Electrochemical sensors and biosensors for identification of viruses: a critical review.

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Electrochemical Sensors and Biosensors for Identification of Viruses: A Critical Review

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ABSTRACT

Due to their life cycle, viruses can disrupt the metabolism of their hosts, causing diseases. If we want to disrupt their life cycle, it is necessary to identify their presence. For this purpose, it is possible to use several molecular-biological and bioanalytical methods. The reference selection as performed based on electronic databases (2020–2023). This review focused on electrochemical methods with high sensitivity and selectivity (53% voltammetry/amperometry, 33% impedance, and 12% other methods) which showed their great potential for detecting various viruses. Moreover, the aforementioned electrochemical methods have considerable potential to be applicable for care-point use as they are portable due to their miniaturizability and fast speed analysis (minutes to hours), and are relatively easy to interpret. A total of 2011 articles were found, of which 86 original papers were subsequently evaluated (the majority of which are focused on human pathogens, whereas articles dealing with plant pathogens are in the minority). Thirty-two species of viruses were included in the evaluation. It was found that most of the examined research studies (77%) used nanotechnological modifications. Other ones performed immuno-logical (52%) or genetic analyses (43%) for virus detection. 5% of the reports used peptides to increase the method's sensitivity. When evaluable, 65% of the research studies had LOD values in the order of ng or nM. The vast majority (79%) of the studies represent proof of concept and possibilities with low application potential and a high need of further research experimental work.

KEYWORDS

Emergency sensors and biosensors; viruses; Ebola; SARS; COVID-19; influenza; African swine fever; nanomaterials; bioanalytical approaches

GRAPHICAL ABSTRACT

Methodology of review

Flow chart for reference selection was performed based on was performed based on electronic databases (2020–2023). Electrochemical detection is a very suitable method for the sensitive and selective databases of both proteins and nucleic acids.[1–3] The primary interest is its use for direct highly sensitive determination of viral nucleic acid (78%). Analysis of research studies focused on the detection of viral pathogens. The percentage of experimental studies focused on detecting a selected viral pathogen and the data was related to the total number of experimental works (n = 141) shown in Figure 1. This review provides an overview of the various electrochemical techniques developed to detect different virus species.

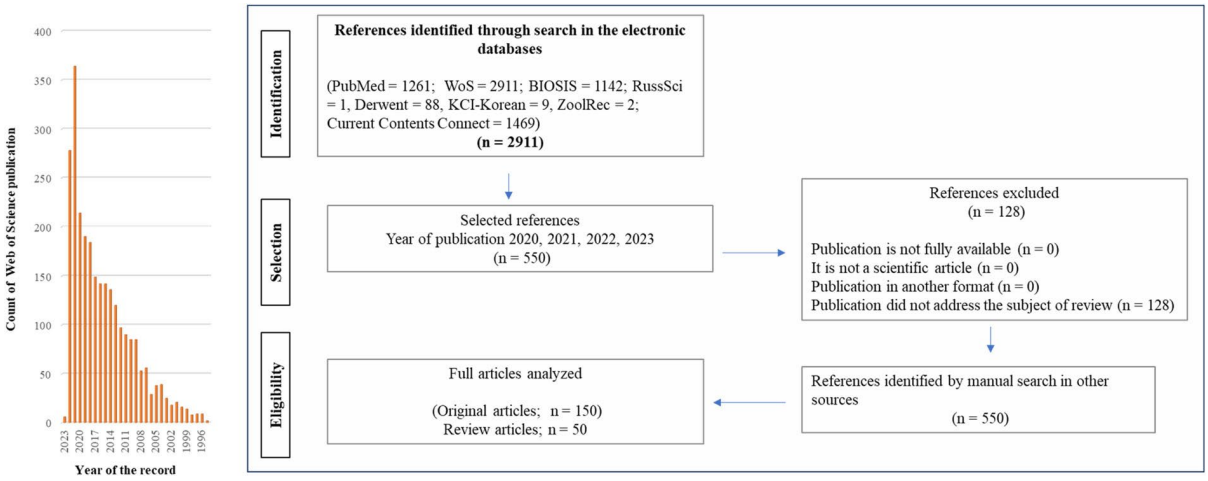

Figure 1. Flow chart for reference selection in the electronic databases (1945–2023). Keyword: electrochemical detection of a virus. Each record was manually checked for compliance with the specified criteria. The analysis of individual records (total number of records 5830, after removing duplicates in 2010) was performed based on publications from 2020, 2021, 2022, and 2023. Non-compliant records were discarded, except for review articles and articles on virus detection in plants. The remaining articles were evaluated and suitable reports (n = 30) and all original papers (n = 82) were selected.

Introduction

Dangerous diseases significantly impact on public health, the world economy, and global security. The ongoing epidemic of serious viral infections (caused by Ebola virus, HIV, influenza viruses, SARS, Lassa virus, Marburg virus, Crimean-Congo hemorrhagic fever virus, South American hemorrhagic fever viruses, MERS-Cov, Hantavirus, Rabies virus, yellow fever virus, COVID-19)[4–7] leads to the need for rapid detection of viral pathogens.[8,9] Viruses in evolutionary warfare are subject to intense mutations, and early identification is necessary to ensure appropriate treatment and vaccine development.[10] Bioanalysis and biomedical use of nucleic acids with the use of new molecular-biological approaches is growing significantly.[2,10] For this purpose,
there are several detection approaches,[11–16] e.g., PCR,[17] lateral flow immunoassays, LAMP-based methods, ELISA, electrochemical methods, aptamers,[18–20] fluorescence spectroscopy, AFM, SPR and SERS spectroscopy, silver staining, CRISPR-Cas, bio-barcode detection, and resonance light scattering,[12] use of electrochemical biosensors,[21,22] electrochemical immuno-sensors,[23] transparent (ITO) electrodes and collision-based immunosensing,[24] electrochemical detection using microfluidic redox neutral electrochemical platform.[25,26] To improve the electrochemical signal, new layout concepts (geometric factors) of the electrochemical detection system are proposed.[27] In addition, the

significant miniature ability of individual electronic devices brings other uses and applications such as nanopores (solid-state, biological nanopore, nanopore sequencing).[28] The signal from the nanopores is recorded and changes depending on the size of the molecules passing through these nanostructures.[29] To further improve the stability of the used biosensors (geno-sensors), modifications of the used oligonucleotide sequences, such as PNA and others, are advantageous.[30] An extension of the portable biosensor technological concept was demonstrated on an aerial sampling system. The obtained and modified sample (virus) is applied in a microfluidic arrangement (electrostatic interaction of the virus with the surface of the carrier) on a suitable working electrode. The device's sensitivity is relatively good and can detect hundreds of virus particles.[31,32] The main aim of this review article was to provide information on the possibilities of electrochemical virus detection by focusing on detection methods, electrode modifications and their sensitivity. Furthermore, it aims to show the potential of electrochemical methods in the analysis of real biological samples.

A strategy for the electrochemical detection of viruses

Biosensensoring technology is widely expanded into common use including medical devices.[33] The approach's utility is significant in tissue engineering, 3D printing, or drug administration.[34] A very important strategy is the use of real-time detectable chemical sensors. Combined with multiplex scanning, it is an effective tool for researching new biomarkers leading to precise medicine targeting. A typical biosensor consists of two functional units: bioreceptor, e.g., enzyme, DNA, or RNA responsible for specific/selective bonding to the target analyte. The second compound is a physicochemical miniature ability electrochemical, optical, or mechanical, translating the response to a useful and evaluable signal. From a historical point of view those sensors were first used in controlled conditions, lately they have become single-use home tests. The next step is adding features for getting more data for assessment. The main goal of biosensor detection of the virus pathogen is to be simple, quick, and specific (Figure 2). For the bioreceptor part, there are many ways to prove the presence of the pathogen e.g. nucleic acid or its parts. The disadvantage of this method is the necessity of getting a free nucleic acid chain when the analyte must be modified before the analysis (physically, chemically, or biologically). Another option is the identification of viral proteins. This option also requires the preparation of the sample before the analysis. Probably the most efficient, quick, and easy-to-use sensor is the identification of intact virion present in the sample (Figure 3). The analyte (virus) reacts with the sensor's surface (recognition part). Some biomolecules on the sensor's surface specifically react with the analyte (nucleic acids, antibodies, imprinted polymers, etc.). It is necessary to modify the reaction surface to prevent nonspecific binding and the generation of false-positive or negative responses. After the interaction, the (physicochemical) signal is transferred via a transducer to the detector. The measured signal is then mathematically processed.

The samples analysis is linked to which biological matrices are being used (e.g. tissue, biopsy, swab) followed by aiming the biosensor at the specific analyte. Thus, it is possible to change selectivity and sensitivity by modifying the sample, including homogenization, special deproteinization, separation, precipitation etc. Important is furthermore the method of detection and type of detector used. In the case of electrochemical detection, the options are, e.g., voltammetry, potentiometry, amperometry, or chronopotentiometry. The next step in developing biosensors is the connection with an electronic box communicating with a miniaturized processor like a mobile phone. The data are evaluated instantly and can be compared with accessible data in data storage (Figure 3).

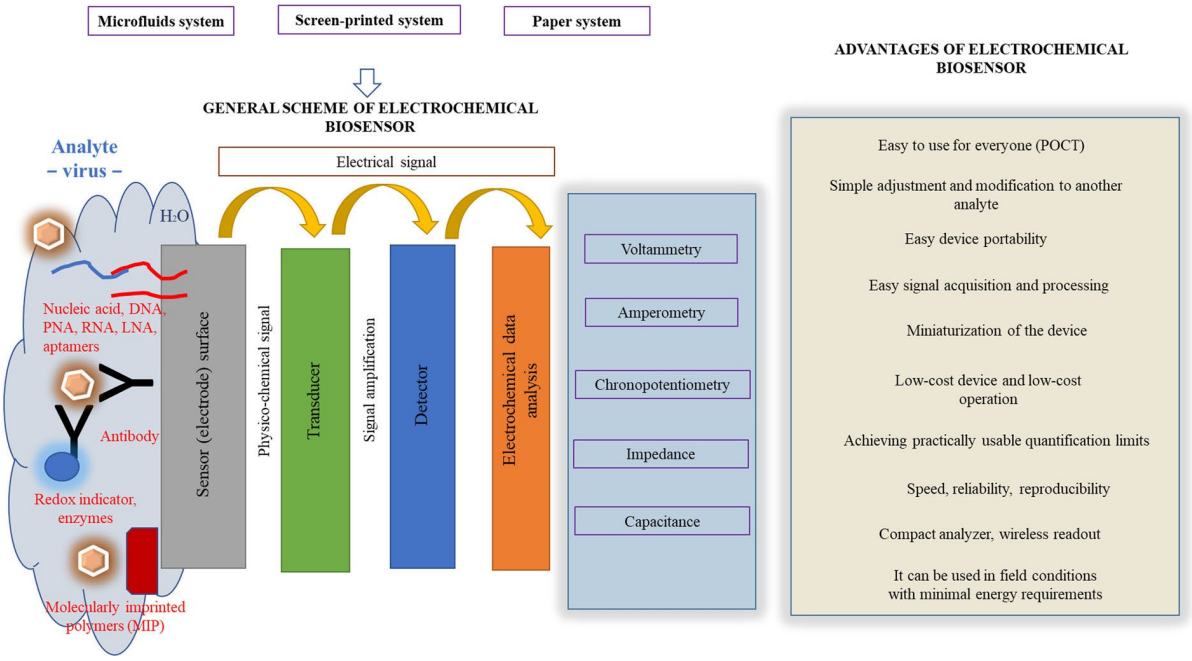

Figure 2. The simplified scheme of a nucleic acid biosensor. The analyte (virus) reacts with the sensor's surface (recognition part). On the sensor's surface, some biomolecules specifically react with the analyte (nucleic acids, antibodies, imprinted polymers etc.). It is necessary to modify the reaction surface to prevent nonspecific binding and the generation of false-positive or negative responses. After the interaction, the (physicochemical) signal is transferred via a transducer to the detector. The measured signal is then mathematically processed.

A strategy for the electrochemical detection of nucleic acids of viruses

It is known that there is a great diversity of viruses in the biosphere.[5,7] Absolutely direct evidence of the presence of a given virus is practically impossible with the currently available technologies (in 2023). Knowing where the virus binds and eventually multiplies is necessary to identify them.[9] We took our samples, which we can cultivate with small amounts of virus particles in cell culture. The most common method for detecting the presence of a virus is PCR. The sensitivity and selectivity of this method are very good and a small amount of sample is needed for analysis. The primary condition for using PCR is the isolation of nucleic acid (NA), which represents the greatest source of uncertainty and possible errors. Subsequent NA amplification ranges in units of NA copies in well-defined samples. Direct detection methods attempt to circumvent the need for NA isolation and amplification (enzymes, conditions, an impossibility of use in field conditions, etc.).

