Int. J. Electrochem. Sci., 7 **(2012)** 10779 - 10801

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Review Paper

Electrochemical Sensors and Biosensors for Influenza Detection

Ludmila Krejcova[1], *David Hynek*[1,2], *Vojtech Adam*[1,2], *Jaromir Hubalek*[1,2] *and Rene Kizek*[1,2,*]

[1] Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic, European Union
[2] Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic, European Union
[*]E-mail: kizek@sci.muni.cz

Received: 3 September 2012 / *Accepted:* 28 September 2012 / *Published:* 1 November 2012

World Health Organization, United Nations and national governments are doing detailed monitoring of influenza viruses due to the fact that there is considerable concern about the emergence of new global pandemics with significant socio-economic impact. These concerns are based on known epidemiological data and knowledge, because antigens (hemagglutinin and neuraminidase) of influenza viruses can easily and quickly changed. Result of mutational changes is the emergence of a new subtype of the virus, which can cause a worldwide pandemic with a high fatality rate due to its virulence properties (Spanish Flu 1918, Asian Flu 1957, Hong Kong Flu 1968). Assessment of pathogenicity and virulence is the key to taking appropriate health actions in the outbreak of several tens of hours. Methods used for detection of viruses demand on equipment and personnel, and, moreover, confirmation (diagnosis) of infection lasts hours to days. Given the above, finding methods for rapid, sensitive and selective detection of the virus in the environment, body fluids and tissues is still challenging. A promising area of nanotechnology seems to using nanoparticles in combination with electrochemical detection. The aim of this review is to describe and discuss the previously known facts in the detection of influenza viruses and to outline the challenges and trends in the field of electrochemical detection. In this paper, there are described and discussed appropriate strategies for detection viral nucleic acid, specific viral proteins and virions. The strategies are divided into two main parts as sensors and biosensors.

Keywords: influenza virus; electrochemical detection; biosensor; sensor; nucleic acid; viral protein; viral genome; magnetic nanoparticle; quantum dots; voltammetry

1. INTRODUCTION

Influenza viruses belong to the family *Orthomyxoviridae*, which is the member of the group ssRNA viruses with negative polarity. This family contains three genera: Influenza A, Influenza B and Influenza C [1-3]. These genera differ from each other by the presence species-specific nucleo-protein

antigens, the number of gene segments, host specificity and clinical manifestations. Size of influenza virion is 80-120 nm and its schematic structure, which is similar for all genera, is shown in Fig. 1.

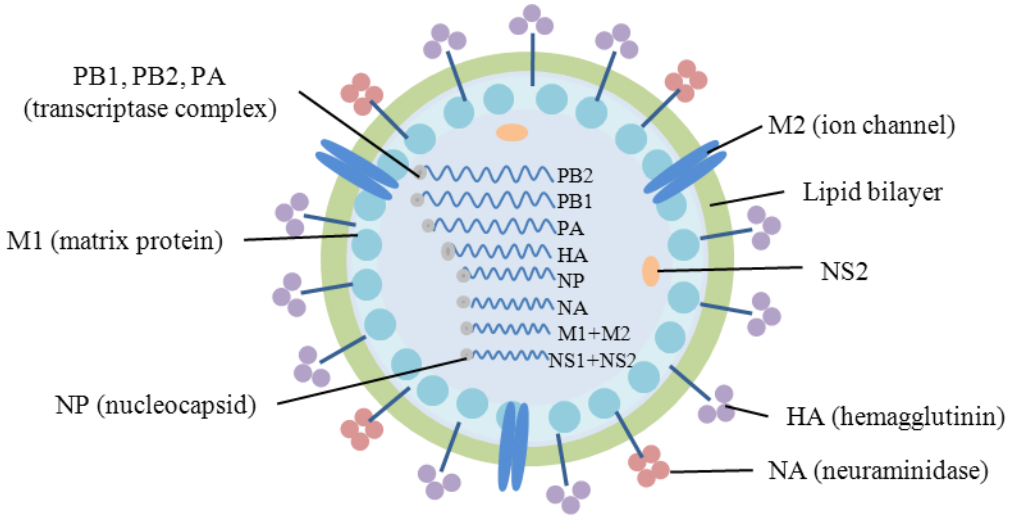

Figure 1. Structure of influenza virion. The influenza virion (as the infectious particle is called) is roughly spherical. It is an enveloped virus – that is, the outer layer is a lipid membrane, which is taken from the host cell, in which the virus multiplies. Inserted into the lipid membrane are 'spikes', which are proteins – actually glycoproteins, because they consist of protein linked to sugars – known as HA (hemagglutinin) and NA (neuraminidase). These are the proteins that determine the subtype of influenza virus (A/H1N1, for example). The HA and NA are important in the immune response against the virus; antibodies against these spikes may protect against infection. M2 protein is anchored in the lipid membrane. Beneath the lipid membrane is a viral protein called M1, or matrix protein. This protein, which forms a shell, gives strength and rigidity to the lipid envelope. Within the interior of the virion are the viral RNAs – 8 of them for influenza A viruses. These are the genetic material of the virus; they code one or two proteins. Each RNA segment, as they are called, consists of RNA joined with several proteins shown in the diagram: B1, PB2, PA, NP. These RNA segments are the genes of influenza virus. The interior of the virion also contains another protein called NS2.

The enveloped influenza A virions have three membrane proteins: hemagglutinin (HA), neuraminidase (NA) and ion channel (M2). There is a matrix protein (M1) just below the lipid bilayer, a ribonucleoprotein core (consisting of 8 viral RNA segments and three proteins: PA, PB1, PB2), and the NEP/NS2 protein [2]. Influenza B virions have four proteins in the envelope: HA, NA, NB, and BM2. Like the M2 protein of influenza A virus, the BM2 protein is a proton channel that is essential for the uncoating process. The NB protein is believed to be an ion channel, but it is not required for viral replication in cells [3]. The enveloped virions of influenza C viruses have hexagonal structures on the surface and form long (500 μm) cordlike structures as they bud from the cell. Like the influenza A and B viruses, the core of influenza C viruses consists of a ribonucleoprotein made up of viral RNA

and four proteins. The M1 protein lies just below the membrane, as in influenza A and B virions. A minor viral envelope protein is CM2, which functions as an ion channel. The major influenza C virus envelope glycoprotein is called HEF (hemagglutinin-esterase-fusion) because it has the functions of both the HA and the NA. Therefore the influenza C virion contains 7 RNA segments, not 8 RNAs like influenza A and B viruses [4,5].

Wild aquatic birds are the natural hosts for a large variety of influenza A. Occasionally, viruses are transmitted to other species and may then cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics. It is not surprising that the type A viruses are the most virulent human pathogens among the three influenza types and cause the most severe disease. The influenza A virus can be subdivided into different serotypes based on the antibody response to these viruses. The serotypes that have been confirmed in humans, ordered by the number of known human pandemic deaths, are [6]:

- H1N1, which caused Spanish Flu in 1918, and Swine Flu in 2009
- H2N2, which caused Asian Flu in 1957
- H3N2, which caused Hong Kong Flu in 1968
- H5N1, which caused Bird Flu in 2004
- H7N7, which has unusual zoonotic potential
- H1N2, endemic in humans, pigs and birds
- H9N2
- H7N2
- H7N3
- H10N7

The type B virus only circulates among human beings, and it can make people ill in a geographically limited area, often, when the type A influenza is subsiding. Type B influenza breaks out less frequently than influenza A, so when people fall ill with influenza B, it is often more serious because only a few will have developed antibodies. Influenza C is less common than the other types and usually only causes mild disease (in children and old and altered immunity people) [6]. Nearly all adults have been infected with influenza C virus, which causes mild upper respiratory tract illness. Lower respiratory tract complications are rare. There is no vaccine against influenza C virus.

Antigenic equipment of influenza viruses can be easily and quickly changed. These changes are probably responsible for the degree of virulence. Influenza viruses have on their surface two types of glycoprotein antigens: *hemagglutinin* (responsible for the ability of the virus entering into the host cell and replication) and *neuraminidase* (which is used by the release of newly formed virus particles from host cells) [7]. HA is a trimeric glycoprotein expressed on the influenza virus membrane [8]. HA of influenza viruses binds to host cell surface complex glycans via a terminal sialic acid (Sia) with α2-3

and α2-6 linkages, and this is the first key step in the process of infection [9-13]. NA acts as the receptor destroying enzyme in virus release [14]. The process is shown in Fig. 2.

