% (anti-gp41) and 64.5% (anti-p24) within 5 min. Amperometric sensors were also utilized to measure the concentrations of zidovudine fabricated using silver nanofilm and multiwalled carbon nanotubes immobilized on glassy carbon electrodes. This amperometric reported a linear range for zidovudine (0.37 μM–1.5 mM) concentrations from 0.1 to 400 ppm with a detection limit of 0.04 ppm (0.15 μM) (Rafati and Afraz 2014). Tombelli et al. (2005) reported the aptamer-based biosensor, which was immobilized on the gold surface of piezoelectric quartz crystals for the diagnosis of HIV-1 tat protein, based on the binding of a biotinylated aptamer on a layer of streptavidin. Two aptamer-based sensors have been established, and RNA aptamer specific for HIV-1 tat protein ranges from 1.25 to 2.5 ppm. Recently Yeter et al. (2021) reported an electrochemical label-free DNA impedimetric sensor with gold nanoparticle-modified glass fiber/carbonaceous electrode for the detection of HIV-1 DNA. The ssDNA was assessed using an electrochemical impedance biosensor. The correction of the sensor was achieved between 0.1 pM and 10 nM. The limit of detection was calculated using signal-to-noise ratio of 3 (S/N = 3) as 13 fM. An electrochemical label-free DNA impedimetric sensor was successfully developed, which is highly sensitive and relatively low-cost. A sensitive electrochemical assay was developed to monitor the electrophysiology of HIV-infected cells and treated cells with anti-HIV drugs (Kaushik et al. 2016).

6.3.7 Zika Virus

Zika virus infection is spread by the mosquito bite; it is a viral infection related to neuro-disorders and microcephaly. It is a most severe medical problem globally. Zika virus is similar to that of other flaviviruses (Kostyuchenko et al., 2016). The current approaches to diagnosis of Zika are testing the nucleic acid level at RNA, RT-PCR, and IgM Zika antibody arrest ELISA in serum, cerebrospinal fluid, or urine samples (Huzly et al., 2016). The electrochemical immunosensor made by layer by layer immobilization, of ZIKV-engulf protein antibody together as a single layer of dithiobis deposit on interlinked with microelectrode for the detection of ZIKV. The signal was recorded using the gold electrode, and concentration range

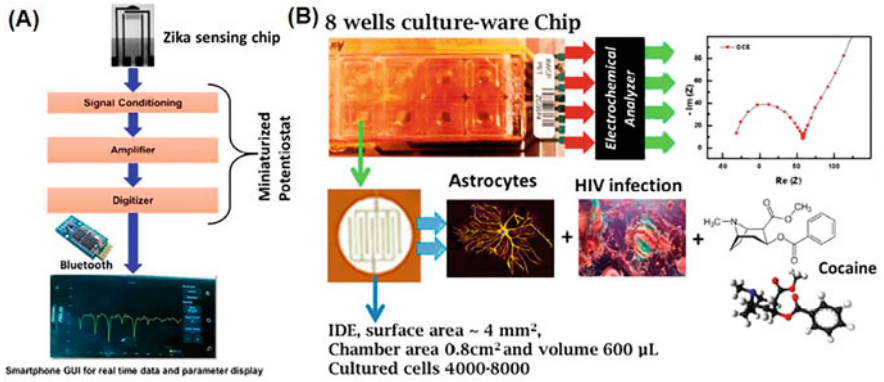

Fig. 6.5 The electrochemical Zika virus immunosensing chip for the detection of Zika-virus envelope protein at 10 pM level (**a**) (Kaushik et al. 2018). The chip-based electrochemical system monitors the electrophysiology of cells during infection and treatment (**b**) (Kaushik et al. 2016)