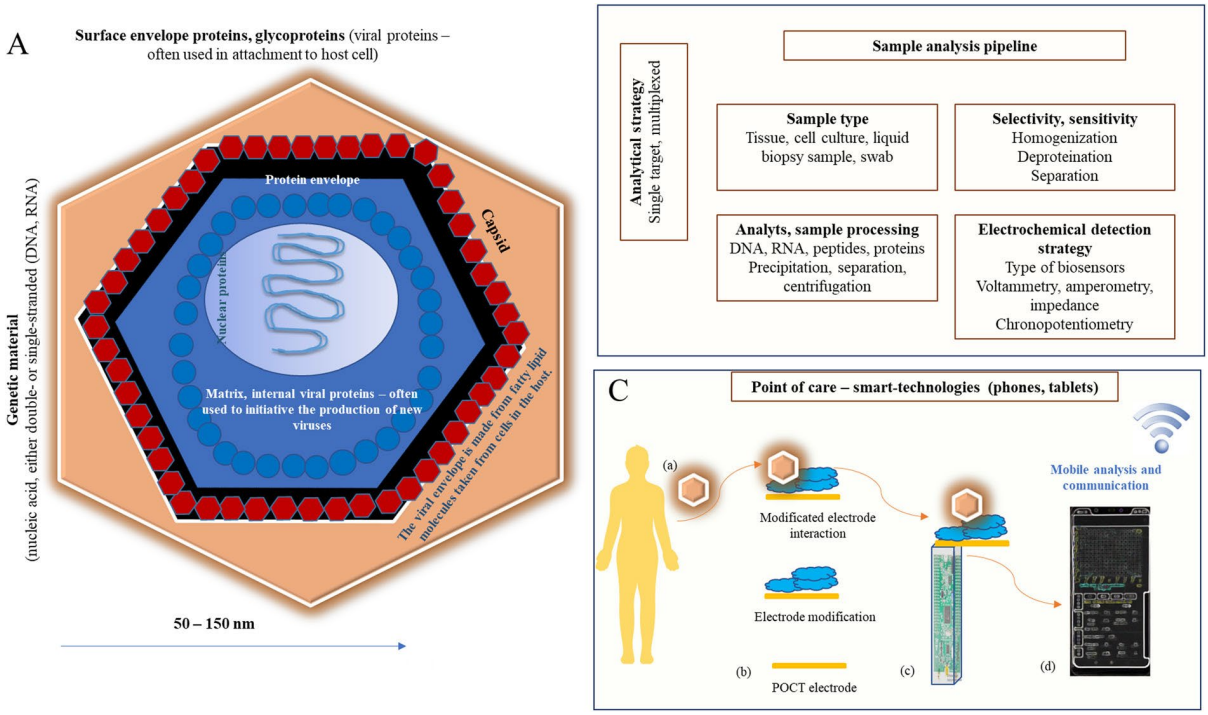

Figure 3. General scheme of a viral particle invading primarily eukaryotic cells. Individual viral molecules can be targets for molecular-biological and bioanalytical approaches (A). Analytical strategy according to individual potential target (detected) molecules (B). The following analytical strategy can be used for specific pathogen detection POCT systems (a). The sample in a suitable form is placed on a suitably modified electrode (b). The analyte (virus, viral nucleic acid) is connected to a suitable miniaturized potentiostat (c). The processor processes the resulting signal in the mobile phone, which already has well-built systems for determining the location, sharing data to suitable storages, and, when using appropriate evaluation algorithms, can be used to propose a diagnosis (d)[110] (C).

Nanomedicine

Nanotechnologies open up new possibilities. For these purposes, a number of different nanomaterials have been proposed: the most used type is gold nanoparticles (AuNPs), due to their good electron transfer, easy modification of the surface with different functional groups (-SH, -NH2, -COOH, -CN and other). Carbon nanomaterials, such as graphene oxide, reduced graphene oxide, carbon nanotubes and carbon dots, have very good electron transfer properties. Carbon nanomaterials allow a relatively easy modification for nanotrasporter modifications. Other types of nanoparticles such as quantum dots, protein structures (apoferritin) and molecular imprinted polymers bring further possibilities to improve the actual detection process. Overall, a completely specific group of nanoparticles is magnetic nanoparticles (MNPs). The great advantage of MNPs is the large working surface and the possibility to modify their surface structure with different chemical groups and also biomolecules.[35] The manipulation of MNPs provides fast and reliable separation of bound and unbound molecules using an external magnetic field[33,36]

Target biological molecules can be nucleic acids (dsDNA, ssDNA, dsRNA, ssRNA, various structures of NA) or amplified sections of NA (using various aplification approaches). For their identification either direct detection, intercalating electrochemical indicators or electrochemical tags (inorganic, organic including enzymatic) can be used. Alternatively, we can use antibodies/aptamer/peptides for label-free or label-based electrochemical detection. In addition, specific protein/receptor interactions with viral antigens, such as glycoproteins (Fetuin A, glycans), can be exploited.[33]

A significant complication is the biological matrix containing a variety of interferences (NA, proteins, cells, cell structures, etc.) which significantly affects the detection process. Pretreatment of the sample prior to analysis can remove potential interferents and improve the process sensitivity and specificity of the assay. As suggested previously, MNPs are very suitable for these purposes. A strategic

approach is shown in Figure 4. Despite this, a protein corona can form on the nanoparticles used, which subsequently interferes with the entire analytical process. Very little is known about its origin, significance and interference behavior.[37]

After surface treatment, different types of magnetic particles will allow fast and selective capture of the several magnetic gold nanoparticles suitable for NA binding. Cations bind to their envelope, giving the particle a positive charge. Nanoparticles can bind NA directly or use various types of antibodies or NAs (aptamers).[19] Moreover, it is a multiplexing detection that aims to confirm the analyte using several different signals or methods. One possibility is the binding of the viral particle, which is subsequently disrupted and the released NA is determined directly electrochemically.

Paleček showed in his oscillopolarographic study that DNA produces redox signals.[38] The NA can be sensitively determined with different methods, such as e.g., CV, DPV, SWV, ESI and CPSA with high sensitivity.[39,40] The presence of NA is compared to samples without NA. The cutoff limit of the positive signal can be determined statistically. NA can be analyzed directly by electrochemical methods.[9,41–44] After absorbing the analyte, the nonspecifically bound portion of the sample is washed away in the washing step (Figure 4).

If a biosensor/genosensor is designed, the detection area (electrode, nanoparticle) must usually be modified by a suitable protein, or polymer.[45,46] The electrode surface is blocked after binding the detection oligonucleotide (usually a short oligonucleotide strand). After the NA has hybridized, the physico-chemical signal is transferred via a transducer to the detector.[47] The measured signal is then mathematically processed. The probe is bound to the electrode surface in various ways, most often via the -SH group,[46] or AuNPs or CNT. The nucleic acid signal is changed after hybridization.[45] The record usually shows a decrease in signal. In the case of hydrolysis, an increase in signal is observed. An electroactive label (methylene blue, heavy metals (Cd, Pb, Ag, Ru), QDs, ferrocene, thionine) shows an increase in an electrochemical response. After hybridization, the signal of the electroactive label changes. An electroactive label probe is used to confirm the presence of the captured sequence. The probe is labeled with biotin to which streptavidin/avidin with bound enzyme and with AP, HRP bind, or there is a carrier of an electroactive element.[45] A carrier of other gold nanoparticles with a detection sequence and a labeling sequence binds to the hybridized nucleic acid; the labeling sequence contains a label that is subsequently detected. Among other things, changes in the structure of the NA (aptamers, quadruplexes, hairpins, microRNAs, etc.) are used in the DNA hybridization detection process (Figure 5).

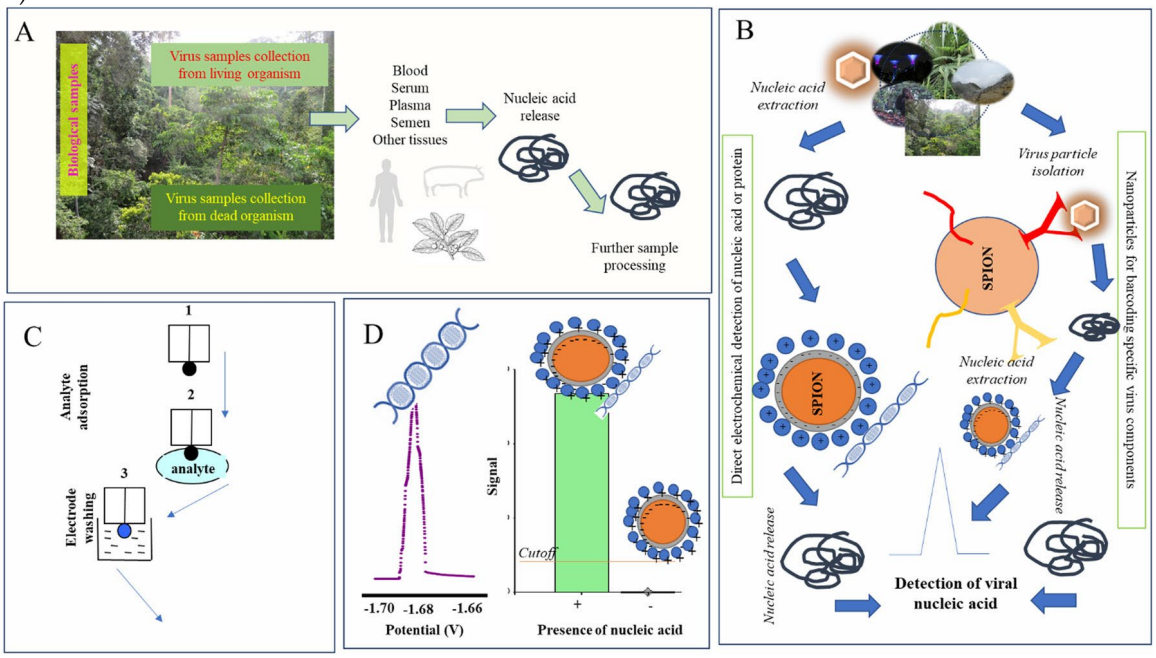

Figure 4. Approach strategies for electrochemical analysis of nucleic acids from a biological sample. Electrochemical nucleic acid detection and the possibility of using a nanotechnological approach to capture nucleic acid (A). Potential strategic approaches for bioanalytical detection of the presence of viral nucleic acid. There is a great diversity of viruses in the biosphere (A). Absolutely direct evidence of the presence of a given virus is practically impossible with the currently available technologies (in 2020). Knowing where the virus binds and eventually multiplies is necessary to identify it. In this study, we took our own samples, which we can cultivate in cell culture with really small amounts of virus particles. The most common method for detecting the presence of a virus is PCR. The sensitivity and selectivity of

this method are very good and a small amount of sample is needed for analysis. The primary condition for using PCR is the isolation of NA, which represents the greatest source of uncertainty and possible errors. Subsequent NA amplification ranges in units of NA copies in well-defined samples. Direct detection methods attempt to circumvent the need for NA isolation and amplification (enzymes, conditions, an impossibility of use in field conditions, etc.). Nanotechnologies open up new possibilities for addressing this. After surface treatment, different types of magnetic particles will allow fast and selective capture of the analyte sought. Magnetic gold nanoparticles are suitable for NA binding. Cations bind to their envelope, giving the particle a positive charge. Nanoparticles can bind nucleic acid directly, or use various types of antibodies or nucleic acids (aptamers). Moreover, it is a multiplexing detection, where the goal is to confirm the analyte using several different signals or methods. One possibility is the binding of the viral particle, which is subsequently disrupted and the released nucleic acid is determined directly electrochemically, (B). The nucleic acid can be sensitively determined, e.g. by CPSA with high sensitivity. The presence of nucleic acid is compared to samples without NA. The cutoff limit of the positive signal can be determined statistically (D). Nucleic acid can be analyzed directly by electrochemical methods. After adsorbing the analyte, the nonspecifically bound portion of the sample is washed away (C).

Various portable sensors and biosensors are challenges for current bioanalytical research.[2,43,48] Recombinase protein uses reverse primer with specific single-stranded binding proteins. Subsequently DNA polymerase allows amplification of NA in situ. Amplificated present viral NA is sensitive to the detection of DNA using voltammetry.[49] Aptamers are very convenient for their specificity to the targeted structure, much better stability and acquisition cost. In principle, an oligonucleotide with a complementary sequence to the part of the aptamer is anchored to the surface of the electrode.[19] The aptamer structure specifically binds to the target viral antigen. This change is then recorded. The aptamer is released from the oligonucleotides attached to the surface of the electrode and binds to the surface antigen of the virus. The space for the enzyme-substrate will be freed up and subsequently, the enzyme reaction will start, which is recorded electrochemically.[50] Another possible way for sensitive and selective viral pathogen detection might be nanopores. Schematic processes for detecting a selected analyte using a nanopore can be used, where an individual signal is subsequently recorded as a specific cluster.[29] A chimeric peptide-DNA nanoprobe made from a substrate peptide for papain was prepared. After digestion with a suitable protease, a specific DNA fragment is released. The fragment is hybridized with a probe immobilized on the surface of the working electrode. Subsequently, a suitable electrochemical marker is used to obtain the signal.[51] The concept shows the use of two types of nanoparticles: SPIONs for capture to the magnet and detection nanoparticles for enhancing the electrochemical signal (thionein)[52] (Figure 6).

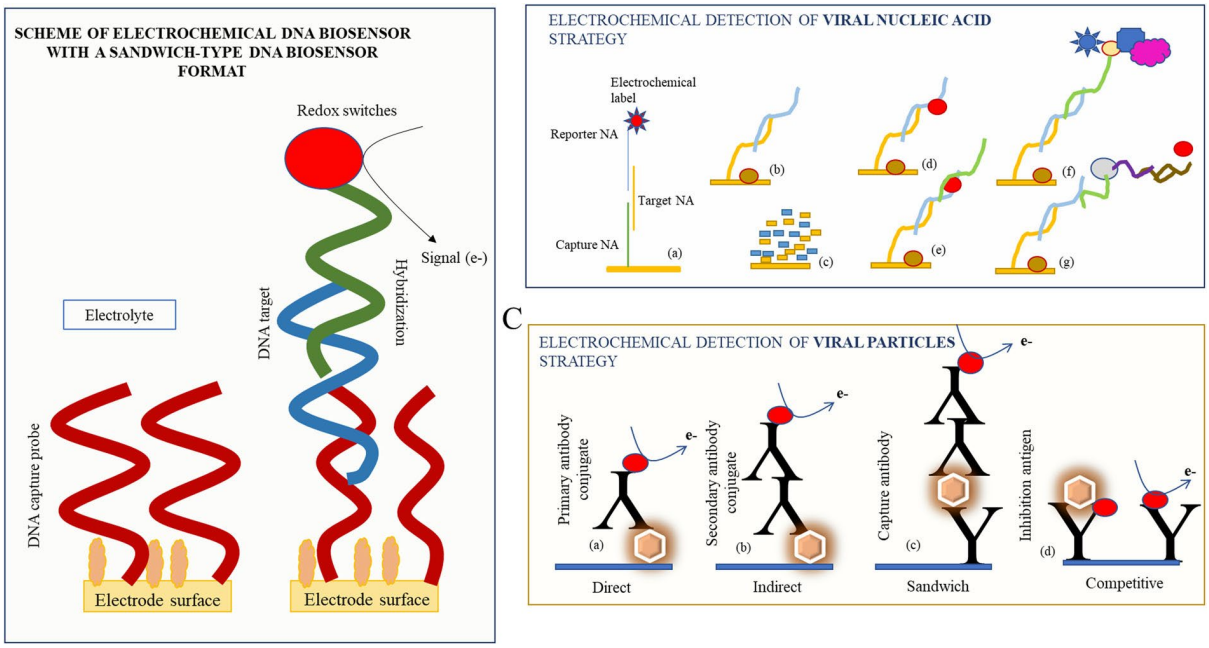

Figure 5. The simplified scheme of an electro-hybridization biosensor. Electrochemical detection of viral nucleic acids strategy. The electrode surface is blocked after detecting oligonucleotide (usually a short oligonucleotide strand) binding. After the nucleic acid has hybridized, the physico-chemical signal is transferred via a transducer to the detector. The measured signal is then mathematically processed (A). Possible methods of electrochemical nucleic acid detection (B). The probe is bound to the electrode surface in various ways, most often via the -SH group, AuNPs or CNT. The nucleic acid signal is a change after hybridization (a). The record usually shows a decrease in signal (b), in the case of hydrolysis, an increase in signal is observed (c). An electroactive label (methylene blue, heavy metals (Cd, Pb, Ag, Ru), QDs, ferrocene, thionine) shows an increase in an electrochemical response. After hybridization, the signal of the electroactive label changes (d). An electroactive label probe is used to confirm the presence of the captured sequence (e). The probe is labeled with biotin to which

streptavidin/avidin with bound enzyme and with alkaline phosphatase (AP), HRP bind, or there is a carrier of an electroactive element (f). A carrier of other gold nanoparticles with a detection sequence and a labeling sequence binds to the hybridized nucleic acid; the labeling sequence contains a label that is subsequently detected (g). Among other things, changes in the structure of the NA (aptamers, quadruplexes, hairpins, microRNAs, etc.) are used in the DNA hybridization detection process (B). Electrochemical detection of viral particles strategy by electro-immuno detection (C). The antibody is labeled with a specific label, which can be directly electroactive (heavy metals: Cd, Pb, Ag, Ru; QDs, etc.) or an enzyme that cleaves the substrate, which is subsequently electroactive (AP, etc.). Technically, the detection of the viral particle can be direct, using labeled primary (monoclonal) antibody (a); indirect antibody when first a polyclonal antibody binds to the virus and then a labeled monoclonal antibody binds to this antibody (b); a sandwich antibody is based on the binding of the polyclonal antibody to the support. Subsequently, the viral particle is captured, to which the polyclonal antibody binds and to which the labeled monoclonal antibody subsequently binds (c); a competitive antibody is based on the binding competition of the labeled antibody (monoclonal) (d); (C).