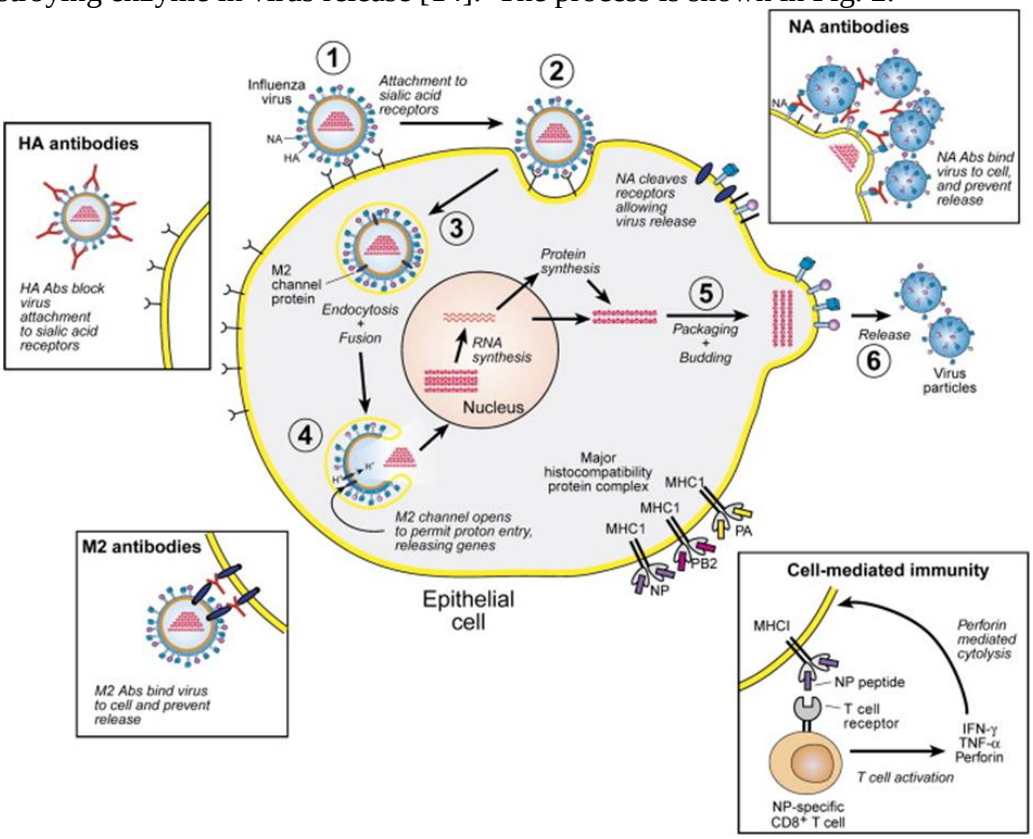

Figure 2. Life Cycle of Influenza Virus and Role of the Adaptive Immune Response during Infection (adopted from [15]. Influenza virus attaches to the epithelial cell surface through binding of the viral hemagglutinin (HA) protein to cell surface sialic acid receptors (1, 2). The virion is internalized through endocytosis and fusion (3). Opening of the M2 channel allows proton flow across the viral membrane (4), triggering fusion of viral and endosomal membranes and release of viral genes into the cytoplasm, from where they travel to the nucleus. Viral proteins produced in cytoplasm assemble with viral genes and bud from the cell membrane as progeny virions (5). Release of new virus particles (6) requires the viral neuraminidase (NA) protein, which cleaves sialic acid receptors from the cell membrane. Antibodies (Abs) to the HA protein block virus attachment (inset, upper left), thereby decreasing the number of cells infected. They can also function to prevent fusion (4). Abs to the NA protein (inset, upper right) bind virus to the cell, preventing release of new virions. Abs to theM2 protein bind virus to the cell and prevent release of viral particles into the extracellular fluid (inset, lower left). Cell-mediated immunity contributes to resistance when CD8+ T cells specific for viral proteins such as nucleoprotein (NP) or polymerase proteins (PB2 and PA recognize viral peptides presented by MHC class I proteins, resulting in the release of cytokines with antiviral activity (IFN-g and TNF-a) and perforins that mediate cytolysis of the infected cell (inset, lower right). Lysis of the infected cell decreases the amount of virus released by the cell. The latter three mechanisms, NA Abs, M2 Abs, and CD8+ T cells, operate after a cell becomes infected. Only HA Abs prevent infection; this is likely to be why they are the most effective *in vivo*. Reprinted with the permission.

Subtypes of influenza viruses are classified just according to NA and HA forms. 16 subtypes of HAs with differences in their primary sequences have been identified [16]. Among these 16-subtypes, only three HA-subtypes H1N1, H2N2, and H3N2, have successfully adapted to humans [17]. 9 subtypes of NA surface antigens were described. Only 103 of all 144 possible HA × NA combinations have been found yet [18].

Possible rapid spread of potential new pandemic subtype of influenza viruses is the great threat. Global civilization, the whole world airplane transport and other ways of global transport create the suitable conditions for extremely rapid spreading of potential pandemic. Simple instrumentation, quick and low cost detection of influenza viruses is of great interest in this case, because such method could reveal the threat before its spreading. However, most frequently used methods for the detection of viruses are laborious, time consuming, expensive, need specialized facility and trained staff. Cultivation of viruses in cell culture, immunofluorescence, polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) and serological methods belong to these methods [19-21].

Cultivation of viruses in cell culture is considered as the gold standard for virus detection. Conventional classic laboratory diagnosis of influenza is based on virus isolation and serologic testing. Primary rhesus monkey (PMK) or Madin-Darby canine kidney (MDCK) cells are mostly used for the isolation of viruses. Cell cultures are examined for cytopathic effect and screened by hemadsorption with confirmation by immunofluorescent monoclonal antibody against influenza A or B. This method is laborious and slow, usually takes 5-7 days [22,23]. Some laboratories replace conventional culture with shell-vial culture. Shell-vial assays that use R-mix cells, a combination of mink lung cells and human adenocarcinoma cells (strains Mv1Lu and A549, respectively), coupled with immunofluorescent staining demonstrates faster results and sensitivity that approaches based on conventional virus culture. Turn around time for shell-vial assay using R-mix cells is 1.4 days, compared to a turn around time of 5.2 days for conventional virus culture [24]. For some viruses, which have not cytopathic effect, this method is not appropriate. Some viruses are difficult to cultivate or uncultivable in cell culture. In these cases, cultivation in chick embryos or in experimental animals is used.

Besides cultivation assay, immunofluorescence of clinical specimens using monoclonal antibodies against influenza virus antigen is a reliable and relatively rapid technique for the detection of influenza [25]. Influenza A virus may also be detected rapidly in nasal secretions by ELISA [26]. The rapid detection of both types of influenza infections would allow appropriate antiviral therapy and is particularly important, since agents active against both influenza A and B are now available [27]. At present, six rapid diagnostic tests for influenza are used [24]. All tests, besides one, are based on directed influenza virus antigens determination. ZstatFlu test (Zyme Tx, Inc., Oklahoma City, OK, USA) detects influenza virus due to NA enzyme activity. In contrast to cell culture, rapid diagnostic tests do not detect influenza B as efficiently as influenza A virus [24]. *Boon et al.* applied mouse monoclonal antibody or anti-influenza nucleoprotein MoAb for detection of the virus [28]. In another

work *Chen et al.* detected avian H5N1 influenza virus using five monoclonal antibodies 8H5, 8G9, 13D4, 2F2, and 3G4 raised against H5N1 virus strains Chicken/HK/YU22/2002 [29].

PCR, as another method used for virus detection, is very sensitive to target molecule, but it is also very sensitive to contamination of the template nucleic acid and give sometimes false positive result. The most commonly used is reverse transcription-polymerase chain reaction (RT-PCR) [30,31], RT-PCR with detection by ELISA [32], real-time reverse transcription-polymerase chain reaction (RRT-PCR) [33,34], and nucleic acid sequence-based amplification (NASBA) [35,36]. RT-PCR, ELISA RT-PCR, and RRT-PCR are very similar to each other. Difference lies in PCR product detection. RT-PCR detects the PCR product by using electrophoresis in an agarose gel to separate the PCR product by size, which allows a presumptive identification. As a more specific alternative, ELISA can be used instead of agarose gel electrophoresis. RRT-PCR test uses a fluorescently labelled probe to detect the increase in PCR product while the test is being performed: i.e., the results are reported in real time [37]. The other test used for avian influenza is NASBA, which directly amplifies and detects RNA [35,36]. All these methods can provide fast and sensitive diagnostic results, and many published tests using different primers and probes have been reported [38-40]. *Pipper et al.* reported new model of microfluidic platform that can detect highly pathogenic avian influenza virus H5N1 in a throat swab sample by using magnetic forces to manipulate a free droplet containing superparamagnetic particles. In a sequential process, the viral RNA was isolated, purified, preconcentrated by 50,000% and subjected to ultrafast real-time RT-PCR [41]. Compared to commercially available tests, the bioassay is equally sensitive and is 440 % faster. Serological methods of influenza infection is based on demonstration of a four-fold or greater rise in specific antibody titre between acute and convalescent serum samples, measured by hemagglutination inhibition, complement fixation or neutralization tests [24,42]. Serological methods require seroconversion and therefore are not able to detect acute infection.

The most important prerequisite in the fight against influenza virus and possible pandemic is early isolation and detection of the viral nucleic acid presence. Broadening of the range of applicable methods for influenza detection has a great importance. It is necessary to change the conditions and properties of detection (requirements of easy-to-use and well portable instrumentation, rapidity of test and low cost). In our review we focus on sensors and biosensors for influenza virus detection, which can be meet the above mentioned criteria. Sensors and biosensors connected with electrochemical detection seem to be the best way for influenza virus detection. Therefore, these are mainly discussed.

2. SENSORS

The sensor is an essential part of the measuring device that converts the input signal to the quantity suitable for the measurement and interpretation. Electrochemical sensors can be divided into sensors with liquid electrolyte or solid electrolyte [43]. Electrochemical sensors can operate in

potentiometric or amperometric way. The interface between the electrode and the analysed environment here serves as a sensitive layer. The working electrode is actual physical transducer. A good sensor obeys the following rules: good specifity and sensitivity, insensitivity to any other property likely to be encountered in its application, and does not influence the measured quantity [44,45].

2.1 Mercury electrodes

The most common applied sensors for influenza virus detection are different types of electrodes. Electrochemical determination of different characteristic oligonucleotides (ODNs) is most common way of detection [46-49]. First electrochemical method called oscillographic polarography used for detection of DNA was suggested by Jaroslav Heyrovsky in 1941, but commercially available instruments became available in the first half of the 1950's [50,51]. Usually derivative curves were recorded, such as dE/dt against E. Both redox and adsorption/desorption phenomena were reflected by this method. The method was simple and fast, possessing advantages of its cyclic mode, later appreciated in cyclic voltammetry. In spite of the fact that Berg claimed DNA is not electroactive [52], Palecek showed that it was wrong presumption caused by using inappropriate electrochemical method [51], because these compounds are electroactive as it was shown by oscillographic polarography of DNA measured by Palecek [53,54]. Since than, there have been done great progress and development in electrochemistry of nucleic acids at various electrodes [55]. In the case of using of mercury electrode as a working one, the attention has been aiming at various electrochemical methods including linear sweep and cyclic polarography/voltammetry [56] (elimination polarography/voltammetry [57,58]), differential pulse polarography/voltammetry [59], square wave polarography/voltammetry [60,61], AC polarography/voltammetry [62] and chronopotentiometry [63,64] for analysis of DNA. Coupling of adsorptive transfer stripping technique (AdTS) to the above-mentioned methods is very promising for nucleic acid studying and is discussed [43]. In addition, coupling of separation (paramagnetic micro and nano particles) and detection tools (mercury electrode) brings numerous advantages including simplicity, easy-to-use and sensitivity [55,65-72].