was between 10 pM and 1 nM. Kaushik et al. (2018) designed a biosensor for diagnosis of ZIKV with the detection limit of 10 pM and has been successfully integrated within the mobile for storage data and at point-of-care data analysis. The ssDNA-based biosensor was designed to detect ZIKV using an oxidized form of glassy carbon electrode adapted with silsesquioxane function immobilized with gold nanomaterial. The biosensor showed a detection limit of 0.82 pmol/L, with a linear way of 1.0×10^{-12}–1.0×10^{-6} mol/L with actual human serum samples (Afsahi et al., 2018). Tancharoen et al. (2019) reported that the complete structure virus is used for immobilization on a gold nanoparticle interlace electrode with graphene oxide and forms a gel polymer. The device detection limit was 1.0×10^{-20} mol/L. Recently, a biosensor based on synthetic ZIKV DNA oligonucleotide immobilization with gold-adapted polyethylene terephthalate (PET) electrode was reported. It showed a limit of 25×10^{-9} mol/L (Faria and Zucolotto, 2019). Steinmetz et al. (2019) reported a similar sensor to Kaushik et al. (2018) using a label-free impedimetric DNA biosensor for ZikaV diagnosis. Again, a similar report of label-free biosensor showing a limit of detection of 25 nM was reported by Faria and Zucolotto (2019). A mobile-based testing approach for quick recognition of Zika, dengue viruses, and chikungunya has been reported. Direct collection of ZikV from human clinical samples such as blood, urine, and saliva was performed by Priye et al. (2017). An effort was made to detect the Zika virus over an electrochemical immunoassay platform (Fig. 6.5) with a real sample for early-stage diagnostic on the site of the epidemic (Kaushik et al. 2018).

6.4 Conclusion and Future Direction

The application of electrochemical assay over miniaturized platform offers several advantages, such as inexpensive and label-free signal amplification. Since the commercial point-of-care application of glucometer, there has been extensive growth in the field of biosensors followed by their utility in various virus sensing applications. Despite the numerous advantages over other probes, the downside of the electrochemical assay, such as poor stability and variability using an antibody as a probe, cannot be neglected and needs to be resolved to have better activity in future research and development. Although engineered and some of the advanced bioprobes such as aptamers, affimers, or small fragment antibodies have remarkable stability, enhanced activity, and low cost compared to other probes for sensing, there is still a need to improve their specificity for high performance. In recent years, nanozyme (nanomaterial with enzymatic properties), DNAzyme, and ribozyme have emerged as suitable and novel systems for sensing applications, but still, these are in their naïve stage and need extensive effort to flourish in the field of biosensing. The functional groups on the surface of enzymes play a vital role in the catalytic activity, especially in the electron transfer process. Modification of their surface properties using different strategies can be used as a target binding without affecting their native properties. Another essential feature of the sensor for virus detection is a better understanding of the underlying mechanism of catalytic activity. The currently available techniques for detecting virus infection are time-consuming or unsuitable as a point-of-care system to fulfill WHO ASSURED guidelines. POC systems have been a much-needed diagnostics approach because of their user-friendliness, easy operation, accessibility in disease sites, and quick diagnostic in the remote areas that lack suitable clinical laboratory setup and expertise. This attribute can be attained by prudently understanding the current clinical need and selecting the suitable bioprobe and transducer platform for sensor design followed by novel surface modification strategies. The toxicity of various components (redox mediators, dyes, or nanomaterials) of the sensor is one of the main challenges that need to be addressed, especially for biomedical applications and health, environmental, and safety concerns. For the implantable and wearable sensor, despite the study in an animal model, its suitability should be carefully tested in the human model with respect to time frame.

The improvement in the current virus sensor by integration with integrated circuits and cutting-edge technologies will enable them for a better point-of-need or point-of-care system for detection of virus infection. The advancement of cutting-edge technology such as artificial intelligence (AI) and the Internet of things (IoT) has also been unified with biosensors nowadays, has enabled the sensors for real-time monitoring of biomarkers to generate bioinformatics needed for disease monitoring, and has provided therapy to optimize in time treatment.

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