Considerable attention has been paid to electrochemical devices developing sequence-specific DNA hybridization biosensors. These biosensors rely on converting the DNA basepair recognition event into a useful electrical signal. Electrochemical DNA hybridization biosensing meets the size, cost, and performance requirements of decentralized genetic testing.[47] The electrochemical biosensor based on CRISPR/Cas system provides excellent sensitivity and low LOD in the range of attomoles. In addition, this system possesses high specificity to distinguish single base mismatches of nucleic acid.[53] Hejazi et al.[54] developed an electrochemical DNA biosensor based on a gold electrode modified with a self-assembled monolayer composed of a peptide nucleic acid (PNA) probe and 6-mercapto-1-hexanol, that has the LOD of 570 pM. The Cas endonuclease recognizes the RNA transcript according to the target complementary sequence (Cr-RNA). The formation of the Cas-crRNA-RNA transcript tertiary complex turns on its cleavage activities and leads to an increase in the subsequently analyzed signal.[50] Viral nucleic acid is extracted followed by rolling circle amplification (RCA), suitable for amplifying small amounts of nucleic acid. After amplifying specific fragments (searched viral genes), the amplicon is hybridized to the prepared nanoconstruct. It consists of a SPION nanoparticle with an oligonucleotide probe complementary to the specific sequence of the gene being searched for. To visualize the given construct, a detection-labeled oligonucleotide probe complementary to the desired target sequence of the virus is prepared. Washing steps are performed between individual steps.[55] A modified ITO electrode was used for spike protein detection. For specific interactions, cell membranes expressing the appropriate receptors were isolated. After the binding of a suitable target antigen to the receptor, a proper signal is monitored.[56] The DNA nanostructure is modified with an oligonucleotide arm labelled with a redox indicator. After the binding of the target sequence, there is a change in conformation and redox signal transmission.[57] Use of a magnet for LAMP amplification of a selected gene. An oligonucleotide probe is bound to the magnetic particle, NA extracted from the sample is added to it, hybridization with sodium and subsequent LAMP amplification occurs on the magnetic particle, and the resulting amplified nucleic acid is labeled electrochemically by an active molecule, which is subsequently released for electrochemical analysis[58] (Figure 7).

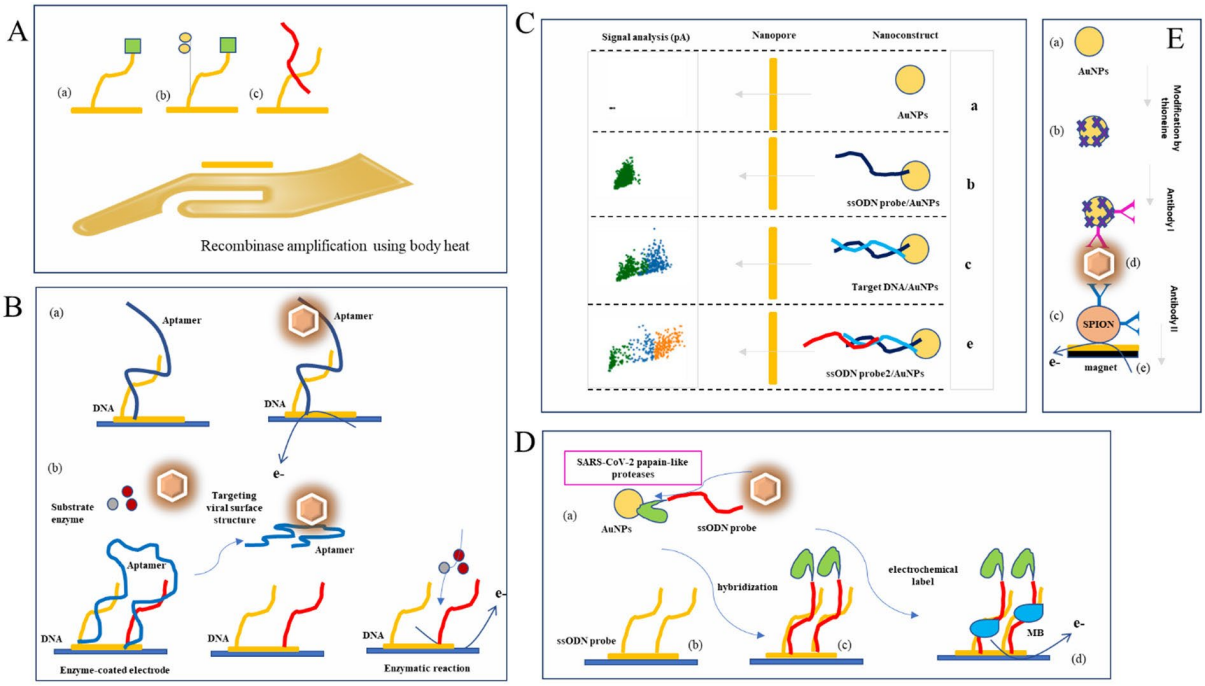

Figure 6. Recombinase polymerase amplification (RPA) on Wes. Recombinase protein, reverse primer (a); Single-stranded binding proteins (b), polymerase (c), sensitive detection of DNA using voltammetry[49] (A). In principle, an oligonucleotide with a complementary sequence to the part of the aptamer is anchored to the surface of the electrode. The aptamer structure specifically binds to the target antigen. This change is then recorded. Aptamer-based bio-nanogate for specific virus detection. The aptamer is released from the oligonucleotides attached to the surface of the electrode and binds to the surface antigen of the virus. The space for the enzyme-substrate will be freed up and subsequently, the enzyme reaction will start, which is recorded electrochemically[50] (B). Schematic process for the detection of a selected analyte using a nanopore, where an individual signal is subsequently registered as a specific cluster: (a) AuNPs; (b) AuNPs/ODN1; (c) AuNPs/ODN1 and target sequence; (d) AuNPs/ODN1/Target/ODN2 [29] (C). A chimeric peptide-DNA nanoprobe made from a substrate peptide for papain was prepared. After digestion with a suitable protease, a specific DNA fragment is released. The fragment is hybridized with a probe immobilized on the surface of the working electrode. Subsequently, a suitable electrochemical marker is used to obtain the signal.[51] The concept shows the use of two types of nanoparticles: SPIONs for capture to the magnet and detection nanoparticles for enhancing the electrochemical signal (thionein).[52]

A strategy for the electrochemical detection of proteins of viruses

The electrochemical detection of viral particles by immuno detection uses antibodies labeled with a specific label, which can be directly electroactive (heavy metals: Cd, Pb, Ag, Ru, QDs, etc.) or an enzyme that cleaves the substrate, which is subsequently electroactive (AP, etc.). Technically, the detection of the viral particle can be direct, using labeled primary (monoclonal) antibody; indirect antibody when first a polyclonal antibody binds to the virus and then a labeled monoclonal antibody binds to this antibody; a sandwich antibody is based on the binding of the polyclonal antibody to the support. Subsequently, the viral particle is captured, to which the polyclonal antibody binds and to which the labeled monoclonal antibody subsequently binds; a competitive antibody is based on the binding competition of the labeled antibody (monoclonal).

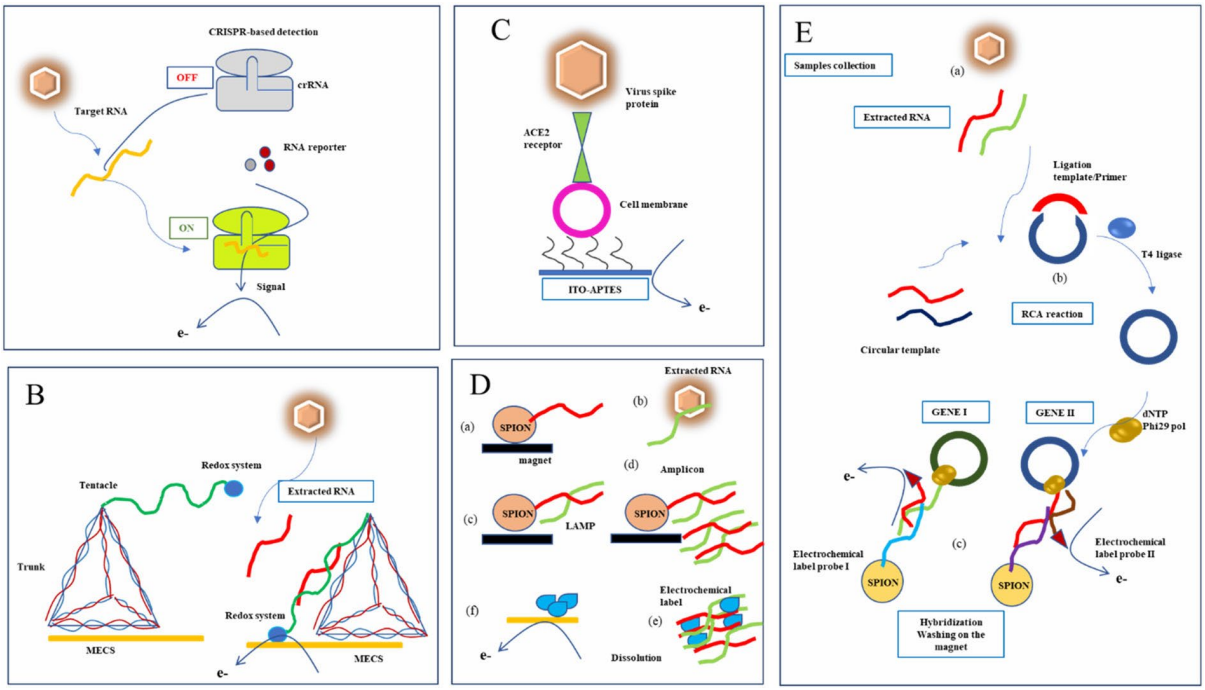

Figure 7. CRISPR-based nucleic acid detection (RNA). The Cas endonuclease recognizes the RNA transcript according to the target complementary sequence (Cr-RNA). The formation of the Cas-crRNA-RNA transcript tertiary complex turns on its cleavage activities and leads to an increase in the subsequently analyzed signal.[50] (A) Viral nucleic acid is extracted (a) followed by rolling circle amplification (RCA), which is suitable for the amplification of small amounts of nucleic acid (b). After amplifying specific fragments (searched viral genes), the amplicon is hybridized to the prepared nanoconstruct. It consists of a SPION nanoparticle with an oligonucleotide probe complementary to the specific sequence of the gene being searched for (c). To visualize the given construct, a detection-labeled oligonucleotide probe complementary to the desired target sequence of the virus is prepared. Washing steps are performed between individual steps[55] (B). A modified ITO electrode was used for spike protein detection. For specific interactions, cell membranes expressing the appropriate receptors were isolated. A proper signal is monitored after binding a suitable target antigen to the receptor.[56] The MECS DNA nanostructure is modified with an oligonucleotide arm labeled with a redox indicator (a). After the binding of the target sequence, there is a change in conformation and a change in redox signal transmission (b)[57] (C). Use of a magnet for LAMP amplification of a selected gene. An oligonucleotide probe (a) is bound to the magnetic particle, nucleic acid extracted from the sample is added to it (b), hybridization with sodium (c) and subsequent LAMP amplification (d) occurs on the magnetic particle, and the resulting amplified nucleic acid is labeled electrochemically by an active molecule (e), which is subsequently released for electrochemical analysis (f).[58]

Overview of experimental works using electrochemical detection for different species of virus detection

In the monitored period (2020–2023), experimental works (more than 200 individual items) on the electrochemical detection of 32 virus types were analyzed. Groups were created and sorted by individual viruses, and electrochemical methods used for their determination, and detected targets using NA, peptides, or immunological accesses. Methods of etection of NA and immunodetection of viruses are similar, with the ratio being 1:1. With the detection of NA, where most often differently modified oligonucleotides are used, the number of oligonucleotides creating aptamers or other unique structures is increasing. Monoclonal and polyclonal antibodies with different modifications were being used for immunological detection. So far special peptide structures have been used at the minimum as detection molecules. The most common peptide structure used is PNA. In more than 70% of records nanotechnological modifications strengthened viral detection. In more than 75% of records, low detection limits of ng or even nanomolar concentrations has been achieved. Electrochemical methods mostly used were voltammetric methods, predominantly DPV a SWV. Another very intensely used electrochemical approach is impedance spectroscopy. Other methods like amperometry, potentiometry, or chronopotentiometry are not used frequently. The total time of analysis (counted with preparation) was within hours. The measurement and evaluation itself takes usually few minutes. In most studies, the authors tried to apply their procedures and methods to analyze real samples. Electrochemical testing sensors were used on more than 650 recorded samples. Within more than 80% of studies, stability and interference were studied. Most of the methods presented probably have very

good stability. More than 50% of studies showed fundamental research without obvious applicable potential. Only 2% of analyzed and studied data were evaluated with significant potential (Figure 8).