2.2 Amalgam electrodes

It was reviewed few times that amalgam electrodes (AE) can successfully substitute mercury electrodes [73-75]. Amalgam electrodes coupled with AdTS were successfully used for investigation of DNA, various DNA bases, and oligonucleotides (ODNs), making an easy detection of picogram amounts of the modified DNA or ODN [76-78]. DNA possesses intrinsic electrochemical activity due to the presence of electrochemically reducible or oxidizable nucleobases and exhibits characteristic, structure-sensitive adsorption/desorption behaviour at amalgam electrodes. Thus, label-free electrochemical DNA sensing is in principle possible [66].

2.3 Carbon electrodes

Heterogeneous carbon electrodes are electrochemical sensors containing carbon as an electrically conductive material, which is placed in the matrix (binder). Binders can be liquid (carbon paste electrodes) or fixed (printed carbon electrode). The advantage of these electrodes is the possibility of modifying by chemical or biological agents to a mixture of carbon and a binder to enhance the selectivity and specificity of the assay [79]. Other advantages of carbon electrodes are wide range of working potentials (depending on the type of carbon from the environment and - 1.7 V to + 1.2 V), low background current and low cost [80,81].

Concerning carbon paste electrodes (CPE), they are composed from carbon powder and liquid binder in an appropriate ratio. Paste is filled into the suitable electrode housing. Pastes mixtures contain highly conductive graphite and electrically non-conductive, chemically inert and water insoluble liquid (paraffin, mineral and silicone oils). Stability of these electrodes is several weeks [81-83].

Carbon nanotubes (CNT) that become one of the most extensively studied nanostructures because of their unique properties belong to another popular materials used for electrodes fabrication. There are exist two variants – singlewalled (SWCNT) and multiwalled (MWCNT) carbon nanotubes. Both types offered a porous structure with a large effective surface area, highly electrocatalytic activities and conductivity. CNT can enhance the electrochemical reactivity of important biomolecules. The remarkable sensitivity of CNT enables us to use them as highly sensitive nanoscale sensors. These properties make CNT extremely attractive for a wide range of electrochemical sensors ranging from amperometric enzyme electrodes to DNA hybridization biosensors, because these electrodes have been successfully modified by nucleic acids and other types of biomolecules [84]. Noncovalent interaction along the CNTs sidewalls via physical adsorption or entrapment and covalent binding via carboxylate chemistry or nonselective attack of nanotube sidewalls by highly reactive species give an overview of the functionalized CNTs methodologies for DNA, antigen-antibody, cells, and other molecules sensing [85]. For DNA determination various ways are possible to be used. One of them is determination of DNA due to oxidation of guanine or adenine residues of ssDNA [86].

2.4 Screen printed electrodes

This type of electrodes is prepared by screen printing technique (Screen Printed Electrodes). If the used material is carbon, then this is Screen Printed Carbon Electrodes (SPCE). However, name Thick-Film Electrodes (TFE) better characterizes the dimensions of the electrodes as tens micrometers film. The polymer paste containing carbon nanoparticles is printed through the patterned screen on the ceramic or plastic carrier (substrate). After curing binder (solvent evaporation at room temperature or higher, typically 60-120 °C, or using UV-radiation), the electrode is ready for use. The whole process

can be repeated several times. In the next phase more electrodes including reference and auxiliary can be applied [87,88].

In general, detection of viral ODNs on screen printed electrode (SPE) is connected with hybridization reaction [65,89-91]. Two ways of influenza virus detection using SPE is applied as i) direct using of SPE and ii) using of SPE with modified surface. First way is connected with the usage of SPCE. In this case, various carbon structures as nanowires and nanotubes (singlewalled, multiwalled) with different dimensions are employed. Comparison of determination of viral nucleic acid by SPCE to carbon paste and mercury electrodes was discussed [65].

The second way as utilization of modified surfaces of SPE for viral nucleic acid detection is more frequent. Mostly use surfaces are based on carbon, which is modified with various particles as chitosan/Fe_3O_4 nanoparticles and gold nanoparticles. The most attractive feature of chitosan/Fe_3O_4 nanoparticles modified electrodes is a suitable microenvironment (Fe_3O_4 nanoparticles), which could contribute to electron transfer and thus sensitivity enhancement when using methylene blue (MB) as an external mediator and square wave voltammetry (SWV) as determination method [89]. Such modified SPE had a low detection limit (as low as 50 pM), acceptable stability and good reproducibility. On the other hand, gold nanoparticles, which are formed *in situ* by applying a constant current intensity during a fixed time, acts as an immobilization and transduction surface. Immobilization takes place through thiol-gold interaction in a relatively fast way, and the genosensor response is found to be linearly to the biotinylated viral nucleic acid concentration between 2.5 and 50 pM. Detection limit of 2.5 pM was estimated [90].

3. BIOSENSORS

A typical biosensor is constructed from three main parts (Fig. 3) as a recognition element (enzyme, antibody, DNA, etc.), a signal transducing structure (electrical, optical, or thermal), and an amplification/processing element [92].

The method of transduction depends on the type of physicochemical change resulting from the sensing event. Often, an important part of a biosensor is a membrane that covers the biological sensing element and has the main functions of selective permeation and diffusion control of analyte, protection against mechanical stresses, and support for the biological element [93,94]. The most commonly used sensing elements are enzymes [84,95,96], antibodies [85,97] and oligonucleotides [85,98,99]. Common used electrochemical transducers are potentiometric [100,101], amperometric [102] and conductometric [103,104]. In some applications piezoelectric transducers are used [105-108]. Short overview of biological elements and transducers commonly used in the fabrication of biosensors are shown in Table 1.

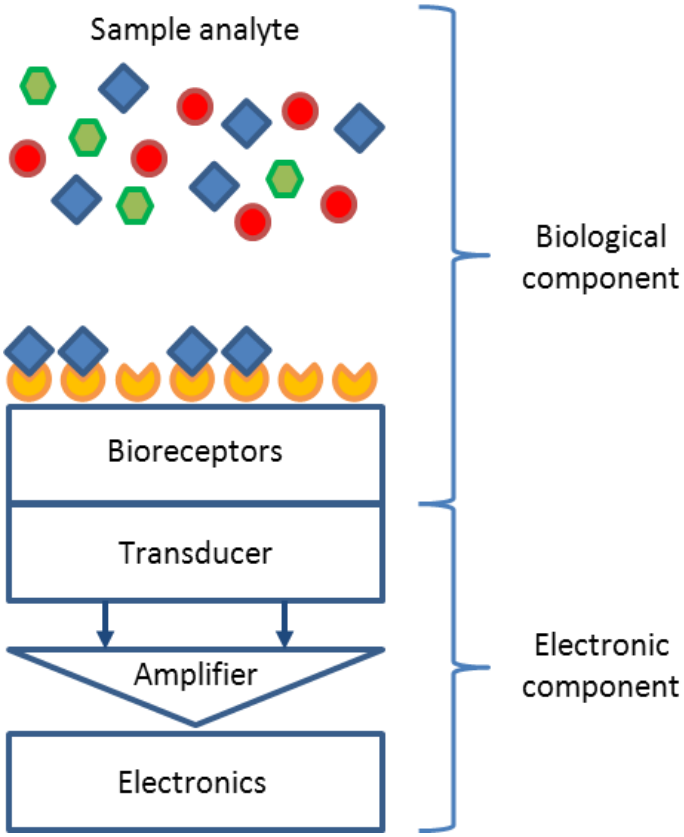

Figure 3. General structure of biosensor. Transducers are made as an electrochemical (potentiometry, amperometry) or optical (adsorption, fluorescence) parts. There are immobilised enzymes, microorganisms, DNA, antibodies and so one as bioreceptors applied.

Table 1. Biological elements and transducers. Adapted from [109].

Biological elements	Transducers
Enzymes	Electrochemical
Antibodies	Amperometric
Receptors	Potentiometric
Cells	Conductometric
Membranes	Optical
Tissues	Fibre optic
Organisms	Surface plasmon resonance
Organelles	Calorimetric
Nucleic acids	Heat conduction
Organic molecules	Isothermal
	Acoustic
	Surface acoustic wave
	Piezocrystal microbalance

3.1 Hybridization of Nucleic Acids on Working Electrodes

The surface-immobilization of ssODN probe on the electrode is a key step to fabricate the electrochemical oligonucleotide biosensor. It is not surprising that various electrodes have been modified and tested for DNA biosensing. SWCNTs array electrode was fabricated as a DNA hybridization biosensor based on the direct current response of guanine [110]. This biosensor gave, under optimum conditions, the response proportional to the concentration of target DNA within the range from 40 to 110 nM with a detection limit of 20 nM. Application of enzyme labels is another way of DNA detection. Heller's group applied this way and demonstrated that a highly sensitive amperometric monitoring of DNA hybridization (down to 5 zmol) could be achieved in connection with an horseradish peroxidase (HRP)-labelled target [111]. The enzyme labels to generate electrical signals are also extremely useful for ultrasensitive electrochemical bioaffinity assay of DNA as it is shown by Wang's group, which used CNTs for amplifying alkaline phosphatase (ALP) enzyme-based bioaffinity electrical sensing of DNA with a low detection limit of app. 1 fg/ml [112].