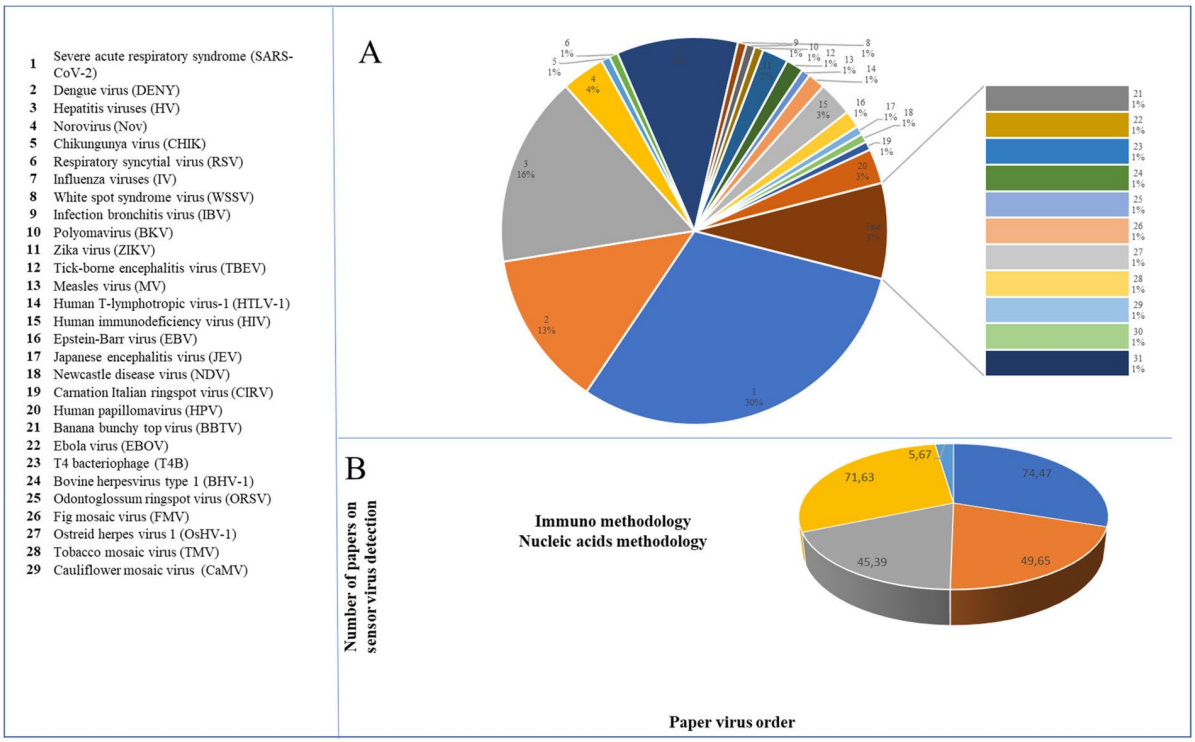

Figure 8. Analysis of research studies focused on detecting viral pathogens (2020–2023). Percentage of experimental studies focused on detecting a selected viral pathogen, data related to the total number of experimental works (n=86). In the monitored period, experimental results on detecting 29 types of viruses were presented (A). Diagram of the number of works focused on immune and NA detection strategy. They were sorted by individual viruses (B). The pie chart summarizes the representation of particular groups of works with the following criteria: 1 (65.52%)—sensitivity of the detection method with LOD in ng or nM; 2 (52.87%)—immunological detection methods; 3 (42.53%) NA detection; 4 (78.16%) use of nanoparticles; 5 (4.60%)—the use of peptides or peptide-derived structures. For more details, see the Methodology section.

Ebola virus (EBOV)

A sensor for EBOV detection using a rolling circle amplification (RCA) technique was designed. The RCPs were generated on magnetic particles, and subsequently, oligonucleotide was connected via streptavidin-biotin bonds to GOx (MB/RCP/GOx/ODN). The enzymatic catalysis of glucose by the bound GOx allows for indirect electrochemical measurement of the DNA target using Prussian blue. The proposed system uses enzymatic amplification of the GOx signal.[59]

Dengue virus (DENV)

Lee et al. focused on developing a sensor using the CRISPR/Cpf1 system, which recognizes the target sequence and randomly cleaves single-stranded DNA. The MB indicator in conjunction with AuNPs on the AuE could recognize 100 fM of the specific oligonucleotide.[60] Immunosensor detection of NS1 protein was performed on an antibody-modified pencil graphite electrode modified with AgNPs. After binding to NS1 protein on the electrode, the oxidation signal of AgNPs (0.5 ng/mL) was measured.[61] Rashid et al. prepared AuNPs modified with a specific aptamer forming a quadruplex structure in the presence of NS1.[62] Modification of the electroactive nanoscale film-gold electrode was used for sensitive recognition of the presence of NS1 protein.[63] Shinghal et al. prepared modified paper electrodes set for detecting DENV as a field sensor, which could increase the reliability of the test. After hybridization, MB was used as a redox indicator.[64] In the study by Wu et al., the probe DNA/MHA/MCH electrode was modified to hybridize to a viral NA.[65] Palomar et al. dealt with voltammetric detection of NS1 protein on Au nanoparticle-decorated multi-walled carbon nanotube composites.[66] Kang et al. used the reduction of 4-nitrobenzene diazonium tetrafluoroborate, which is bound to AuE.[67] Nucleic acid is extracted from the filter paper and transferred to the PCR thermal

block. In the detection part, the ion-exchange nanomembrane NA detection system is located in a reservoir (counter and/or sensing). The membrane is modified by ODN. After hybridization to the modified membrane, the recording of the voltammetric curves (E/I) changes. The interaction is determined from the differences in curves.[68] Dutta et al. designed a simple NS1 DENV protein detection system using a polyaniline-based electrochemical sensor with an NS1 antibody on the CE surface. The generated biosensor responds to the presence of NS1 protein. EIS was used for the analysis itself.[69] The gold electrode was modified with ferrocene with a specific antibody bound. Upon binding to the antibody, the signal changes. The proposed system could distinguish the amount of NS1 in ng/mL.[70] Santos et al. developed an impedimetric immunosensor for the detection of anti-Non Structural 1 (anti-NS1) DENV, based on the specific nonstructural Protein 1 (rNS1) antibodies immobilized over poly(4-aminobenzoic acid)-modified screen-printed electrodes (SPEs).[71] Arshad et al. suggested a molecularly imprinted polymer (NS1 imprinted dopamine) impedimetric sensor on SPCE (modified with polysulfone nanofibers). The specificity of the polymer to NS1 protein was demonstrated.[72] Another immunosensing for NS1 protein recognition using ruthenium bipyridine complex (Ru(II)) and graphene oxide (GO) nanosheets was used by Kanagavalli and Veerapandian. The structure of GO-Ru (II) was modified with the protein-G followed by mAb NS1 and the intensity of the redox signal was evaluated. The interaction specificity was ensured by an antibody to the NS1 protein.[73] Siew et al. prepared SPCE modified by graphene/titanium dioxide for immunosensing of DENV.[74] A label free electrochemical immunosensor for detecting the E-protein of the dengue virus was constructed by Sangili et al.[75] This platform is based on L-cysteine-functionalized AuNP on reduced graphene oxide. The fabricated sensor achieved a low LOD of of 1.6pg mL^{-1}. The immunosensor has been used to detect the viral E-protein in patients' serum samples.[75]
Rashid et al. designed a hybridization biosensor on SPCE-modified AuNPs for DENV detection with LOD around 2.8 mg/L.[76]

Influenza viruses (avian influenza virus)

The use of aptamers[77] and aptasensors[78] is suitable for detection. Ravina et al. designed a hybridization biosensor based on SPE/Au modification coated with Cys and subsequently probe-bound to swine influenza A (H1N1) HA by the probe. The electrochemical label MB detected the hybridization process. The procedure was used on real samples—nasal swabs.[42] Akaksha et al. prepared a specific antibody-modified graphene field-effect transistor (GraFET). An LOD of around 10 fM was achieved in the system. However, the practical applicability of the sensor is still debatable, as no suitable biological samples have been tested.[79] Another sensor is the H1N1 biosensor based on preparing paper electrodes, which uses antibodies to the nucleoprotein and HA.[80] A detection system using an AIV-specific aptamer with a methylene blue redox indicator was prepared. Two SPEs were used for detection, one for positive and one for negative signals. The proposed detection system was used to detect real samples of influenza viruses with a very good response.[81] Joshi et al. designed an immuno-sensor with thermally-decomposed rGO (TrGO) at the ITO electrode. The chemical binding of PBSE anchored antibodies to H1N1. The sensor showed good stability and reproducibility when used on real samples—diluted saliva.[82] Luo et al. tested an electrochemiluminescent biosensor using $Ru(bpy)_3^{2+}$ to detect H9N2 AIV. The mAb-modified magnetic particles serve to separate the virus. A sandwich is formed and H9N2 AIV binds between the two particles. Various viruses were tested by the method with a good selectivity [83] Lee et al. designed a self-calibrating electrochemical aptasensing platform for external interference correction. An aptamer for the nucleoprotein was prepared. The signal was determined as a redox change in MB.[81] Matsubara et al. proposed a method utilizing a boron-doped diamond electrode for virus detection. A peptide dendrimer that binds to viral HA was used on the electrode surface. Different specific peptides have been used for different subtypes of influenza viruses.[84] Dunajova et al. designed an impedimetric biosensor mAb on SPCE.[85] Kim et al.[86] developed an analytical assay for detecting H1N1 viral proteins (hemagglutinin—HA and neuraminidase—NA) and the complete H1N1 virus. An electrochemical sensor uses peptides specific to HA or NA. A gold-binding peptide was used to anchor the complex. Good reproducibility was obtained on the gold electrode using square wave voltammetry (SWV). The proposed sensor showed a low LOD (1.52 PFU/mL) and high selectivity for the H1N1 virus. Reddy et al.[87] fabricated an ultra-sensitive peptide-based nanobiosensor to detect active influenza viruses and viral proteins. The sensor uses a nickel oxide (NiO)–reduced graphene oxide (rGO)/MXene nanohybrid platform. Sensing is

based on the specific interaction between H1N1 and H5N1 binding peptides anchored on the nanocomposite/glassy carbon electrode (GCE) surface. The proposed biosensor allows highly sensitive and specific detection of influenza viruses and viral proteins with a very low LOD (3.63 and 2.39nM for H1N1 and H5N1, respectively). A very simple potentiometric sensor was designed to detect selected viruses (H1N1, H3N2). The specificity was achieved by using a 3D molecular imprinted Au substrate. The LOD for the sensor was around 100 pg/mL.[88] A similar concept for detecting viral antigens was proposed by labeling graphene oxide with gold and platinum complexes with peroxidase activity.[89]

A paper-based electrochemical immunosensor for labelfree detection of several avian influenza virus antigens (H5N1, H7N9, and H9N2) using flexible screen-printed carbon nanotube-polydimethylsiloxane electrodes was developed by Lee et al.[90] The sensor contains three working electrodes, on each of which 1 of 3 antibodies has been immobilized, which allows for the separate measurement of three different hemagglutinin (HA) proteins. LODs ranged from 54.0 pg/mL (H9N2 HA) through 55.7 pg/mL (H5N1 HA) to 99.6 pg/mL (H7N9 HA) antigens in phosphate-buffered saline. The immuno-sensor exhibits rapid measurement time (20–30 min). The magnetic nanoparticles were modified with a specific aptamer to H7N9. An ssDNA detection probe was designed for this aptamer, released in the presence of the virus, and hybridized to a detection particle with ssDNA2 labeled with AuNPs. After adding the Nicking endonuclease Nt.AlwI, the AuNPs are released into the solution, where they are subsequently determined electrochemically. In this determination, the concentration of H7N9 was 0.2 pg/mL to 200 ng/mL with good linear dependence and LOD 24.3 fg/mL. It was possible to experiment with an artificial serum sample.[91]

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) and Middle east respiratory syndrome coronavirus (MERS-CoV)

Electrochemical approaches in developing portable point-of-care devices that can be combined with common methods for detecting various coronavirus strains have been published in different papers[14,92–96] and voltammetric and impedimetric approaches were discussed by Antiochia.[97] New low-cost approaches for miniaturizable virus detection systems have been proposed. It was thus possible to prepare a miniaturized impedimetric biosensor that could be used for laboratory diagnostics.[98] The design of a very fast sensor with CPE and immuno-functionalization was proposed by Borberg et al.[44] Due to the emergence of a new coronavirus—severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—causing the coronavirus disease 2019 pandemic (COVID-19), research efforts have focused on the development of detection tests, including the development of nano-biosensors, that could be highly effective in its detection.[41,96] Laghrib et al. summarized SARS-CoV-2 detection approaches, including the possible use of a smartphone platform connected to sensors and the use of RTLAMP protocol.[99] G-quadruplex-based biosensors are described in the review by Xi et al.[100] Eissa's research group is focused on the S1 protein of MERS-CoV on a multiplexed electrode array.[101,102] Fabiani's study demonstrated the use of modified magnetic nanoparticles with a polyclonal and subsequently a monoclonal antibody. Finally, the antibody is labeled with an enzyme (alkaline phosphatase), which cleaves 1-naphtyl phosphate to 1-naphthol, which is electrochemically active and provides a very good voltametric and/or amperometric signal. The method had good reproducibility and was applied to real saliva samples.[103] The study by Hashemi et al. focused on the SARS-CoV-2 detection strategy based on SPE modified with GO and AuNPs. The viral glycoprotein was analyzed voltammetrically.[104] An array was prepared with four AuNPs modified with specific ODNs for the viral sequence. This probe was transferred to a paper-based electrochemical platform (gold electrode). Sensitivity was demonstrated primarily to the SARS-CoV-2 sequence in a relatively short time window of minutes.[105] In the study by Vadlamani et al., a simple concept of amperometric detection of S-RBD protein on Co-TNTs was proposed.[106] The CPE was modified with GO and 1-pyrene butyric acid N-hydroxysuccinimide ester (PBASE) linker antibody to the virus surface. The redox signal was analyzed via a ferri/ferro system with the detection of 260nM recombinant protein and 5.5 · 105 PFU/mL.[107] An immuno-sensor (antibody to virus N protein) on SPCE was prepared. SWV was used for detection. The sensor was then applied to the analysis of samples without the need for special sample treatment.[108] Diazonium salt was attached to the electrode surface by electroreduction. Subsequently, the N protein was bound to the surface of the electrode. An antibody to the N protein was added to the reaction mixture. The antibody binds directly to the N protein if the SARS virus is absent. If a sample with SARS is added to the solution, the viral protein binds to the antibody. The antibody can no longer bind to the

N protein. In the case of competition with the antibody, the monitored signal corresponds to the presence of the virus.[108] The electricalelectrochemical vertical device point-of-care biosensor was designed using binding to RBD bound to the graphite surface. LOD of the method was around 1 pg/mL.[109] Using smartphones as POCT approaches has been demonstrated with LOD of around 2 pg/mL of viral protein.[110] Chitosanmodified TiO2 nanoparticles bonded to the GCE surface were prepared. A sample is applied to the modified electrode. After the interaction, the antibody was added and the signal was measured.[111] A simple impedance biosensor with an aptamer structure was tested for viral antigen with a detection limit of around 0.4 pg/mL.[112] In another concept, the SPCE was modified with AuQDs and polyhydroxy butyrate to which the viral antigen binds. A detection antibody is applied to the electrode surface modified in this way, and the resulting electrochemical signal is monitored. Yousefi et al. described a sensor for SARS-CoV-2 detection using gold film as a working electrode.[113] A DNA linker was bound to the electrode surface and bound to an anti-SARS-CoV-2 antibody. After binding the virus to the antibody, the chronopotentiometry response changes.[114] Ahuja et al.[115] introduced a cost-effective ENIG PCB-based electrochemical sensor for detecting viruses from environmental water samples. They detected Phi6 phage as a surrogate for SARS-CoV-2 and other pathogenic RNA viruses. This phage has a size comparable to SARS-CoV-2 (80–100 nm), as well as the presence of lipid membrane and spike proteins.[116, 117]

RNA was isolated from the phage particles and then used as a template for cDNA synthesis, and cDNA was subsequently used as a template for PCR. Two DNA fragments of 117 and 503 bp were amplified. The biosensor uses a DNA intercalating redox dye (methylene blue) to detect the target amplicon rapidly.[115] Recently, Mehmandoust et al.[118] introduced a novel and selective method based on SiO2@UiO-66 using a label-free electrochemical immunoassay for rapid SARS-CoV-2 spike protein detection. The electrochemical sensor demonstrated a wide dynamic range (from 100.0 fg/mL to 10.0 ng/mL) with low-limit detection. It allowed point-of-care testing (within 5.0 min, in nasal samples with satisfactory recovery).[118] Ayankojo et al.[119] reported a molecularly imprinted polymer-based electrochemical sensor for detecting SARS-CoV-2 spike protein subunit S1 (ncovS1). The sensor uses the covalent interaction between 1,2-diols of the highly glycosylated protein and the boronic acid group of 3-aminophenyl boronic acid. The sensor has a reaction time of 15 min and shows LOD of 15 and 64 fM in phosphate-buffered saline and nasopharyngeal samples, respectively. The proposed platform possesses great potential for point-of-care testing. Najjar et al.[120, 121] introduced 3D printed lab-on-a-chip multiplexed electrochemical sensor for simultaneous detection of SARS-CoV-2 RNA (single-molecule CRISPR/Cas-based molecular detection) and host anti-SARS-CoV-2 antibodies (serological detection of antibodies against the three immunodominant viral antigens) from saliva samples in two hours. This sensor is suitable as a point-of-care platform. Białobrzeska et al.[122] fabricated immunosensors based on the anti-RNA binding domain of nucleocapsid protein antibodies of SARS-CoV-2 that are grafted onto various surfaces (glassy carbon/diamond/gold). Biosensors enabled rapid (less than 10 min) detection of the SARS-CoV-2 virus with an LOD of 0.227–0.362 ng/mL according to the surface used (glassy carbon/diamond/gold). In addition, sensors showed high specificity for SARS-CoV-2 compared to other viruses attacking the respiratory tract. Carbon nanotube thin film immunosensor was designed to detect virus surface antigens. With the system used, it was possible to analyze 24 ag/mL of viral protein.[123] Genetic engineering was used to express selected antibodies. An antibody from Nicotiana benthamiana was thus prepared, which showed good properties with an LOD in the order of fg/mL.[124]

Nitrogen-doped holey graphene (N-HRGO), a nanocarrier decorated with thionine (TH) molecules as electrochemical indicators, was attached to the CCE. Antibodies to the S protein were used to capture the viral antigen. The electroanalytical determination (DPV) ranged from 1 pg/mL to 10 ng/mL with LOD of 0.3 pg/mL.[125] Another immunosensor using impedance detection was prepared with LOD of 0.2 ng/mL of nucleocapsid protein. The approach was tested on saliva samples of patients with the confirmed disease with very good results.[122] A new type of printed electrode was prepared that was modified with an antibody for the impedance detection of SARS-CoV with a LOD of 56 fg/mL.[126] In experimental work with a modified electrode (FTO/GNR/RBD-Ab), a LOD of 0.73 fM[127] was obtained. The concept of a voltammetric immunosensor was tested on a sample of 28 patients with good sensitivity and specificity ROC 0.911, relative to RT-PCR.[128]

A genosensor bound to AuSPE was designed for several selected SARS-CoV2 genes, which was compared with LAMP assays on strips.[129] A quantum tag modified with methylene blue was used for the electrochemical tag of nucleic acid hybridization.[130] The genosensor method was focused on the

papain-like cysteine protease (PLpro) for the SARS-CoV marker. In principle, papin-like cysteine protease cleaves the fragment by reacting with the oligonucleotide probe. Electrochemical detection (SVW) uses MB and a ferro/ferri redox system. An LOD of 1 pg/mL was achieved[51] (Figure 5). Ramírez-Chavarría et al.[131] introduced a sensitive, accurate, and affordable electrochemical sensor to detect N and ORF1ab genes of the SARS-CoV-2. The sensor is based on the reverse transcription loop-mediated isothermal amplification (RT-LAMP) reaction and consists of screen-printed electrodes acting as a disposable test strip. It was developed for detecting SARS-CoV-2 in wastewater samples. The sensor showed an LOD of 38 pg/mL. SARS-CoV-2 label-free electrochemical (ESI) nanohybrid molecular imprinting polymer (MIP)-aptasensor based on nickel-benzene tricarboxylic acid-metal–organic framework (Ni3(BTC)2 MOF) was developed by Rahmati and Roushani.[132] This platform represents one of the most sensitive biosensors capable of detecting intact SARS-CoV-2 virus in real samples. The sensor showed an LOD of 3.3 ± 0.04 PFU/mL. An ultra-fast electrochemical (chronoamperometry) sensor for continuous indoor air monitoring of SARS-CoV-2 was designed by Lu et al.[133] The sensor can test 250 L of air in less than 5 min, which is much faster than both the rapid antigen (<20 min) and RTPCR test (<90 min). The LOD was 0.004 cp/L in the air. In the work by Li et al., a portable device was designed that enabled real-time nucleic acid amplification (LAMP) using electrochemistry (potentiometry). The device enabled the detection of the presence of nucleocapsid protein in artificial samples with a sensitivity of 2 x 102 copies in 25 min.[134] Another concept of using viral nucleic acid amplification on a magnet was verified on a plasmid DNA model (RdRp gene). LAMP amplification detected around 2500/mL virus copies (4 aM).[58,135] The system could be beneficial for home testing of the SARS-CoV-2 virus. Deng et al. created a hairpin structure to form a DNA duplex with free 3-OH ends that can be recognized and catalyzed by terminal deoxynucleotidyl transferase (TdT) to form a long DNA chain. The structure is subsequently labeled with a ruthenium complex. The created complex enables a catalytic reaction to achieve a LOD of 45 fM with a wide range (0.1–3000 pM). Testing was performed in biological samples of diluted saliva, including real patient samples.[136] A biosensor was designed using NPAOM-Si-S-Aunano-Aptamer membrane modification using $Fe(CN)_6^{4-}$ as and a redox probe. The studied range was from 2.5 to 40 ng/mL with a LOD of 0.8 ng/mL. The sensor was stable for 21 days.[137] A specific CRISPR-Cas12a aptamer was used using a modified gold electrode. A specific impedance and fluorescence signal was obtained in the 0.05 to 125 ng/mL range with a 77 pg/mL LOD.[138] Similarly, a biosensor was designed on SPCE modified by a CRISPR ssDNA reporter. RT-RPA followed by Cas12a-crRNA with CeO_2 [139] was performed on the printed electrodes. The obtained electrochemical (ESI) signal provided a limit of 270 copies/mL.[139] Modification of streptavidin conjugated with HRP was used to enhance the electrochemical signal. The redox reaction of the TMB substrate generated the electrochemical (chronoamperometry) signal.[140] Thanks to the designed concept, achieving an LOD of around 2.8 fM was possible.[140] A simple gene-sensor was designed by modifying GONCs particles with an oligonucleotide probe. After the hybridization of the target sequence, there was a change in the voltammetric and impedance signal.[141] The mentioned concept could be used to distinguish virus variants, achieving good sensitivity and specificity (n=16).[142] A sensitive and specific sensor for the diagnosing SARS-CoV-2 was designed on based on boron nitride quantum dots/flower-like gold nanostructures signal amplification. LOD was in attomolar concentration (0.48 aM). While testing clinical samples (120), RNA was isolated from the smears and then analyzed with the biosensor mentioned above. The obtained ROC curves showed 100% sensitivity and specificity.[143] Recently, Kim et al. reported the recombinase polymerase amplification (RPA)-coupled electrochemical biosensor for rapid (in less than 20 min) and sensitive detection of SARS-CoV-2. This platform provides LOD of 0.9 ng/mL (RdRp gene) and 3.9 ng/mL (N gene) and can be potentially used as a point-of-care test.[49] A highly applicable proof of concept for the detection of viral RNA was designed using modified SPIONs. After binding the viral RNA to the SPION particles, this complex was hybridized with modified graphite oxide with gold particles and thymol blue. Magnetic SPION nanoparticles allow the complex to be captured on a magnet, and after heating, the detection graphite oxide complex is released, which is then analyzed using DPV. This way, LOD of 3 aM of about 100 copies of RNA was achieved. The technique was tested on several different samples (sputum, throat swab, urine, feces, plasma, serum whole blood, oral swab, saliva) with good detection ratios.[144] Another interesting method of detecting the presence of a specific nucleic acid was proposed for the S and N genes. In principle, nucleic acid (RNA) is first extracted from the obtained sample. Using the RCA reaction, a Padlock probe is created to which the target sequences (S or N gene) are subsequently anchored. SPION nanoparticles complementary to the target viral sequences are used

for detection. Subsequently, labeled complementary sequences to the target gene are added to the reaction. The actual detection occurs after washing the SPION construct (as shown in Figure 4).[55] Cell membrane structures with expressed receptors were isolated from cell cultures. The specific ACE2 receptor to which the Spike protein binds was chosen for detecting SARS-CoV2. The resulting simple sensor uses impedance detection. The LOD obtained were 10 pg/mL.[56] The detection of viral nucleic acid using an aptasensor for the SARS-CoV2 receptor was done using SWV on a laser-engraved graphene electrode. The proposed procedure achieved LOD of 0.8 ng/mL.[145] MECS-modified graphene microelectrode based on DNA nanostructures mimics the hydra architecture. The DNA nanostructure is equipped with a suitable electrochemical label, and in the case of hybridization with the target viral nucleic acid, the conformation changes and thus the monitored electrochemical (DPV) signal changes as well. The proposed concept made it possible to identify units of viral copies (50/mL).[57]

African swine fever virus (ASFV)

Even though it is a very serious hemorrhagic virus with considerable mortality, its detection is not given adequate attention. Twenty-five years ago, Stiene and Bilitewski published a paper on the electrochemical (amperometry) detection of ASFV.[146] In 2002, an electrochemical biosensor using an antibody to ASFV was designed.[147] In 2018, Biagetti et al. prepared an optical chimeric DNA/LNA biosensor.[148] It was not until 2020 that our group performed the basic electrochemical investigation of ASFV DNA and prepared an oligonucleotide ASFV sensor. The ODN probe was bound to SPION and the complementary probe was labeled with QDs. The signal was evaluated as a redox signal (Cd).[39,40] A disposable chip for p54 ASFV with LAMP amplification and electrochemical (CV) detection was designed. SPCE modified with AuNPs was used. The LOD of the method was around 0.3 pg/mL.[149]

Rabies virus (RV)

An electrode modified with graphene oxide and an immobilized detection probe was designed. A detection limit of around 0.1 mg/mL was obtained. The method was tested on real samples of bat nasopharyngeal swabs.[150]

Other significant PATHOGENIC viruses for humans

Epstein-Barr virus (EBV), Zika virus (ZIKV)

Huang et al. designed an electrochemical (SWV) sensor based on binding a specific antibody to the EBV surface capsid. The electrochemical label was lead.[151] This amplification system was verified on model samples and serum samples from 18 patients and its practical application can be assumed.[151] Yu et al.[152] reported a highly sensitive label-free electrochemical immunosensor for the sensing of EBV. A single layer of 1,6- hexane dithiol (HDT) was fabricated on the screen-printed electrode (SPE) surface. AuNPs were modified on SPE by the Au-S bond. Then protein A was attached to AuNPs. Targeted immobilization of EBV antibody was achieved by high-affinity binding of protein A to the Fc segment of the antibody. Upon specific binding of the antigen to the antibody, the formation of immune complexes blocks the [Fe(CN)6]4-/3- electron transfer, which is captured by cyclic voltammetry/electrochemical impedance spectroscopy (CV/EIS). The proposed sensor showed an LOD of 0.1 pg/mL.