Avidin-biotin conjugation was also employed for influenza virus (type A) detection [113]. An electrochemical DNA biosensor was fabricated by avidin-biotin conjugation of a biotinylated probe DNA (5′-biotin-ATG AGT CTT CTA ACC GAG GTC GAA-3′) and an avidin-modified glassy carbon electrode (GCE) to detect the influenza virus (type A). An avidin-modified GCE was prepared by the reaction of avidin and a carboxylic acid-modified GCE, which was synthesized by the electrochemical reduction of 4-carboxyphenyl diazonium salt. The current value of the electrochemical DNA biosensor was evaluated after hybridization of the probe DNA (5′-biotin-ATG AGT CTT CTA ACC GAG GTC GAA-3′) and target DNA (5′-TTC GAC CTC GGT TAG AAG ACT CAT-3′) using cyclic voltammetry (Fig. 4). The current value decreased after the hybridization of the probe DNA and target DNA [113].

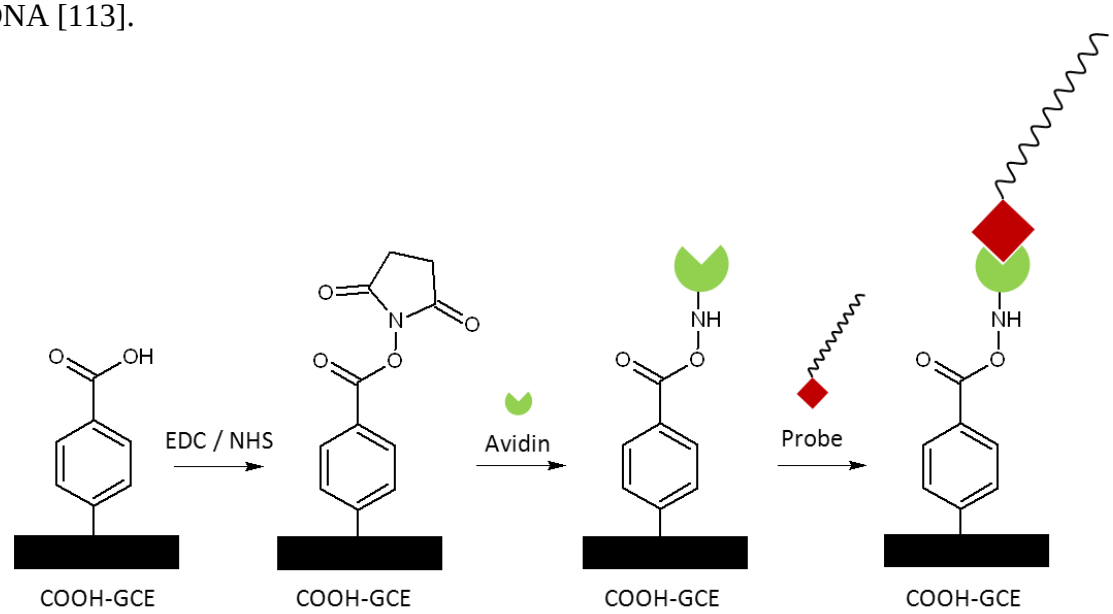

Figure 4. Scheme of immobilization of probe DNA on the surface of the COOH-modified GCE. (adopted from [113]).

In addition, biosensor that relies on the adsorption immobilization of the 18-mer single-stranded nucleic acid related to dengue virus gene 1 on activated pencil graphite was developed [100]. Hybridization between the probe and its complementary oligonucleotides (the target) was investigated by monitoring guanine oxidation by DPV. The electrochemical detection of annealing between the DNA probe immobilized on the modified electrode and the target was achieved. The target can be quantified in a range from 1 to 40 nM with good linearity and a detection limit app. 1 nM.

Determination of influenza viruses by electrochemical immunoassay also belongs to the possible ways of detection [114-116]. Usage of carbon nanotube electric immunoassay for the detection of swine influenza virus H1N1 was performed et al. [114]. The assay was based on the excellent electrical properties of SWCNTs. Antibody-virus complexes influenced the conductance of underlying SWCNT thin film, which has been constructed by facile layer-by-layer self-assembly. Pristine SWCNTs were functionalized to graft hydrophilic carboxylic groups for the stable dispersion to water. Polyelectrolytes used for layer-by-layer assembly as a polycation and polyanion were poly(diallyldimethyammonium chloride and poly(stylenesulfonate). Poly-L-lysine was used to immobilize the anti-virus antibody physically on the surface of SWCNTs. The antibody titre was determined by haemagglutinin inhibition assay [114]. This CNT-based immunoassay also has the potential to be used as a sensing platform for lab-on-chip system (Fig. 5).

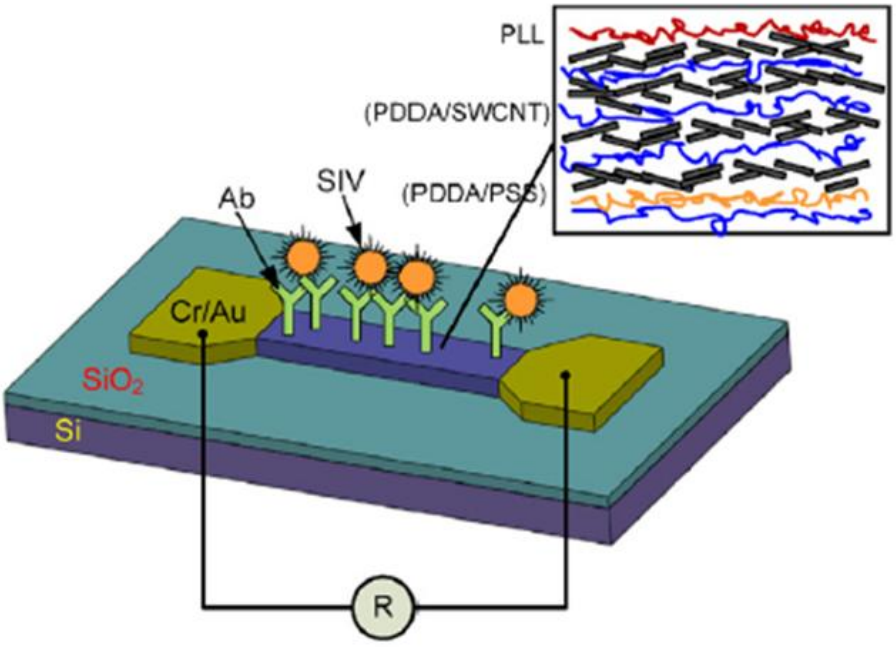

Figure 5. The fabricated carbon nanotube thin film immunochips: schematic of an individual immunochip with close-up hierarchy of SWCNT multilayer (adopted from [114]). Reprinted with the permission.

Gold electrodes provide an ideal material for labelling of ODNs due to their affinity with thiol groups. The Self Assembled Monolayer (SAM) is usually formed using mercaptopropionic acid. Thiolated ssODNs become the basis of wide range of biosensors, which are used for influenza detection too [117]. Immobilization of specific influenza antibodies onto bio-functionalized gold electrode is also possible [118]. Other way represent usage of gold nanowires [119] or planar nanogap electrodes [120]. Modifications of gold electrodes by carbon nanotubes [121], thiol groups [122,123] and enzymes [96] have been also published. The principle of biosensors is mostly putted up on hybridization reaction. Effectiveness of this reaction can be enhanced by usage of peptide nucleic acid (PNA). The enhanced of effectiveness is due to neutral character of PNA probe opposite negatively charged DNA probes [122,124].

Microarray

DNA microarray (DNA chip or gene chip) is based on parallel sample hybridization with specific probes (spots) on a solid surface (glass slide or silicon film). Custom probe specificity is determined by their sequences and by the sequences of anchored oligonucleotides. The basic division is according to the number of probes*:* low-density (the tenth hundreds specific probes) and high-density (thousands millions to specific probes). The low-density microarray is used for analysis of files of biomarkers and oligonucleotides. High-density is used for genomic analysis (sequencing DNA sections) or in RNA expression analysis. Most studies about influenza and microarray is based on detecting the presence [125] or typing and subtyping [7,126-130] or identification [131] of influenza, especially new strands of influenza virus. Procedures are almost based on virus-specific oligonucleotides determination [125,127-129]. The microarray utilizes mostly a panel of primers for multiplex PCR amplification of the HA, NA and MP genes of influenza viruses.

There have not been published numerous papers on the using of electrochemical microarrays for detection of influenza, but there is great potential. There was used the microarray silicon chip for the detection of influenza A virus [131]. The chip has 12 544 electrodes, each with a size of 44 μm in diameter, oligomers of 35-40 bases were synthesized at each electrode. After the *in situ* synthesis of microarray, the oligonucleotide probes on the chip were phosphorylated with T4 polynucleotide kinase for 30 min at 37 °C. The samples were first added to monolayers of Madin-Darby canine kidney cells and incubated for 1 h at 37°C to allow viral adsorption to the cells. The genotyping results showed that the device identified influenza A hemagglutinin and neuraminidase subtypes and sequenced portions of both genes, demonstrating the potential of integrated microfluidic and microarray technology for multiple virus detection. In addition, HRP as a biomarker for DNA hybridization detection was used in combination with the aforementioned electrochemical microarray [132]. The detection was based on electrochemical reduction of the enzymatic oxidization product of 3,3’,5,5’-tetramethylbenzidene (TMB). TMB oxidation was catalysed by HRP biomarker linked to a DNA target molecule (Fig. 6). Each target DNA oligomer was tagged with a biotin molecule and after hybridization the biotin is was with a streptavidin-HRP conjugate. Total RNA isolated from virus-containing material was reverse-

transcribed into cDNA, which was then PCR amplified to produce biotinylated single-strand DNA. Biotinylated-ssDNA was hybridized on 2 arrays containing probe specific sequences unique to Influenza A subtypes HA1 to 16 and NA subtypes 1 to 9 [132].

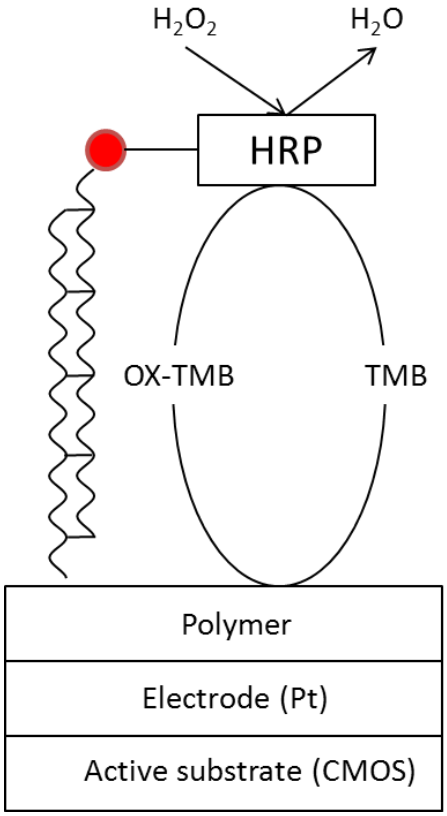

Figure 6. Diagram of the electrode surface after DNA probe hybridization and labelling of biotin with streptavidin-HRP conjugate. TMB is oxidized by HRP in the presence of H2O2 and subsequently reduced at the electrode surface during electrochemical detection (adopted from [132]).

3.2 Quartz Crystal Microbalance

Using quartz crystal microbalance (QCM) as an immuno biosensor for the detection of influenza virus has been carried out [105-108]. All of these procedures are used for detection of specific influenza antibodies and differ only in method of attachment of particles on the gold surface. The basic framework of the experiment is determination of influenza antibodies and then individual differences lie in the procedures for attaching the electrode surface. QCM influenza determination is possible without and/or with gold nanoparticles. The determination without gold nanoparticles was practised on influenza A virus (VR-544, H3N2) detection, which was based on the determination of H3N2 virus antigen and specific binding by polyclonal IgG antibodies against H3N2 on a crystal

surface [107]. On the other hand, determination with gold nanoparticles is carried out due to the increasing the sensitivity of the QCM immunosensor for the influenza viruses detection. This way of determination is applicable in connection with specific protein detection [106,108] and specific ODN-sequence detection [133]. These procedures have been applied on three types of influenza viruses H3N2 [107], H5N1 [105,108] and H1N1 [106].

3.3 Nanoparticles

Advanced materials represent a way how to enhance sensitivity and selectivity of electrochemical detection of nucleic acids. The materials can be divided into metal particles and quantum dots (QDs). Application of combination of MWCNTs and gold nanoparticles opened new possibility in nucleic acids detection by electrochemistry [115,116]. Various ways of determination are presented and differ from nanoparticle material and detected target. Two types of nanoparticles material are commonly used, gold [123,134-137] and iron [138]. Gold nanoparticles have been functionalized with virus specific antibodies or oligonucleotides. In each of these constructs, AuNPs act as both an easily conjugated scaffolding system for biological molecules and a powerful fluorescence quencher. Gold nanoparticles can be also immobilized and used as electrochemical transducers. Moreover, gold-coated magnetic beads were employed as the platforms for the immobilization and immunoreaction process, and horseradish peroxidase was chosen as an enzymatic tracer. The proteins (antibodies or immunocomplexes) attached on the surface of magnetic beads were found to induce a significant decline in their electric conductivity [115]. Modification of carbon electrodes with oxide nanoparticles (ZnO and SnO_2) was also tested for electrochemical monitoring of nucleic acid hybridization [139,140]. Kamikawa et al. dealt with the determination of influenza at gamma iron(III) oxide nanoparticles [138]. This way of determination was based on HA detection. Electrically active magnetic nanoparticles, consisting of aniline monomer polymerized around gamma iron(III) oxide (γ-Fe_2O_3) cores, served as the basis of a direct-charge transfer biosensor developed for detection of HA from the Influenza A virus. H5N1 preferentially bound alpha 2,3-linked host glycan receptors. Nanoparticles were immunofunctionalized with antibodies against target HA.

Usage of QDs for influenza detection in combination with electrochemical determination is rare. Determination of influenza virus made through combination of magnetic particles (MPs) and QDs with targeted DNA is presented by Krejcova et al. [141]. This way of detection was based on hybridisation reaction between anti-H5N1 and H5N1 ODNs chain, which was labelled with quantum dot. Differential pulse voltammetry was used for detection of cadmium(II) ions and square wave voltammetry for detection of cytosine-adenine peak in ODN-SH-CdS complex [141]. Similar approach was done in the study with influenza virus determination by using of paramagnetic particles modified with glycan, which can selectively bind to specific viral A/H5N1/Vietnam/1203/2004 protein labelled quantum dots [142]. Optimized detection of cadmium sulphide quantum dots (CdS QDs)-protein

complexes connected to paramagnetic microbeads was performed using differential pulse voltammetry on the surface of HMDE and/or GCE [142].

There was CdSe nanostructures applied for biosensor construction [143]. Based on these CdSe nanostructures, ssDNA/CdSe/GCE electrochemical DNA sensor was constructed. A novel method for detection the DNA sequences of avian influenza virus was presented (Probe DNA: 5′-GGA ATG GTA GAT GGA TGG TAT-3′; Target DNA: 5′-ATA CCA TCC ATC TAC CAT TCC-3′). This method was based on the change of electrochemical signal with the different combining ability of ssDNA and dsDNA with CdSe nanostructures. Methylene blue (MB) was used as the hybridization indicator and the response signal of MB was measured using differential pulse voltammetry after the working electrode was immersed in B–R buffer (pH 7.0). After DNA modification on the CdSe hollow spheres/GCE it was worth to note that the peak current of ssDNA/CdSe/GCE has dramatically decreased. This was due to the formation of the DNA layer on the surface of the CdSe nanostructures that hinds the electron-transfer process between MB molecules and CdSe/GCE surface, indicating that DNA has been successfully loaded on the CdSe/GCE and therefore the peak current was low correspondingly. After hybridization with complementary target DNA sequences, the signal increased obviously. This was because that hybridization would take place after target DNA sequences were immersed into complementary DNA sequences, which caused the target ssDNA sequences to form dsDNA. During the process, the structure of DNA changed dramatically. Before hybridization, the ssDNA can uncoil sufficiently to expose its bases. After hybridization, the dsDNA has a stable double-helix geometry, which makes the bases in the DNA lie inside the double-helix geometry of dsDNA, and negatively charged phosphate backbones be exposed outside. This special structure of dsDNA would greatly weaken the conjugated ability of dsDNA and CdSe. Therefore, after hybridization, the formed dsDNA would release from the CdSe/GCE surface. For the non-complementary DNA sequences, the signal almost unchanged because this DNA could not be hybridized with the probe DNA [143]. Other authors chose the following targets: human papillomavirus [96], mosaic virus [144] and Epstein-Barr virus [145].

3.4 Aptamers

The composition of aptamers is simple, generally has a few dozen nucleotides (less than 100), and thus the design of the aptamers is relatively simple. Due to the advantages of aptamers, their using for designing biosensors for identification and detection of pathogens is of extremely importance [146-148]. Application of aptamers for influenza detection is possible in several ways. First possible way is selection of aptamers against influenza virus hemagglutinin [149]. The best aptamer can be selected by surface plasmon resonance as an efficient methodology for selecting aptamer that has high affinity to HA of human influenza virus. This procedure allowed monitoring and selecting the target-bound aptamers specifically and simultaneously. Second possible way is detection of viral gene sequence using a DNA aptamer immobilized onto a hybrid nanomaterial-modified electrode [121]. The modified

electrode is assembled with MWNT, polypyrrole nanowires and gold nanoparticles (GNPs). This electrode offers a porous structure with a large effective surface area, highly electrocatalytic activities and electronic conductivity. The biosensor is based on the hybridization and preferred orientation of a DNA aptamer immobilized onto a modified electrode surface with its target (H5N1 specific sequence) present in solution [121]. Gopinath et al. reported another aptamer selection process of influenza A virus (H3N2). They used the whole-cell of H3N2 as targets to obtain the aptamers that specifically bound the HA protein on the surface of H3N2. The affinity between screening aptamers and HA proteins is 15-fold higher than the affinity between monoclonal antibodies and HA proteins [150]. Besides detection and determination of influenza virus, aptamers have other great feature, which is a position as a promising candidate for treatment and prophylaxis of influenza virus infections [151]. There were found DNA aptamers that specifically targeted the H5N1 influenza virus and their antiviral activity *in vitro* was also described. These aptamers would be expected to disrupt virus entry, and thus slow the infection process so the host immune system has time to respond. This is important in the absence of new vaccines to prevent the emergence of new viral infections.

3.5 Impedance

Impedance measurement is another way of electrochemical detection of influenza viruses. There are two basic methods: an impedance spectroscopy [152,153] and electrochemical impedance immunosensors [154-156] which are typically constructed using an mSAM base layer [157,158]. Upon hybridisation between the bioreceptor and the viral antigen, there is a measurable response in conductivity across the immunosensor surface, which is translated into a change in the resistance. Hassen et al. reports a quantitation of influenza A virus in samples containing large amounts of extraneous bovine serum albumin (BSA), foetal bovine serum (FBS) and hepatitis B virus (HBV) vaccine. Detection was carried out using electrochemical impedance spectroscopy with an antibody-neutravidin-thiol architecture immobilized on the surface of gold electrode [153]. Another method was based on an oligonucleotide DNA probe, complementary to the target H1N1 virus sequence, which was immobilized onto the electrode surface by covalent binding. Two different protocols (direct hybridization with the DNA target and a sandwich scheme) were employed and compared. In both cases the resulting hybrid was biotin-labelled to allow the additional conjugation with streptavidin gold nanoparticles (strept–AuNPs) [154].

Impedance imunnosensors were employed for the influenza detection due to HA [155] or DNA sequence [154] differentiation. Impedance immunosensor based on an interdigitated array microelectrode with antibodies against HA was developed. Polyclonal antibodies against AI virus H5N1 surface antigen HA were oriented on the gold microelectrode surface through protein A. Target H5N1viruses were then captured by the immobilized antibody, resulting in a change in the impedance of the IDA microelectrode surface [155].