In the study by Cajigas et al., a screen-printed carbon electrode decorated with hierarchical gold nanostructures (SPCE/Au), with Ru3þ as an electrochemical reporter was used to identify ZIKV. AuNPs were modified with ODN to bind to the target viral sequence. The generated AuNPs/ODN were attached to SPCE via ODN capture. The formed SPCE/ODN1/ODN2/ODN3/AuNPs complex was marked with a redox indicator to amplify the electrochemical (DPV) signal.[153] In another study, a paper-based potentiometric sensor (silver paint as an electrode) modified with a ZIKV virus-specific aptamer (Zika SF9 envelope protein) was prepared. In the detection system, it was possible to distinguish the presence of SF9 protein after binding to a specific aptamer.[154] Streptavidin-coated magnetic particles were modified with a biotinylated capture probe to the ZIKV target sequence and a Dig-labeled signal probe was modified to bind the labeled target antibody (HRP). TMB was

enzymatically oxidized and detected on SPCE. Using i/t curves, the presence of the target search sequence was evaluated.[155] The sensor field was used for EIS immunodetection of ZIKV with a LOD of 1 ng/mL.[156] An immunosensor modified by SPCE for ZIKV detection was prepared. Using the proposed technology, LOD 12.31 pg/mL was achieved.[157]

Human immunodeficiency virus (HIV) and human T-lymphotropic virus-1 (HTLV-1)

Until now, various techniques have been used for the early detection of HIV.[158] A review by Mozhgani et al. summarizes the research results on nano-genosensors for detecting HIV-1 and HTLV-1 viruses based on electrochemical, optical, and photoelectrochemical platforms.[159] Nandi et al. prepared a review on nanosensors capable of quantitative and qualitative detection of HIV, which are highly specific and sensitive and provide fast reproducible results. Techniques in combination with various elements of biological recognition (aptamers, antibodies, oligonucleotides) are available for identification.[160] Tamayo et al. designed a biotin self-assembled monolayer for an impedimetric genosensor to detect HIV-1 directly. They used gold rods with biotin-modified ODN as a probe for detection.[161] Li et al.[162] used a differential pulse voltammetry (DPV) platform for sensing of the HIV gene. The ssDNA was attached to the NH2-rGO/b-CD modified GCE surface. Methylene blue (MB) as an electroactive label interacted with ssDNA to give a well-developed obvious DPV signal. The method showed good selectivity. Testing of the biosensor was possible in the presence of human serum samples.[162] Yeter et al.[163] developed a label-free DNA impedimetric sensor with gold nanoparticles (AuNP)-modified glass fiber/carbonaceous electrode to detect HIV-1 gene.[163] A sensor using lead ion-imprinted polymer (Pb-IIP) nanoparticles and an electrochemical probe were designed. The polymer was applied to CPE to form an AuNPs/Pb-IIP-CPE nanocomplex. Pb redox was monitored as a signal. The system was used to detect the HIV-1 pol gene.[164] Sensors for the detection of HTLV using SPCE modifications were designed. Rapid DNA biosensor based on a synthesis of nanocomposite of reduced graphene oxide, polypyrrole, and AuNPs (rGO-PPy-(L-Cys)-AuNPs/oligo) for the HTLV-1 Tax gene detection was proposed. The designed DNA biosensor was deposited on the surface of a screen-printed carbon electrode (SPCE).[165] In another study, the SPCE was modified with a nanocomposite of rGO, polypyrrole, and AuNPs (rGO-PPy-AuNPs). Anthraquinone-2-sulfonic acid monohydrate sodium salt (AQMS) was used as an electrochemical label. The sensor was highly sensitive to a given sequence in the order of aM.[166] Al-Douri et al. designed a biosensor based on the inter-digitated electrode (IDE) designed with silver and intergraded on Si-AZO. This IDE(Ag)/AZO/Si platform was functionalized with ODN.[167] Au NPs-AgInS2 was used for the photoelectrochemical analysis of the HIV sequence. The designed ITO sensor enabled the detection of concentrations around 3 fM.[168]

A microfluidic device with a two-electrode configuration was used to simultaneously detect two different DNA markers, human immunodeficiency virus-1 (HIV-1) cDNA, through specific interaction between PNA probes and target DNA. The integrated system requires a small sample volume. The results show potential for HIV-1 analysis.[169] A simple microfluidic system using glucometer technology based on CRISPR technology was prepared. HIV DNA and HIV RNA were thus analyzed with the detection of around 200 copies of the nucleic acid.[170]

Human papillomavirus (HPV)

Avelino et al. prepared metal-polymer hybrid nanomaterial for the impedimetric detection of HPV. Impedance detection was performed on AuE/AuNPs/PANI/ODN.[171] The method was applied to infected patients with HPV11 and HPV16 genotypes. Electrochemiluminescent analysis was designed for HPV detection. Cu(I) nanoparticles were immobilized on GCE. The electrode was further modified with arm-DNA/cDNA and pDNA. The pDNA was subsequently labeled with a QD-DNA probe; QD (Zn-doped MoS2). This nanoconstruct emits radiation and catalyzes hydrogen peroxide, which can be measured. When target HPV-16 DNA was captured by cDNA, the arm-DNA was bound to the QD-DNA. Under the incubation of T7 exonuclease, the bonding between QD-DNA and arm-DNA was cut-off, releasing the arm-DNA as the DNA walker to bind to the next QD-DNA.[172] Farzin et al. designed a carbon nanotube/amine-ionic liquid functionalized rGO nano-platform for HPV-16 detection. Using the GCE/MWCNT/NH2-IL-rGO modification method, it was possible to determine the ODN concentration at 1.3 nM.[173] Mahmoodi's work describes the development of an electrochemical biosensor for the early detection of HPV-18. A nanocomposite of rGO and multi-walled carbon

nanotubes (MWCNT) were electrodeposited on a SPCE. Then, Au nanoparticles (AuNPs) were deposited on a modified SPCE. A single-stranded DNA probe (ssDNA) was then immobilized on the modified electrode. Linkage was connected between AuNP and ssDNA probes provided by L-cysteine via AuNP functionalization (L-Cys-AuNP). Differential pulse voltammetry (DPV) determination was also used for electrochemical measurements. The measurement was based on the oxidation signals of AQMS. Due to the hybridization of SPCE-modified DNA with extracted DNA from clinical samples, the biosensor achieved good efficiency.[174]

Hepatitis viruses

Ganganboina et al. prepared magnetic nanoparticles with QDs modified with anti-HEV. Impedance spectroscopy on Au/rGO modified anti-HEV was used for electrochemical detection. The biosensor was used for virus analysis in the cynomolgus monkey with good agreement with PCR analysis. Electrochemical results could be confirmed by QD/Fe2O3/Anti-HEV fluorescence analysis.[175] Commercial oligonucleotide dynabeads/STV with oligonucleotide were prepared for the same virus. PbS modified with complementary ODN were prepared. After hybridization and separation, the PbS particles were hydrolyzed and the Pb was determined.[176] Ngamdee et al. designed a detection system based on the redox behavior of Au3þ on SPCE. Spiked samples of the PCR fragment of the real HEV sample were analyzed.[177] Alzate et al. designed a 3HEV genotype genosensor. The hybridization sensor was designed to label the probes with biotin and the detection probe with digoxigenin. An HRP-labeled antibody recognized the digoxigenin. The actual electrochemical detection used the TMB electrochemical indicator. A linear range from 300pM to 2.4nM with LOD of 1.2pM was obtained. The sensor was tested on wastewater samples with good results.[178] A DNA biosensor for the detection of HBV was developed by Lin et al.[179] GCE was modified with ErGO followed by Cu-MOF to amplify the electrochemical (DPV) signal. Hybridization to an oligonucleotide probe detected the target viral nucleic acid. However, the practical applicability of the proposed concept is still low.[179] A decrease in the oligonucleotide probe signal is observed to distinguish the hybridization process. GCE was modified with rGO/AuNPs and a specific aptamer to the HBV sequence. MB was used as a redox indicator. After virus binding, structural changes occur and the MB signal changes.[180] In the study by Srisomwat et al., a 3D microfluidic paper-based analytical device platform was designed. The detection system consisted of a pyrrolidinyl peptide nucleic acid (acpcPNA), which provides considerable system stability. Plasmid DNA was used as a sample. This is an interesting experimental concept of a biosensor with high sensitivity. However, its use in practice is still debatable.[181] In the study by Upan et al., SPCE was modified with AuNPs-CNT/AgNPs/antibody to detect HBV surface antigen. The analysis is based on the change in the electrochemical (DPV) signal. The proposed procedure could be a suitable basis for preparing the detection of a large number of biological samples.[182] Sheta et al. prepared a novel HCV electrochemical biosensor based on a polyaniline/Ni-MOF nanocomposite. Subsequently, the nanocomposite was modified with an oligonucleotide probe. The signal was obtained as an EIS with limited detection (0.75 fM) around the tested HCV oligonucleotide. It is a funtional concept but it will certainly show a number of difficulties with its practical use.[183] Zhao et al. proposed an electrochemical (DPV) biosensor based on Cu3(PO4)2-BSA-GO nanoflowers. AuE was modified with nanoflower with AuNPs and ODN. A probe with the electrochemical label Fc demonstrated hybridization.[184] A sensor was prepared based on tin-doped WO3/In2O3 nanowires as heterojunction photoelectrode. The effect of irradiation has been studied, but it is unclear how the binding and bound ODNs are affected.[185] An immunosensor for HBe Ag antigene of HBV was designed using PdCuTP functionalized porous graphene as a nanoenzyme. This nanocomplex was shown to have better properties than horseradish peroxidase (HRP). The hydrogen peroxide signal was measured when peroxidase activity was blocked after HBV binding to the antibody.[186] The proposed method of detection has been successfully applied to serum. Further optimization and study of the behavior of the nanocomplex are necessary for further use.[186] Qian et al. proposed an ultrasensitive HBV detection method using a hollow HP5-Au/CoS nanobox WP5 (water-soluble pillar[5] arene) was bound to rGO and modified. The probe DNA was immobilized onto the electrode surface through the host-guest interaction between WP5 and methylene blue (MB) labeled DNA. The prepared nanobox then binds to the nucleic acid of interest. Nanobox exhibits peroxidase activity, which is measured in amperometric mode.[187] Jiang et al. designed a biosensor for HCV detection using cucurbit[7]uril (CB[7]) and methylene blue as an indicator. After hybridization, the MB-labeled probe was released using

exonuclease III. The signal was analyzed on a modified electrode.[188] In the study by Wei et al., an immunosensor based on a GO/Fe3O4/Prussian blue (PB) was designed. Subsequently, AuNPs were generated, and specific antibodies bound. The PB signal was measured, which decreases with increasing HBsAg concentration.[189] Akkapinyo et al. prepared a biosensor on SPCE-modified anti-HBsAg.[190] The article proposes a magnetic reduced graphene-oxide-copper nanocomposite (mrGO-CuNC) with an ODN probe. Copper ions can accelerate the oxidation of o-phenylenediamine. The electrochemical (amperometry) signal of its oxidized product is used to characterize HCV DNA.[187] Srisomwat et al. described an automated paper device eLFA for quantitative determination of HBV. A time delayed microfluidic strategy on paper enabled automated and accurately sequenced solution transmission by loading a single sample was enabled. A metal metallization strategy was used for electrochemical detection of target DNA. As a probe, pyrrolidinyl peptide nucleic acid (acpcPNA) was used. The total operation is very fast (within 7 min of sample loading). The proposed sensor has been successfully used to detect HBV DNA in the serum of patients without the need for any amplification step.[191] Chowdhury et al. fabricated a pulse-triggered ultrasensitive electrochemical sensor using graphene QDs and gold-embedded polyaniline nanowires. A biosensor electrode using specific anti-HEV antibody-conjugated to nitrogen- and sulfur-codoped graphene quantum dots (Anti-HEV-N, S-GQDs) and gold-embedded polyaniline nanowires (AuNP-PAni) as the electrode matrix was prepared. After antigen binding (HEV), the signal was analyzed by EIS. The method was used to detect the presence of HEV in infected monkeys.[192] Antipchik et al. designed a molecularly imprinted polymer to recognize envelope protein E2 (E2-MIP). This way, a 4.6 fg/mL LOD was achieved.[193] A novel highly sensitive electrochemical genosensor based on by electrochemically Pd(II)-Al(III)-layered double hydroxide (LDH) (Pd-Al LDH) for detecting DNA of HBV in human serum was developed by Heidari et al.[194] LOD of the sensor proposed was 1 fM. The designed sensor is a potential candidate for fabricating a portable diagnostic kit.[194] An immuno biosensor for the detection of HCV was designed. A modification of AuNPs with a 10 pg/mL detection limit[195] was used. Particles modified with thionein and a specific antibody and other particles with magnetic particles were designed. A complex of nanoparticles bound in this way to the virus will be formed. Subsequently, this complex is electrochemically (DPV) analyzed. A detection limit of 0.3 ag/mL was achieved.[52]

Norovirus (NoV)—formerly called Norwalk-Like virus

Ganganboina et al. published a new strategy for fabricating magneto-fluorescent nanoparticles for NoV sensing. They demonstrated dual-modality (fluorescence and electrochemical) sensing using QD-encapsulated magnetic hollow sphere nanoparticles (QD@MHS NPs) with magnetic separation ability.[175] Guo et al. published work on NoV capsid protein VP1 using CdS QDs modified with antibody and bound to ITO (indium tin oxide) electrodes.[196] Baek et al. designed a biosensor based on specifically capturable peptides functionalized AuNPs decorated tungsten disulfide nanoflower (WS 2 NF/AuNP). The virus sample was bound to WS 2 NF/AuNP-peptide particles. Subsequently, the complex was immobilized on the SPCE and the signal was determined by impedance.[197] A dual detection system with peroxidase activity has also been proposed using V2O5 nanoparticles-encapsulated liposomes (VONP-LPs). This system was designed in conjunction with magnetic particles. This concept indicates the possibility of continuous signal evaluation electrochemically and photometrically.[198] An impedance biosensor with a 60 ag/mL LOD was prepared by modifying AuE with a streptavidin/biotin-labeled antibody.[199]

Hantavirus

3D printing is intensively used in various fields, including bio-applications. Its use in sensor techniques is also becoming very significant. Both sensor platforms, chips, and possibly electrodes are printed. In work by Martins et al., the immunodetection of hantavirus was proposed using the 3D printing technique. The electrode was prepared from ABS polymer around PLA carbon black and the electrical contact was with copper wire. The proposed concept showed its possible use in hantavirus detection. The LOD was around 22 mg/mL.[200]

Yellow fever virus

A biosensor utilizing MWCNTs with an immobilized virus detection probe was prepared. LOD was around 0.1 mM.[201]

Other viruses pathogenic to animals

White spot syndrome virus (WSSV), infectious bronchitis virus (IBV), BK polyomavirus (BKPV)

The WSSV sensor consisted of carbon quantum dots modified with AuNPs and a virus-specific antibody. Impedance after virus binding was analyzed. The system was tested on real shrimp in aquaculture samples with a limit of 48 copies of viral nucleic acid.[202] The analysis strategy of IBV is based on virus disruption and the released nucleic acid hybridizes with the probe. After heating, the probe is removed and hybridized to the probe bound to the gold electrode. An AuNPs label with a complementary probe and a bound RuHex indicator is prepared. The obtained indicator is determined electrochemically. However, further research will be necessary for applicability. [196] GCE was modified with the prepared hydroxyapatite to bind an oligonucleotide probe complementary to the target sequence of the BKPV. The duplex formed was electrochemically (ESI) measured as a redox signal MB. The method was applied to the measurement of selected urine samples from patients. The method is feasible but the relationship to the virus present in the sample is unclear.[203]

Japanese encephalitis virus (JEV), respiratory syncytial virus (RSV)

Roberts et al. prepared a specific antibody-modified graphene field-effect transistor (GraFET) to detect JEV. The applicability of the sensor is still debatable for practical use. No suitable biological samples have been tested.[79] Recently, Roberts et al. introduced electrochemical (DPV) detection of JEV NS1 secretory protein using gold nanorods as a signal enhancer.[204] LOD was 0.36 fM and 0.53 fM in buffer and spiked serum, respectively. The electrode did not reveal any nonspecific binding with Dengue Virus, West Nile Virus, or Yellow Fever Virus NS1-Ag. SPEs modified with Au and GCE were prepared. Antibody F protein was bound after their modification with 4-amino thiophenol (4-ATP) and/or bovine serum albumin (BSA). The change in signal after RSV binding was measured directly by impedance. The system is buffered and applied to the cell culture.[205] An immunobiosensor based on SPCE for the nonstructural NS1 protein of the JEV was designed. The system was miniaturized using a smartphone. LOD was 0.45 fM. The method showed very good sensitivity and specificity.[206]

Tick-Borne encephalitis virus (TBEV)

The electrochemical behavior of silver nanoparticles (AgNPs) conjugated with antibodies against tick-borne encephalitis virus in various phases of electrode modification was studied. GCE with electrochemically generated AuNPs was used as a platform for antibody immobilization. The voltammetric signal was recorded by detecting the oxidation of silver ions on unmodified GCE. [207] Khristunova et al. proposed to modify AgNPs anti-Human IgG antibodies to TBEV. The sample was applied to a plate and monitored electrochemically after Ag was released from the antibodies. [208,209] In the work by Ivanov et al., the possibility of detecting viral antigen on the eutectic gallium indium (eGaIn) alloy/hydrogel interface was suggested.[210]

Measles virus (MV), chikungunya virus (CHIKV)

The proposed sensor for MV detection was prepared by modification of AuE NHC and antibody. Virus binding to the sensor was detected by a change in impedance.[211] To detect CHIKV, a liposome was prepared, filled with electrochemical (methylene blue, MB) and fluorescent (quantum dots, QDs) labels and modified with an antibody to recombinant CHIK-VP. APTES Fe_3O_4 was used for separation. After capture, the liposome was broken up with chloroform and subsequently analytes were determined.[212] A SPCE-modified immunosensor for CHIKV detection (DPV) was designed. This technology provided an LOD of 1.33 pg/mL.[157]

Newcastle disease virus (NDV)

A sensor was prepared using as detection part chitosan modified with Cu(I)/Cu(II)-Chi-Gra system and antibody to virus capsid (BSA-Pab/NDV-Cu(I)/Cu(II)-Chi-Gra). GCE the AuNP- was modified with a Chi-Gra antibody. In the case of binding of the reporter part, the Cu signal was detected.[213]

Carnation Italian ringspot virus (CIRV)

A sensor focused on the p19 protein using interactions with RNA/miRNAs was designed, and the complex formed on SPCE was analyzed by EIS.[214]

Bovine herpesvirus type 1 (BHV-1)

An impedimetric biosensor using a BHV-1 antibody was prepared to ensure selectivity. GCE was activated and subsequently immobilized with an antibody. The sensor was tested for biological samples of serum, nasal secretions, seeds, and urine.[215]

Porcine epidemic diarrhea viruses (PEDv)

Victorious et al.[216] introduced a dual-electrode electrochemical chip and a barcode-releasing electroactive aptamer for rapid on-farm detection of PEDv. An aptasensor specific to the viral N-protein was designed to detect the virus from pig saliva. The nucleic acid (aptamer) was hybridized with an electrochemically labeled (methylene blue) probe. In the presence of the N-protein, the aptamer nucleic acid binds to the protein, releasing the labeled nucleic acid. This released labeled nucleic acid binds to the complementary sequence on the second detection surface. The proposed procedure significantly increases the selectivity and sensitivity of viral protein determination. The biosensor showed an LOD of 6 nM (0.37 lgmL^{-1}), a sensitivity of 83% and specificity of 100 %, and an analysis time of one hour. The procedure was applied to several (n=12) real samples.[216]

Ostreid herpes virus 1 (OsHV-1)

An electrochemical biosensor for detecting OsHV-1 based on isothermal recombinase polymerase amplification (RPA) has been developed. Amperometric detection of the amplicon was based on a sandwich hybridization assay with an immobilized thiolated capture probe and a reporter probe labeled with horseradish peroxidase (HRP). The detection limit of 207 target copies of OsHV-1 was reached.[217]

Viruses pathogenic to plants and bacteria

Very little attention is paid to the issue of viral sensors in plants.[218] Konwarh and Sharma prepared a detailed summary of plant virus detection using available bioanalytical approaches, including electrochemical.[219]

Banana bunchy top virus (BBTV)

An improved electrochemical ELISA was developed using electronic signals to detect BBTV. Streptavidin-conjugated CdSe QDs were used as signal amplifiers. The antibody was labeled with biotin, and a QDs/STV/biot/Anti complex was formed. Resistance was measured.[220]

Odontoglossum ringspot virus (ORSV)

Wang et al. 2020 used semiconductors with nanohybrid structured electrodes on a silicon wafer. The monolayer procedure was then used to modify the electrode surface and the anti-ORSV antibody sequentially. Experimental results have shown that the concentration of ORSV in virus infected orchid leaves can be effectively detected. [221]

Table 1. An overview of the biological properties of viral pathogens that were determined using electrochemical detection.

Virus	Structure of genome	Clinical symptoms	Target organisms	Methods	Selected ref.
African Swine Fever Virus (ASFV)	Double-stranded DNA virus	Early symptoms: high fever, lethargy loss of appetite. Late symptoms: vomiting, diarrhea, abortions, and swollen red eyes.	Pigs	CV, amperometry	[39,146–148,230]
Banana Bunchy Top Virus (BBTV)	Single-stranded DNA	Chlorosis of the leaf blade, dark discontinuous streaks. Late symptoms: dwarfism of the leaves. Fever,	Banana	Resistance	[220]
BK Polyomavirus (BKPV)	Double-stranded DNA virus	upper respiratory symptoms, and cystitis. Fever,	Primates	DPV	[203,231]
Bovine Herpesvirus Type 1 (BHV-1)	A double-stranded DNA virus	depression, leucopenia, profuse nasal and ocular discgarg.	Bovine	EIS	[215,232]
Carnation Italian Ringspot Virus (CIRV)	a positive-strand RNA virus	Mottling, chlorotic ringspots, and distortion of lea margins.	carnation	EIS	[214,233]
Cauliflower Mosaic Virus (CaMV)	double-stranded DNA, circular	Mosaic mottling, puckered or distorted leaves, yellow or white leaves.	cauliflower	DPV	[222,234]
Chikungunya Virus (CHIKV)	An RNA virus with a positive-sense single-stranded genome	Most common symptoms: fever and joint pain. Less common symptoms: headache, muscle pain, swelling or rash.	Primates	DPV	[212,235]
Dengue Virus (DENV)	A single positive-stranded RNA virus	High fever, severe headache, severe joint and muscle pain, vomiting, anorexia, and insomnia.	Primates	SWV, DPV, CV, EIS, impedance, voltammetry, chronopotentiometry, ESI	[66,69–71,73,75]
Ebola Virus (EBOV)	Single-stranded, noninfectious RNA genome	Fever, chills, headache, myalgia, and general malaise for a few days followed by anorexia, muscle pain, and appetite loss	Primates	Chronopotentiometry (CP)	[8,236]
Epstein-Barr Virus (EBV)	A double-stranded DNA virus	Fatigue, fever, ras, inflamed throat, swollen spleen, lymph nodes in the neck and liver.	Primates	SWV, CV	[152]
Fig Mosaic Virus (FMV)	A segmented, negative sense, single-stranded RNA virus	Foliar chlorosis, deformation, and mosaic patterns.	Fig	DPV	[225]
Hepatitis Viruses (HVs)–HBV	Double-stranded DNA, hepatitis B virus	Inicial symptoms: anorexia, nausea, vomiting, abdominal pain, and jaundice. Sometimes jaundice, hepatic encephalopathy, ascites, gastrointestinal bleeding secondary to esophageal varices, and coagulopathy.	Primates	DPV, CV, EIS, amperometry, lateral flow electrochemical device/SWV	[237]
Hepatitis Viruses (HVs)–HCV	A single positive-stranded RNA, hepatitis C virus	Fatigue or malaise. In more developed cases: arthralgia, myalgia or cirrhosis.	Primates	EIS, SWV, DPV, resistance	[195,238]
Hepatitis Viruses (HVs)–HEV	CIRCULAR SINGLE-STRANDED RNA, HEPATITIS E (DELTA)	Malaise, anorexia, nausea/vomiting, abdominal uncomfortable, jaundice, and fever.	Primates	impedance, SWV, DPV, amperometry, ESI EIS,	[52,239]
Human Immunodeficiency Virus (HIV)	SINGLE-STRANDED, POSITIVE-SENSE, ENVELOPED RNA VIRUS	2 groups of symptoms: Without AIDS symptoms (CD4 cell count $<$200 cells/microL): Fatigue, muscle pain, skin rash, headache, swollen lymph nodes. With AIDS symptoms (any AIDS-defining condition regardless of the CD4 cell count): Bacterial infections, pneumonia, cervical cancer and many other infections with stronger effect on body.	Primates	DPV, it	[11,12,158–164,168,220]
Human Papillomavirus (HPV)	DNA virus	Usually no symptoms. Sometimes: painless lumps around vagina, penis or anus which can turn into cancerous bodies.	Human	EIS, ECL, DPV	[171,173]
Human T-lymphotropic Virus-1 (HTLV-1)	Positive-sense RNA	Muscle weakness in the legs, stiffness and spasms, problems with controlling of the bladder and bowels; sometimes erectile dysfunction in men.	Human	DPV	[159,165,240]

(*continued*)

Table 1. Continued.

Virus	Structure of genome	Clinical symptoms	Target organisms	Methods	Selected ref.
Infectious Bronchitis Virus (IBV)	Single-stranded RNA with positive-sense	Gasping, couching and nasal discharge.	Primates	LSV	[241]
Influenza Viruses (IVs) Avian Influenza Virus (AIV)	A negative-sense, single-stranded RNA genome that is segmented Single-stranded negative sense RNA virus	Fever, musccle pain, chills and sweats, headache, cough, runny nose, weakness and vomiting. Fever, dry cough, body aches, nauzea, inflammation of the lower respiratory tract.	Primates, pigs, birds	DPV, impedance, EIS, SWV, CV, potentiometry. Graphene field effect transistor (GraFET), electrochemiluminescence, CV, EIS, DPV, it	[12,42,77–85,87–91,137,143,230,242]
Japanese Encephalitis Virus (JEV)	The positive sense single-stranded RNA genome	Usually no major symptoms. Sometimes: fever, seizures, headache and vomiting.	Primates	Graphene field effect transistor (GraFET), DPV	[204,243]
Measles Virus (MV)	A single-stranded, negative-sense, enveloped, non-segmented RNA virus	Flu-like symptoms (fever, tiredness, cough, red eyes), white spots in the mouth and rash occur.	Primates	EIC	[211,244]
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	Single-stranded RNA	Fever, cough, pneumonia and gastrointesinal symptoms.	Primates	Potentiometry, impedance	[5,19,49,72,96,138,154]
Newcastle Disease Virus (NDV)	A negative-sense, single-stranded RNA virus	Loss of appetite, coughing, gasping for air, nasal discharge, bright green diarrhea, neurological signs such as paralysis and convultions. Nausea,	Primates	DPV	[214,245]
Norovirus (NoV)	Single-stranded positive-sense RNA	vomiting, cramps, diarrhea, fever and muscle pain, anorexia and lethargy. Chlorotic	Primates	Impedance, ESI, DPV EIS	[196–199,246]
Odontoglossum Ringspot Virus (ORSV)	A positive-sense single-stranded RNA genome	streak with necrotic spots at the leaves and flowers.	Orchids	Amperometry	[221,247]
Ostreid Herpes Virus 1 (OsHV-1)	A linear double-stranded DNA	Reduced growth and feeding, weak swimming with probability of losing the ability.	Ostreid	SWV	[217]
Porcine Epidemic Diarrhea Viruses (PEDv) Respiratory Syncytial Virus (RSV)	A single-stranded RNA virus	Vomiting, anorexia, dehydration and weight loss.	Pigs	ESI	[216,248]
	A negative-sense, single-stranded RNA virus	Runny nose, decrease in appetite, coughing, sneezing, fever and wheezing.	Primates	DPV, amperometry, SWV,	[205,249]
Severe Acute Respiratory Syndrome (SARS-CoV-2)	A positive-sense single-stranded RNA virus	Fever with chills, cough, loss or change of the smell and tase sense, exhaustion, headache, bodyache, diarrhea. [12–14,30,44,49–51,55–58,88,94,97,98,101, 103–108,111–113,116–124,126–134,136–138, 140,143,200,250]	Primates	chronoamperometry (CA), potentiometry, impedance, DPV/SWV, CV, resistance, ESI	
Tick-Borne Encephalitis Virus (TBEV)	A positive-strand RNA virus	Usually no to mild symptoms. Sometimes fever, loss of appetite, headache, nauzea and vomiting.	Primates	Linear sweep anodic stripping voltammetry (LSASV), I-V curve	[207–210]
Tobacco Mosaic Virus (TMV)	A positive-sense single-stranded RNA virus	Yellowing of the foliage, necrotic leaf spots, mosaic symptoms, stunting and leaf distortion.	Tobacco	Amperometry	[226,251]
T4 bacteriophage (T4B)	Single-stranded, positive sense RNA	The cell is lysed and used to product more phages. The virus can also undergo the lytic cycle without killing the host.	Bacteria	DPV	[252]
White Spot Syndrome Virus (WSSV)	Double-stranded, DNA virus	White spots, lethargy, sudden reduction in food consumption, red discoloration of body and appendages and a loose cuticle.	Bats	EIS	[202,253]
Zika Virus (ZIKV)	A positive-sense RNA single-stranded	High temperature, headache, sore, red eyes, swollen joints, joint and muscle pain and rash all over the body.	Primates	DPV, potentiometry, chronoamperometry, ESI	[155,156,241]