4. CONCLUSION

Nanotechnologies together with various types of electrodes with many material-based advantages open many outstanding possibilities to be used in detection of markers of serious diseases, specific DNA sequences as markers for some viral infections and for suggesting and fabricating of biosensors. Main directions of this type of research can be as follows: a) DNA hybridization-based biosensors; b) drugs interaction; c) DNA protein interaction [159-162]. Sensors and biosensors have witnessed an escalating interest nowadays, both in the research and commercial fields. There are many fields, in which these instruments can be applied including those aimed at discrimination of different organism strains or the presence of viral nucleic acid [163].

Abbreviations:
A - adenine; AdS - adsorptive stripping technique; AC - alternating current voltammetry; AE - amalgam electrode; C - cytosine; CV – cyclic voltammetry; DME – dropping mercury electrode; dsDNA – doublestranded deoxyribonucleic acid; ssDNA – singlestranded deoxyribonucleic acid; DPV - differential pulse voltammetry; EVLS - elimination voltammetry with linear scan; G - guanine; HA – hemagglutinin; HMDE – hanging mercury drop electrode; LSV - linear sweep voltammetry; MNP - magnetic nanoparticle; MME - miniaturized mercury electrode; MWNT - multi-walled carbon nanotubes; NA – neuraminidase; ODN - oligonucleotide; mRNA – mediator ribonucleic acid; SPCPE - screen-printed carbon paste electrode; SWV - square wave voltammetry; T – thymine

ACKNOWLEDGEMENTS
Financial support from NanoBioTECell GA CR P102/11/1068, NANOSEMED GA AV KAN208130801 and CEITEC CZ.1.05/1.1.00/02.0068 is highly acknowledged.

References

1. Y. C. Hsieh, T. Z. Wu, D. P. Liu, P. L. Shao, L. Y. Chang, C. Y. Lu, C. Y. Lee, F. Y. Huang and L. M. Huang, *J. Formos. Med. Assoc.*, 105 (2006) 1.
2. A. W. Hampson and J. S. Mackenzie, *Med. J. Aust.*, 185 (2006) S39.
3. A. Beby-Defaux, G. Giraudeau, S. Bouguermouh and G. Agius, *Med. Mal. Infect.*, 33 (2003) 134.
4. M. R. Hillman, *Vaccine*, 20 (2002) 3068.
5. M. C. Zambon, *J. Antimicrob. Chemother.*, 44 (1999) 3.
6. E. Samorek-Salamonowicz, H. Czekaj and W. Kozdrun, *Med. Weter.*, 62 (2006) 488.
7. J. Q. Zhao, S. X. Tang, J. Storhoff, S. Marla, Y. P. Bao, X. Wang, E. Y. Wong, V. Ragupathy, Z. P. Ye and I. K. Hewlett, *BMC Biotechnol.*, 10 (2010) 1.
8. T. W. Lin, G. Y. Wang, A. Z. Li, Q. Zhang, C. M. Wu, R. F. Zhang, Q. X. Cai, W. J. Song and K. Y. Yuen, *Virology*, 392 (2009) 73.
9. J. J. Skehel and D. C. Wiley, *Ann. Rev. Biochem.*, 69 (2000) 531.
10. R. J. Russell, P. S. Kerry, D. J. Stevens, D. A. Steinhauer, S. R. Martin, S. J. Gamblin and J. J. Skehel, *Proc. Natl. Acad. Sci. U. S. A.*, 105 (2008) 17736.
11. H. Brody, *Nature*, 480 (2011) S1.
12. G. J. Nabel, C. J. Wei and J. E. Ledgerwood, *Nature*, 471 (2011) 157.

13. R. Konig, S. Stertz, Y. Zhou, A. Inoue, H. H. Hoffmann, S. Bhattacharyya, J. G. Alamares, D. M. Tscherne, M. B. Ortigoza, Y. H. Liang, Q. S. Gao, S. E. Andrews, S. Bandyopadhyay, P. De Jesus, B. P. Tu, L. Pache, C. Shih, A. Orth, G. Bonamy, L. Miraglia, T. Ideker, A. Garcia-Sastre, J. A. T. Young, P. Palese, M. L. Shaw and S. K. Chanda, *Nature*, 463 (2010) 813.
14. E. Suenaga, H. Mizuno and K. K. R. Penmetcha, *Biosens. Bioelectron.*, 32 (2012) 195.
15. K. Subbarao, B. R. Murphy and A. S. Fauci, *Immunity*, 24 (2006) 5.
16. R. A. M. Fouchier, V. Munster, A. Wallensten, T. M. Bestebroer, S. Herfst, D. Smith, G. F. Rimmelzwaan, B. Olsen and A. Osterhaus, *J. Virol.*, 79 (2005) 2814.
17. R. J. Garten, C. T. Davis, C. A. Russell, B. Shu, S. Lindstrom, A. Balish, W. M. Sessions, X. Y. Xu, E. Skepner, V. Deyde, M. Okomo-Adhiambo, L. Gubareva, J. Barnes, C. B. Smith, S. L. Emery, M. J. Hillman, P. Rivailler, J. Smagala, M. de Graaf, D. F. Burke, R. A. M. Fouchier, C. Pappas, C. M. Alpuche-Aranda, H. Lopez-Gatell, H. Olivera, I. Lopez, C. A. Myers, D. Faix, P. J. Blair, C. Yu, K. M. Keene, P. D. Dotson, D. Boxrud, A. R. Sambol, S. H. Abid, K. S. George, T. Bannerman, A. L. Moore, D. J. Stringer, P. Blevins, G. J. Demmler-Harrison, M. Ginsberg, P. Kriner, S. Waterman, S. Smole, H. F. Guevara, E. A. Belongia, P. A. Clark, S. T. Beatrice, R. Donis, J. Katz, L. Finelli, C. B. Bridges, M. Shaw, D. B. Jernigan, T. M. Uyeki, D. J. Smith, A. I. Klimov and N. J. Cox, *Science*, 325 (2009) 197.
18. E. F. Kaleta, G. Hergarten and A. Yilmaz, *Dtsch. Tierarztl. Wochenschr.*, 112 (2005) 448.
19. R. L. Caygill, G. E. Blair and P. A. Millner, *Anal. Chim. Acta*, 681 (2010) 8.
20. M. L. Landry, *Curr. Opin. Pediatr.*, 23 (2011) 91.
21. G. A. Storch, *Curr. Opin. Pediatr.*, 15 (2003) 77.
22. A. Z. Hu, M. Colella, J. S. Tam, R. Rappaport and S. M. Cheng, *J. Clin. Microbiol.*, 41 (2003) 149.
23. M. Gueudin, A. Vabret, J. Petitjean, S. Gouarin, J. Brouard and F. Freymuth, *J. Virol. Methods*, 109 (2003) 39.
24. J. P. Gavin and B. R. Thomson, *Clin. Appl. Immun. Rev.*, 4 (2003) 151.
25. C. G. Ray and L. L. Minnich, *J. Clin. Microbiol.*, 25 (1987) 355.
26. S. Davison, A. F. Ziegler and R. J. Eckroade, *Avian Dis.*, 42 (1998) 791.
27. D. E. Noyola, B. Clark, F. T. O'Donnell, R. L. Atmar, J. Greer and G. J. Demmler, *J. Clin. Microbiol.*, 38 (2000) 1161.
28. A. C. M. Boon, A. M. F. French, D. M. Fleming and M. C. Zambon, *J. Med. Virol.*, 65 (2001) 163.
29. Y. W. Chen, W. X. Luo, H. J. Song, B. Y. Yin, J. X. Tang, Y. X. Chen, M. H. Ng, A. E. T. Yeo, J. Zhang and N. S. Xia, *PLoS One*, 6 (2011) 1.
30. G. Cattoli, A. Drago, S. Maniero, A. Toffan, E. Bertoli, S. Fassina, C. Terregino, C. Robbi, G. Vicenzoni and I. Capua, *Avian Pathol.*, 33 (2004) 432.
31. E. Starick, A. Romer-Oberdorfer and O. Werner, *J. Vet. Med. Ser. B-Infect. Dis. Vet. Public Health*, 47 (2000) 295.
32. K. Dybkaer, M. Munch, K. J. Handberg and P. H. Jorgensen, *J. Vet. Diagn. Invest.*, 16 (2004) 51.
33. L. Di Trani, B. Bedini, I. Donatelli, L. Campitelli, B. Chiappini, M. A. De Marco, M. Delogu, C. Buonavoglia and G. Vaccari, *BMC Infect. Dis.*, 6 (2006) 1.
34. E. Spackman, D. A. Senne, T. J. Myers, L. L. Bulaga, L. P. Garber, M. L. Perdue, K. Lohman, L. T. Daum and D. L. Suarez, *J. Clin. Microbiol.*, 40 (2002) 3256.
35. R. A. Collins, L. S. Ko, K. Y. Fung, K. Y. Chan, J. Xing, L. T. Lau and A. C. H. Yu, *Biochem. Biophys. Res. Commun.*, 300 (2003) 507.
36. R. A. Collins, L. S. Ko, K. L. So, T. Ellis, L. T. Lau and A. C. H. Yu, *J. Virol. Methods*, 103 (2002) 213.
37. D. L. Suarez, A. Das and E. Ellis, *Avian Dis.*, 51 (2007) 201.
38. G. Vernet, *Expert Rev. Anti-Infect. Ther.*, 5 (2007) 89.

39. P. Neuzil, J. Pipper and T. M. Hsieh, *Mol. Biosyst.*, 2 (2006) 292.
40. P. Neuzil, C. Y. Zhang, J. Pipper, S. Oh and L. Zhuo, *Nucleic Acids Res.*, 34 (2006) 9.
41. J. Pipper, M. Inoue, L. F. P. Ng, P. Neuzil, Y. Zhang and L. Novak, *Nat. Med.*, 13 (2007) 1259.
42. N. J. Cox and K. Subbarao, *Lancet*, 354 (1999) 1277.
43. E. Palecek and M. Bartosik, *Chem. Rev.*, 112 (2012) 3427.
44. A. J. S. Ahammad, J. J. Lee and M. A. Rahman, *Sensors*, 9 (2009) 2289.
45. F. Wang and S. S. Hu, *Microchim. Acta*, 165 (2009) 1.
46. J. Wang, *Anal. Chim. Acta*, 469 (2002) 63.
47. M. Pumera, Nanoparticles and quantum dots as biomolecule labels for electrochemical biosensing, Nova Science Publishers, Inc, Hauppauge, 2009.
48. S. O. Kelley, E. M. Boon, J. K. Barton, N. M. Jackson and M. G. Hill, *Nucleic Acids Res.*, 27 (1999) 4830.
49. J. M. Gibbs, S. J. Park, D. R. Anderson, K. J. Watson, C. A. Mirkin and S. T. Nguyen, *J. Am. Chem. Soc.*, 127 (2005) 1170.
50. E. Palecek, *Electroanalysis*, 8 (1996) 7.
51. E. Palecek, *Talanta*, 56 (2002) 809.
52. H. Berg, *Biochem. Z.*, 329 (1957) 274.
53. E. Palecek, *Naturwissenschaften*, 45 (1958) 186.
54. E. Palecek, *Nature*, 188 (1960) 656.
55. D. Hynek, J. Prasek, P. Koudelka, J. Chomoucka, L. Trnkova, V. Adam, J. Hubalek and R. Kizek, *Curr. Phys. Chem.*, 1 (2011) 299.
56. A. Erdem and M. Ozsoz, *Electroanalysis*, 14 (2002) 965.
57. L. Trnkova, in V. Adam, R. Kizek (Editors), Utilizing of Electrochemical and Bioanalytical Methods in Biological Research, Research Signpost, Kerala, 2007, p. 51.
58. L. Trnkova, F. Jelen, J. Petrlova, V. Adam, D. Potesil and R. Kizek, *Sensors*, 5 (2005) 448.
59. M. Vorlickova and E. Palecek, *Int. J. Radiat. Biol.*, 26 (1974) 363.
60. D. Huska, J. Hubalek, V. Adam and R. Kizek, *Electrophoresis*, 29 (2008) 4964.
61. F. Jelen, M. Tomschik and E. Palecek, *J. Electroanal. Chem.*, 423 (1997) 141.
62. M. Fojta, L. Havran, J. Fulneckova and T. Kubicarova, *Electroanalysis*, 12 (2000) 926.
63. E. Palecek, M. Masarik, R. Kizek, D. Kuhlmeier, J. Hassmann and J. Schulein, *Anal. Chem.*, 76 (2004) 5930.
64. J. Wang, Analytical electrochemistry, Wiley-VCH, New York, 2000.
65. V. Adam, D. Huska, J. Hubalek and R. Kizek, *Microfluid. Nanofluid.*, 8 (2010) 329.
66. M. Fojta, L. Havran, P. Horakova, P. Kostecka, H. Pivonkova, E. Simkova, J. Spacek, Z. Vychodilova and P. Vidlakova, Electrochemical Analysis of DNA: When, How and Why to Use Electroactive Labels and Indicators, Lenka Srsenova-Best Servis, Usti Nad Labem, 2010.
67. L. Havran, P. Horakova, H. Pivonkova, M. Vrabel, H. Macickova-Cahova, J. Riedl, M. Hocek and M. Fojta, What is new in labeling and functionalization of DNA for electrochemical analysis, 2010.
68. J. Vacek, L. Havran and M. Fojta, *Chem. Listy*, 105 (2011) 15.
69. P. Vidlakova, P. Horakova, L. Tesnohlidkova, L. Havran, H. Pivonkova and M. Fojta, Monitoring DNA modification by platinum complexes at mercury electrodes, 2010.
70. F. Jelen, A. Kourilova, P. Pecinka and E. Palecek, *Bioelectrochemistry*, 63 (2004) 249.
71. E. Palecek, M. Fojta and F. Jelen, *Bioelectrochemistry*, 56 (2002) 85.
72. E. Palecek and M. Fojta, *Talanta*, 74 (2007) 276.
73. B. Yosypchuk and J. Barek, *Crit. Rev. Anal. Chem.*, 39 (2009) 189.
74. B. Yosypchuk and L. Novotny, *Electroanalysis*, 14 (2002) 1733.
75. B. Yosypchuk and L. Novotny, *Crit. Rev. Anal. Chem.*, 32 (2002) 141.

76. R. Fadrna, K. Cahova-Kucharikova, L. Havran, B. Yosypchuk and M. Fojta, *Electroanalysis*, 17 (2005) 452.
77. K. Kucharikova, L. Novotny, B. Yosypchuk and M. Fojta, *Electroanalysis*, 16 (2004) 410.
78. B. Yosypchuk, M. Fojta, L. Havran, M. Heyrovsky and E. Palecek, *Electroanalysis*, 18 (2006) 186.
79. C. B. Jacobs, M. J. Peairs and B. J. Venton, *Anal. Chim. Acta*, 662 (2010) 105.
80. I. Svancara, A. Walcarius, K. Kalcher and K. Vytras, *Cent. Eur. J. Chem*, 7 (2009) 598.
81. K. Kalcher, I. Svancara, M. Buzuk, K. Vytras and A. Walcarius, *Mon. Chem.*, 140 (2009) 861.
82. Svancara I., Kalcher K., Walcarius A. and K. Vytras, Electroanalysis with Carbon Paste Electrodes, CRC Press, Taylor &Francis Group, 2012.
83. I. Svancara, K. Vytras, K. Kalcher, A. Walcarius and J. Wang, *Electroanalysis*, 21 (2009) 7.
84. A. Qureshi, W. P. Kang, J. L. Davidson and Y. Gurbuz, *Diam. Relat. Mat.*, 18 (2009) 1401.
85. J. P. Lei and H. X. Ju, *Wiley Interdiscip. Rev.-Nanomed. Nanobiotechnol.*, 2 (2010) 496.
86. Y. K. Ye and H. X. Ju, *Biosens. Bioelectron.*, 21 (2005) 735.
87. M. A. Alonso-Lomillo, O. Dominguez-Renedo and M. J. Arcos-Martinez, *Talanta*, 82 (2010) 1629.
88. S. Centi, S. Laschi and M. Mascini, *Bioanalysis*, 1 (2009) 1271.
89. D. T. Lam, H. N. Binh, V. H. Nguyen, V. T. Hoang, L. N. Huy and X. N. Phuc, *Mater. Sci. Eng. C-Mater. Biol. Appl.*, 31 (2011) 477.
90. G. Martinez-Paredes, M. B. Gonzalez-Garcia and A. Costa-Garcia, *Electroanalysis*, 21 (2009) 379.
91. S. Girousi and V. Kinigopoulou, *Cent. Eur. J. Chem*, 8 (2010) 732.
92. M. N. Velasco-Garcia and T. Mottram, *Biosyst. Eng.*, 84 (2003) 1.
93. O. A. Sadik, A. O. Aluoch and A. L. Zhou, *Biosens. Bioelectron.*, 24 (2009) 2749.
94. A. Merkoci, M. Aldavert, S. Marin and S. Alegret, *TRAC-Trends Anal. Chem.*, 24 (2005) 341.
95. N. Djellouli, M. Rochelet-Dequaire, B. Limoges, M. Druet and P. Brossier, *Biosens. Bioelectron.*, 22 (2007) 2906.
96. H. Zhang, L. Liu, C. W. Li, H. Y. Fu, Y. Chen and M. S. Yang, *Biosens. Bioelectron.*, 29 (2011) 89.
97. T. Q. Huy, T. H. H. Nguyen, P. H. Chung, D. D. Anh, P. T. Nga and M. A. Tuan, *Appl. Surf. Sci.*, 257 (2011) 7090.
98. S. Cosnier and P. Mailley, *Analyst*, 133 (2008) 984.
99. A. Erdem, *Talanta*, 74 (2007) 318.
100. E. Souza, G. Nascimento, N. Santana, D. Ferreira, M. Lima, E. Natividade, D. Martins and J. Lima, *Sensors*, 11 (2011) 5616.
101. F. Liu, K. S. Choi, T. J. Park, S. Y. Lee and T. S. Seo, *BioChip J.*, 5 (2011) 123.
102. E. Komarova, M. Aldissi and A. Bogomolova, *Biosens. Bioelectron.*, 21 (2005) 182.
103. P. Kara, K. Kerman, D. Ozkan, B. Meric, A. Erdem, Z. Ozkan and M. Ozsoz, *Electrochem. Commun.*, 4 (2002) 705.
104. J. J. Gooding, *Electroanalysis*, 14 (2002) 1149.
105. S. P. Liu, G. M. Chen, Q. Zhou and Y. L. Wei, in M. Sasaki, G.C. Sang, Z. Li, R. Ikeura, H. Kim, F. Xue (Editors), ICMIT 2007: Mechatronics, Mems, and Smart Materials, Pts 1 and 2, Spie-Int Soc Optical Engineering, Bellingham, 2008, p. P7943.
106. T. M. P. Hewa, G. A. Tannock, D. E. Mainwaring, S. Harrison and J. V. Fecondo, *J. Virol. Methods*, 162 (2009) 14.
107. T. W. Owen, R. O. Al-Kaysi, C. J. Bardeen and Q. Cheng, *Sens. Actuator B-Chem.*, 126 (2007) 691.
108. S. A. Miller, L. A. Hiatt, R. G. Keil, D. W. Wright and D. E. Cliffel, *Anal. Bioanal. Chem.*, 399 (2011) 1021.