Cauliflower Mosaic virus 35S (CaMV 35S)

In the work by Ye et al., a procedure for detecting the CaMV 35S gene of the cauliflower mosaic virus was proposed. The probe has been labeled with the iron oxide nanocomposite (Fe3O4) containing gold and silver. Electrochemical (DPV) sensing is performed at the interface of electrolytically deposited AuNPs and carboxylated multilayer carbon nanotubes of a modified glassy carbon electrode. The detection sensitivity was improved by the amplified reduction signal of hydrogen peroxide (H2O2), which takes advantage of the increased electrocatalytic activity of Fe3O4-Au@Ag.[222] Ang et al. designed and electrochemically analyzed a PCR product on SPCE/GO on a model Cauliflower Mosaic Virus 35 S promoter sequence.[223]

Tobacco Mosaic virus (TMV) and fig Mosaic virus (FMV)

Guo et al. developed a method for producing platinum (Pt) porous nanotubes coated with interconnected Pt dendrites using tobacco mosaic virus (TMV) as a template. Surface exposed arginine residues of TMV induced selective Pt production. The synergistic effects of TMV/DPtNT structures increased active sites, transport efficiency, and long-range electron transfer, significantly improving catalytic activity. Such nanotubes can be used to detect H2O2 with good sensitivity.[224]

In the work by Haji-Hashemi et al., the construction and characterization of an immunosensor for highly sensitive mosaic virus (FMV) detection have been described. The specific antibody against the nucleocapsid of the virus was immobilized at the surface of a gold electrode modified with 11-mercaptoundecanoic acid (MUA) and 3-mercapto-propionic acid (MPA). Electrochemical detection of FMV was performed using differential pulse voltammetry in ferri/ferrocyanide solution as a redox probe.[225]

Zhang et al.[226] constructed an electrochemical (DPV) biosensor based on an activator regenerated by radical polymerization (ARGET ATRP) with duplex-specific nuclease(DSN)-assisted target recycling for sensitive detection of TMV RNA. DNA is captured and self-assembled on the electrode surface and hybridizes with TMV RNA forming DNA/RNA hybrids. The sensor showed high sensitivity, superior selectivity, excellent stability, and good reproducibility with an LOD of 2.9 fM.

Tobacco ringspot virus (TRSV)

An impedance chip was designed for virus detection with an LOD of around 0.6 mg/mL.[227]

T4 bacteriophage (T4B)

A sensor for T4B detection was designed. T4B was immobilized on AuE and subsequently analyzed by DPV. The effect of the best possible capture of T4B on the AuE surface using chemical modification was studied. [222]

Advantages and disadvantages of electrochemical virus detection

The advantages of electrochemical detection include their miniaturization for the development of portable detection systems (POCT). This implies that the operational cost of performing a single analysis is low. Furthermore, very small sample volumes (on the order of microlitres) can be used for analysis. For the actual determination, various electrochemical methods (voltammetry, amperometry, impedance spectroscopy, etc.) can be used to increase the sensitivity and selectivity of the determination. In addition, self-detection is less time consuming when compared to PCR techniques. Moreover, the techniques allow the detection of very low concentrations of viral nucleic acid or viral protein. Electrochemical approaches can be used in barcoding procedures both for multiple detection of virus and for distinguishing between different viruses present in the samples.

The disadvantage of the electrochemical approach remains the influence of the biological matrix containing the virus. If the virus detection is focused on the presence of nucleic acid, it is necessary to include nucleic acid release steps. This fact leads to an increase in the time required to obtain a result. Moreover, the potential difficulty to prepare the biosensor (different modification methods, use of more complex labeled probes, etc.) can be considered as a disadvantage. Certainly a disadvantage is how to ensure a long term stability of the prepared biosensor. In addition, the technological complexity of the electrode surface preparation must be taken into account in terms of its reproducibility.

It is very difficult to assess the applicability of individual electrochemical methods for detection. Here, CV provides the most physicochemical information, but the sensitivity is quite high. In case that the study is more focused on the actual electroanalytics, techniques such as DPV or SWV enable the analysis of subnanomolar concentrations. However, the vast majority of the published work is in the form of proof of concept and the reported techniques are not ready for deployment as POCT systems. Thus, the methods will have to undergo further detailed optimization procedures.

Conclusion and future outlooks

The global security should be a real priority for the research and development of the twenty first century, while maintaining the freedom of movement. Previously, we have been involved in several viral epidemics (Ebola, SARS, MERS, influenza, CoV-19) that have significantly undermined this priority. Ensuring this priority is indeed a daunting task for the health system, but also for the political system. Therefore, the use of quick, simple and sensitive tools for the identification of such threats will allow to ensure decent health measures quickly enough. Screening can be used in places of high concentration of people (airports, shops, railway stations, squares etc.). A major challenge is the development reliable systems that allow testing in the quiet environment at home, which will increase the overall safety and well-being of the test subject. A big challenge is the combination of a simple application in a personal mobile phone with a sensor. In addition, data can be evaluated in combination with a remote central computer using artificial intelligence. In the near future, a significant rise in smart biosensors technology can be expected.[36]

Virus samples were tested experimentally in the analyzed studies with a good analytical response. The described electroanalytical methods are realistic and potentially applicable. However, further research studies for their practical use are lacking. The disadvantage for practical use is the complexity of the ensor preparation process, but it can be assumed that robotization and unification could yield more reproducible results.

Technologies using rapid miniaturization of electronics are a big challenge for detecting intact viruses.[20, 228, 229] Miniaturized potentiostats/galvanostats are available, which can be connected to portable devices with sufficient computing capacity (mobile phone, tablet, etc.).[229] When designing a suitable application for reading the signal from the measuring sensor/biosensor, we get a very appropriate technology for POCT. It will enable highly sensitive and selective determination of the analyte sought. The obtained signal can be transferred to suitable storage in real-time. In addition, the whole system can propose a possible diagnosis when appropriate algorithms are incorporated.[110, 144]

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Abbreviations

ACE2	angiotensin-converting enzyme 2
acpcPNA	pyrrolidinyl peptide nucleic acid
aDNA	ancient DNA
AFM	atomic force microscopy
AgNPs	silver nanoparticles
AIV	avian influenza virus
Anti-HBsAg	antibody to Hepatitis B Surface Antigen
Anti-HEV,	anti-HEV antibody to Hepatitis E Surface Antigen
Anti-HEV-N,S-GQDs	specific anti-HEV antibody-conjugated to nitrogen- and sulfur-codoped graphene quantum dots

anti-NS1 DENV	antibody to Nonstructural 1 DENV
anti-ORSV	antibody specific Odontoglossum ringspot virus antibody
AP	alkaline phosphatase
APTES	(3-aminopropyl)-triethoxysilane
ARGET ATRP	activator regenerated by transfer radical polymerization
arm-DNA	arm assembly DNA structures
ASFV	African swine fever virus
AQMS	anthraquinone-2-sulfonic acid monohydrate sodium salt
4-ATP	4-amino thiophenol
AuE	gold electrode
AuNP/AuNPs	gold nanoparticle/s
AuNP-PAni	gold-embedded polyaniline nanowires
AuSPE	screen-printed electrodes modified by gold
AuQDs	gold quantum dots
AZO	aluminum nanoparticle doped zinc oxide nanostructure
BBTV	banana bunchy top virus
BHV-1	bovine herpesvirus type 1
BKPV	BK Polyomavirus
BSA	bovine serum albumin
cDNA	complementary deoxyribonucleic acid
CaMV 35S	cauliflower mosaic virus 35S
Cas CRISPR	associated proteins (encoded by CRISPR loci)
Cas12a-crRNA	CRISPR-Cas endonuclease editing systems
CB[7]	cucurbit[7]uril
CCE	carbon composite electrode
CE	carbon electrode
Chi-Gra	chitosan-graphene
CHIK-VP	Chikungunya virus E1 protein
CHIKV	Chikungunya virus
CIRV	carnation Italian ringspot virus
CNT	carbon nanotube
Co-TNTs	cobalt-functionalized TiO2 nanotubes
COVID-19	coronavirus disease 2019 pandemic
CPE	carbon paste electrode
Cpf1	a CRISPR-associated two-component RNA-programmable DNA nuclease
CPSA	chronopotentiometric stripping analysis
Cr-RNA	trans-activating CRISPR RNA
CRISPR	clustered regularly interspaced short palindromic repeats
CV/EIS	cyclic voltammetry/electrochemical impedance spectroscopy
Cys	cysteine
DENV	Dengue virus
DNA	deoxyribonucleic acid
DPtNT	dendritic platinum nanotube
DPV	differential pulse voltammetry
DSN	duplex-specific nuclease
EBOV	Ebola virus
EBV	Epstein-Barr virus
eGaIn	eutectic gallium indium alloy
E/I	potential/current
EIS	electrochemical impedance spectroscopy
ELFA	electrochemical lateral flow assay
ELISA	enzyme-linked immunosorbent assays
E2-MIP	molecularly imprinted polymer for the recognition of envelope protein E2

ENIG	electroless nickel immersion gold
ErGO	electro-reduced graphene oxide
Fc	ferrocene
fM	femtomols
FMV	fig mosaic virus
FTO	fluorine-doped tin oxide
GCE	glassy carbon electrode
GNR	graphene nanoribbon
GO	graphene oxide
GONCs	Graphene oxide nano-colloids
GOx	glucose oxidase
GraFET	graphene field effect transistor
HA	hemagglutinin
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HDT	1,6-hexane dithiol
HEV	hepatitis E virus
H9N2 AIV	avian influenza virus H9N2
HP5-Au/CoS	MOF-derived cobalt sulfide nanobox was prepared to anchor the hydroxylatopillar[5]arene stabilized Au NPs (HP5–Au/CoS)
HPV	human papillomavirus
HRP	horseradish peroxidase
HIV	human immunodeficiency virus
H1N1	influenza A
HRP	horseradish peroxidase
HTLV-1	human T-lymphotropic virus-1
IBV	infectious bronchitis virus
IDE	inter-digitated electrode
IgG	immunoglobulin G
IL	ionic liquid
ITO electrode/s	indium tin oxide electrode/s
JEV	Japanese encephalitis virus
JEV NS1	Japanese encephalitis virus NS1 protein
LAMP	loop-mediated isothermal amplification
L-Cys	L-cysteine
LDH	layered double hydroxide
LNA	locked nucleic acid
LOD	limit of detection
mAb	monoclonal antibody/monoclonal antibodies
MB	methylene blue
MCH	6-mercapto-1-hexanol
MERS-Cov	Middle East Respiratory Syndrome Coronavirus
MHA	mercaptohexanoic acid
MHS NPs	magnetic hollow sphere nanoparticles
MIP	molecularly imprinting polymer
MOF	a metal-organic framework
MPA	3-mercapto-propionic acid
mrGO-CuNC	magnetic reduced graphene-oxide-copper nanocomposite
miRNAs	microRNAs
MUA	11-mercaptoundecanoic acid
MV	Measles virus
MWCNT	multi-walled carbon nanotubes
MXene	class of two-dimensional inorganic compounds, that consist of atomically thin layers of transition metal carbides, nitrides, or carbonitrides

N gene	SARS-CoV-2 nucleocapsid (N) gene
NA	Nucleic acid
NDV	Newcastle disease virus
NHC	N-heterocyclic carbene
N-HRGO	nitrogen-doped holey graphene
NH_2-IL-rGO	amine-ionic liquid functionalized reduced graphene oxide
NH_2-rGO/b-CD	amino-reduced graphene oxide (NH2-rGO) and b-cyclodextrin (b-CD)
$Ni_3(BTC)_2$ MOF	nickel-benzene tricarboxylic acid-metalorganic framework
NoV	norovirus
NS1 protein	nonstructural (NS1) protein
Nt.AlwI	nicking endonuclease
ODN	oligonucleotide
ORF1ab	gene refers collectively to two open reading frames
ORSV	odontoglossum ringspot virus
OsHV-1	ostreid herpes virus 1
p19	protein the 19 kDa protein
Pab	polyclonal antibodies
PAni	polyaniline nanowires
PANI	polyaniline
PB	Prussian blue
PBASE	1-pyrene butyric acid N-hydroxysuccinimide ester
Pb-IIP	lead ion-imprinted polymer
PBSE	1-pyrene butanoic acid succinimidyl ester
PCB	printed circuit board
PCR	polymerase chain reaction
PdCuTP	PdCu tripod
pDNA	probe DNA
PEDv	porcine epidemic diarrhea viruses
PFU	plaque-forming unit
PLA	polylactic acid
PLpro	papain-like cysteine protease
POCT	point of care testing
PPy	polypyrrole
QDs/STV/biot/Anti complex	QDs/STV/biotin-labeled antibody
QD@MHS NPs	QD-encapsulated magnetic hollow sphere nanoparticles
RBD	receptor binding domain
RCA	rolling circle amplification
RdRp gene−RCP/RCPs	rolling circle amplification product/s
rGO	reduced graphene oxide
RNA	ribonucleic acid
ROC	receiver operating characteristic
RPA	recombinase polymerase amplification
rNS1	specific nonstructural Protein 1
RSV	respiratory syncytial virus
RT-LAMP	reverse transcriptase loop-mediated isothermal amplification
RT-PCR	reverse transcription PCR
$Ru(bpy)_3^{2+}$	tris (2, 2′-bipyridine) ruthenium (II)
RuHex	hexaammineruthenium (III) chloride
RV	rabies virus

SARS	severe acute respiratory syndrome
SPCE	screen-printed carbon electrode
SPE/SPEs	screen-printed electrode/s
SPION/SPIONs	superparamagnetic iron oxide nanoparticle/s
SPR	spectroscopy surface plasmon resonance (SPR) spectroscopy
SERS	surface-enhanced Raman scattering
ssDNA	single-stranded DNA
S-RBD	protein spike-RBD protein
STV	streptavidin
SWV	square wave voltammetry
T4B	T4 bacteriophage
TBEV	tick-borne encephalitis virus
TdT	terminal deoxynucleotidyl transferase
TMB	3,3',5,5'-tetramethylbenzidine
TMV	tobacco mosaic virus
TrGO	thermally-decomposed rGO
TRSV	Tobacco Ringspot Virus
VONP-LPs	V2O5 nanoparticles-encapsulated liposomes
WP5	water-soluble pillar[5]arene
WS 2 NF WSSV	tungsten disulfide nanoflower white spot syndrome virus
ZIKV	Zika virus

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