109. S. Subrahmanyam, S. A. Piletsky and A. P. F. Turner, *Anal. Chem.*, 74 (2002) 3942.
110. X. Z. Zhang, K. Jiao, S. F. Liu and Y. W. Hu, *Anal. Chem.*, 81 (2009) 6006.
111. Y. C. Zhang, H. H. Kim and A. Heller, *Anal. Chem.*, 75 (2003) 3267.
112. J. Wang, G. D. Liu and M. R. Jan, *J. Am. Chem. Soc.*, 126 (2004) 3010.
113. D. J. Chung, K. C. Kim and S. H. Choi, *Appl. Surf. Sci.*, 257 (2011) 9390.
114. D. J. Lee, Y. Chander, S. M. Goyal and T. H. Cui, *Biosens. Bioelectron.*, 26 (2011) 3482.
115. F. Li, L. Mei, Y. M. Li, K. H. Zhao, H. C. Chen, P. Wu, Y. G. Hu and S. B. Cao, *Biosens. Bioelectron.*, 26 (2011) 4253.
116. F. Li, R. Zhou, K. H. Zhao, H. C. Chen and Y. G. Hu, *Talanta*, 87 (2011) 302.
117. K. Malecka, I. Grabowska, J. Radecki, A. Stachyra, A. Gora-Sochacka, A. Sirko and H. Radecka, *Electroanalysis*, 24 (2012) 439.
118. M. F. Diouani, S. Helali, I. Hafaid, W. M. Hassen, M. A. Snoussi, A. Ghram, N. Jaffrezic-Renault and A. Abdelghani, *Mater. Sci. Eng. C-Biomimetic Supramol. Syst.*, 28 (2008) 580.
119. X. Z. Song, H. Yu, X. Chen, Y. Lasanajak, M. M. Tappert, G. M. Air, V. K. Tiwari, H. Z. Cao, H. A. Chokhawala, H. J. Zheng, R. D. Cummings and D. F. Smith, *J. Biol. Chem.*, 286 (2011) 31610.
120. H. Cho, S. K. Kim, Y. Jung, J. Jung and B. H. Chung, *Chem. Commun.*, 47 (2011) 5756.
121. X. G. Liu, Z. Q. Cheng, H. Fan, S. Y. Ai and R. X. Han, *Electrochimica Acta*, 56 (2011) 6266.
122. J. Zhang, B. P. Ting, N. R. Jana, Z. Q. Gao and J. Y. Ying, *Small*, 5 (2009) 1414.
123. K. Y. Zhang and Y. Z. Zhang, *Electroanalysis*, 22 (2010) 673.
124. J. B. Raoof, R. Ojani, S. M. Golabi, E. Hamidi-Asl and M. S. Hejazi, *Sens. Actuator B-Chem.*, 157 (2011) 195.
125. X. H. Li, X. Qi, L. Miao, Y. Wang, F. Z. Liu, H. W. Gu, S. W. Lu, Y. H. Yang and F. Y. Liu, *Diagn. Microbiol. Infect. Dis.*, 65 (2009) 261.
126. J. P. Li, S. Chen and D. H. Evans, *J. Clin. Microbiol.*, 39 (2001) 696.
127. X. P. Kang, Y. Q. Li, H. H. Sun, W. L. Wu, H. Liu, F. Lin, C. F. Qing, G. H. Chang, Q. Y. Zhu, W. J. Chen and Y. H. Yang, *Arch. Virol.*, 155 (2010) 55.
128. V. A. Ryabinin, E. V. Kostina, A. A. Neverov, G. A. Maksakova and A. N. Sinyakov, *Russ. J. Bioorg. Chem.*, 36 (2010) 634.
129. E. V. Kostina, V. A. Ryabinin, A. P. Agafonov, V. A. Ternovoi and A. N. Sinyakov, *Russ. J. Bioorg. Chem.*, 37 (2011) 644.
130. A. Kongchanagul, O. Suptawiwat, C. Boonarkart, R. Kitphati, P. Puthavathana, M. Uiprasertkul and P. Auewarakul, *J. Med. Virol.*, 83 (2011) 1410.
131. R. H. Liu, M. J. Lodes, T. Nguyen, T. Siuda, M. Slota, H. S. Fuji and A. McShea, *Anal. Chem.*, 78 (2006) 4184.
132. K. M. Roth, K. Peyvan, K. R. Schwarzkopf and A. Ghindilis, *Electroanalysis*, 18 (2006) 1982.
133. D. J. Li, J. P. Wang, R. H. Wang, Y. B. Li, D. Abi-Ghanem, L. Berghman, B. Hargis and H. G. Lu, *Biosens. Bioelectron.*, 26 (2011) 4146.
134. B. Mu, X. L. Huang, P. C. Bu, J. Zhuang, Z. X. Cheng, J. Feng, D. L. Yang, C. S. Dong, J. B. Zhang and X. Y. Yan, *J. Virol. Methods*, 169 (2010) 282.
135. K. C. Halfpenny and D. W. Wright, *Wiley Interdiscip. Rev.-Nanomed. Nanobiotechnol.*, 2 (2010) 277.
136. P. J. Jannetto, B. W. Buchan, K. A. Vaughan, J. S. Ledford, D. K. Anderson, D. C. Henley, N. B. Quigley and N. A. Ledeboer, *J. Clin. Microbiol.*, 48 (2010) 3997.
137. T. M. H. Lee, L. L. Li and I. M. Hsing, *Langmuir*, 19 (2003) 4338.
138. T. L. Kamikawa, M. G. Mikolajczyk, M. Kennedy, P. Zhang, W. Wang, D. E. Scott and E. C. Alocilja, *Biosens. Bioelectron.*, 26 (2010) 1346.

139. T. Yumak, F. Kuralay, M. Muti, A. Sinag, A. Erdem and S. Abaci, *Colloid Surf. B-Biointerfaces*, 86 (2011) 397.
140. M. Muti, F. Kuralay, A. Erdem, S. Abaci, T. Yumak and A. Sinag, *Talanta*, 82 (2010) 1680.
141. L. Krejcova, D. Hynek, P. Kopel, V. Adam, J. Hubalek, L. Trnkova and R. Kizek, *Electrophoresis*, In press. (2012).
142. L. Krejcova, D. Dospivova, M. Ryvolova, P. Kopel, D. Hynek, S. Krizkova, J. Hubalek, V. Adam and R. Kizek, *Electrophoresis*, In press. (2012).
143. H. Fan, P. Ju and S. Y. Ai, *Sens. Actuator B-Chem.*, 149 (2010) 98.
144. J. H. Chen, J. Zhang, H. H. Yang, F. F. Fu and G. N. Chen, *Biosens. Bioelectron.*, 26 (2010) 144.
145. L. Y. Chen, Z. J. Qi, R. J. Chen, Y. Li and S. Q. Liu, *Clin. Chim. Acta*, 411 (2010) 1969.
146. Y. X. Wang, Z. Z. Ye, C. Y. Si and Y. B. Ying, *Chin. J. Anal. Chem.*, 40 (2012) 634.
147. S. C. B. Gopinath, *Arch. Virol.*, 152 (2007) 2137.
148. S. P. Song, L. H. Wang, J. Li, J. L. Zhao and C. H. Fan, *TRAC-Trends Anal. Chem.*, 27 (2008) 108.
149. T. S. Misono and P. K. R. Kumar, *Anal. Biochem.*, 342 (2005) 312.
150. S. C. B. Gopinath, T. S. Misono, K. Kawasaki, T. Mizuno, M. Imai, T. Odagiri and P. K. R. Kumar, *J. Gen. Virol.*, 87 (2006) 479.
151. C. S. Cheng, J. Dong, L. H. Yao, A. J. Chen, R. Q. Jia, L. F. Huan, J. L. Guo, Y. L. Shu and Z. Q. Zhang, *Biochem. Biophys. Res. Commun.*, 366 (2008) 670.
152. K. Kiilerich-Pedersen, C. R. Poulsen, T. Jain and N. Rozlosnik, *Biosens. Bioelectron.*, 28 (2011) 386.
153. W. M. Hassen, V. Duplan, E. Frost and J. J. Dubowski, *Electrochimica Acta*, 56 (2011) 8325.
154. A. Bonanni, M. I. Pividori and M. del Valle, *Analyst*, 135 (2010) 1765.
155. R. H. Wang, Y. Wang, K. Lassiter, Y. B. Li, B. Hargis, S. Tung, L. Berghman and W. Bottje, *Talanta*, 79 (2009) 159.
156. J. H. Lin, J. Lum, R. H. Wang, S. Tung, B. Hargis, Y. B. Li, H. G. Lu, L. Berghman and Ieee, in 2010 IEEE Sensors, Ieee, New York, 2010, p. 1558.
157. M. Billah, H. C. W. Hays and P. A. Millner, *Microchim. Acta*, 160 (2008) 447.
158. N. K. Chaki and K. Vijayamohanan, *Biosens. Bioelectron.*, 17 (2002) 1.
159. P. Babula, J. Vanco, L. Krejcova, D. Hynek, J. Sochor, V. Adam, L. Trnkova, J. Hubalek and R. Kizek, *Int. J. Electrochem. Sci.*, 7 (2012) 7349.
160. D. Dospivova, K. Smerkova, M. Ryvolova, D. Hynek, V. Adam, P. Kopel, M. Stiborova, T. Eckschlager, J. Hubalek and R. Kizek, *Int. J. Electrochem. Sci.*, 7 (2012) 3072.
161. D. Hynek, L. Krejcova, O. Zitka, V. Adam, L. Trnkova, J. Sochor, M. Stiborova, T. Eckschlager, J. Hubalek and R. Kizek, *Int. J. Electrochem. Sci.*, 7 (2012) 34.
162. D. Hynek, L. Krejcova, O. Zitka, V. Adam, L. Trnkova, J. Sochor, M. Stiborova, T. Eckschlager, J. Hubalek and R. Kizek, *Int. J. Electrochem. Sci.*, 7 (2012) 13.
163. J. Wang and A. Erdem, An overview to magnetic beads used in electrochemical DNA biosensors, Springer, Dordrecht, 2003