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Review

Advanced nanotechnologies in avian influenza: Current status and future trends – A review

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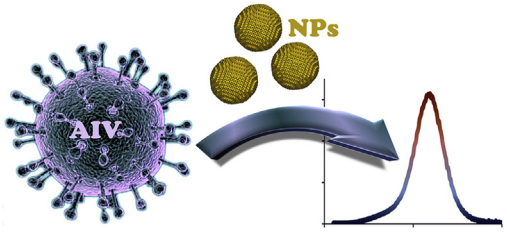
HIGHLIGHTS

- An overview of advanced nanotechnologies to control avian influenza.
- The detection systems using nanomaterials are discussed.
- The future trends in AIV detections will be presented.

GRAPHICAL ABSTRACT



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ABSTRACT

In the last decade, the control of avian influenza virus has experienced many difficulties, which have caused major global agricultural problems that have also led to public health consequences. Conventional biochemical methods are not sufficient to detect and control agricultural pathogens in the field due to the growing demand for food and subsidiary products; thus, studies aiming to develop potent alternatives to conventional biochemical methods are urgently needed. In this review, emerging detection systems, their applicability to diagnostics, and their therapeutic possibilities in view of nanotechnology are discussed. Nanotechnology-based sensors are used for rapid, sensitive and cost-effective diagnostics of agricultural pathogens. The application of different nanomaterials promotes interactions between these materials and the virus, which enables researchers to construct portable electroanalytical biosensing analyser that should effectively detect the influenza virus. The present review will provide insights into the guidelines for future experiments to develop better techniques to detect and control influenza viruses.

Contents

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1. Introduction

The last two decades have witnessed the emergence of highly pathogenic strains of type A avian influenza virus (AIV) that have caused severe disease and economic crises in poultry, including chickens and turkeys, as well as several cases of avian-human transmissions. Highly pathogenic AIV strains are associated with high morbidity and mortality, leading to epidemics and substantial economic losses. For example, in early 2006, a highly pathogenic H5N1 AIV resulted in high mortality at chicken farms in Egypt. The first human case occurred shortly afterwards. Since then, the WHO has reported more than one hundred avian-transmitted AIV human infections and a number of deaths in Egypt [1].

The genetic and phenotypic diversity and the divergence of influenza viruses pose a technological challenge for the development of diagnostics and therapeutics. AIVs are divided into subtypes according to the antigenic properties of their haemagglutinin (HA, H1-H16) and neuraminidase (N1-N9) surface glycoproteins. The only avian subtypes that are reported to cause disease in humans are H5, H7 and H9. The most well-known highly pathogenic avian subtype is H5N1 [2], although the newly reported subtype H7N9 has recently attracted attention. Based on genetic evidence, most influenza strains are traced to aquatic birds. Wild birds are believed to act as 'reservoirs' for these viruses, which cause little or no disease in the wild hosts. However, due to genetic mutations or re-assortment, these strains can become highly pathogenic and adapt to new host species, such as chickens, pigs and humans. Chickens and pigs have been shown to act as 'mixing vessels' for different influenza strains, facilitating genetic re-assortment of the highly pathogenic strains, which have resulted in severe disease pandemics in humans [3–6]. Changes in the host, environment, or vector can lead to an increased incidence of viral infection. Many of the viral diseases that have appeared in the last few decades have now become entrenched in human populations worldwide. Some important known examples are Zika virus, monkey pox virus, severe acute respiratory syndrome (SARS) coronavirus, Hendra virus, Chikungunya virus, Hantavirus, Nipah virus, and the threat of pandemic influenza viruses of avian or swine origin. Changes in climate, the ecosystem and human activities are major factors for pathogen transmission. In particular, the high genetic biodiversity of wild birds combined with the low genetic biodiversity of the domestic bird population promotes the rapid dissemination of the infection in dense farms and live poultry markets [4,7–9]. Unfortunately, the techniques used to detect AIV are not sufficiently developed for the present need. Antiviral therapies have been improved, but display a wide margin of ineffectiveness; therefore, new antiviral agents and improved detection techniques are urgently required to continue the fight against the invading viruses and modulate host responses.

Nanotechnologies that are directly linked to physics, chemistry, biology, material science and medicine are considered a developing field in the applied sciences and include cutting edge technologies that employ the physicochemical properties of nanomaterials to control their surface area, size, and shape to generate different nanoscale-sized materials. Nanoparticles (NPs) have been used to develop NP-based targeted drug carriers, rapid pathogen detectors or biomolecular sensing systems and antivirals that act by interfering with viral infections, particularly during attachment and host cell entry [10–12]. Intensive surveillance of avian influenza in farms, markets and among workers in the agriculture and food industries is a current urgent and unmet need. Improved diagnostic tools with increased sensitivity, high throughput and multiplexing abilities are required. Recent advances in nanotechnology provide several solutions, including high sensitivity, specificity and testing throughput. The present review aims to provide a detailed description of advanced nanotechnological methods that are currently being used to control AIV. The number of papers published in the application of nanotechnologies to avian influenza in the last decade is shown in Fig. 1 according to the year of publication. Recent developments in the use of nanotechnology in biosensors and detection systems and their applicability in AIV diagnostics are explored here and presented with a discussion of probable future trends.

2. Analytical methods for sensing AIV

Influenza viruses have mainly been detected by assessing specific nucleic acids and proteins using analytical methods, such as polymerase chain reaction (PCR) [13,14], enzyme-linked immunosorbent assay (ELISA) and enzyme-linked lectin assay (ELLA) [15], but these methods must be employed with very expensive equipment under specific laboratory conditions by skilled personnel

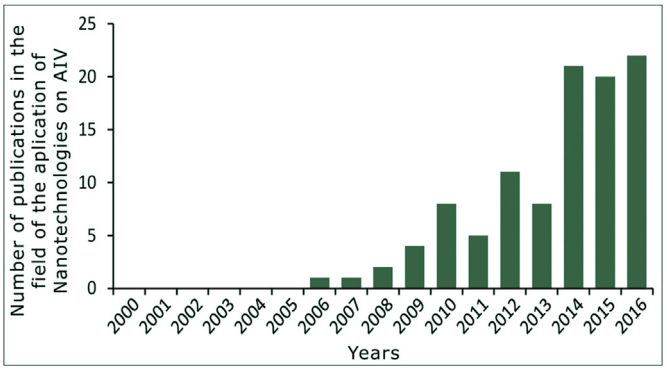

Fig. 1. The number of papers published on the application of nanotechnologies for the detection of avian influenza in the last two decades by year.

[16–19].

Although the above-mentioned techniques provide impressive analytical performance, the few major drawbacks complicate their applications in large epidemiological screenings, where an *in situ* analysis is required to exclude the infection and thus prevent economic losses. The most suitable alternative is the application of nanomaterials that can enhance the performance of common optical or electrochemical biosensors and thus can significantly increase their usefulness for screening AIV in various types of matrices.

2.1. Conventional methods: ELISA and PCR

Western blot, protein and DNA microarrays or PCR followed by DNA sequencing analysis are generally used to diagnose influenza virus infections. In contrast, AIV typing microarray tests can detect and provide additional information about the subtype detected in positive clinical samples compared with that obtained using real-time-PCR [20]. Lab-on-a-chip and fully integrated techniques have been developed for pathotyping and the phylogenetic characterization of influenza A viruses obtained from real samples [21]. Various types of ELISAs, including immunochromatographic strip tests [22] and double antibody sandwich enzyme-linked immunesorbent assay (DAS-ELISA) [23], have been widely used for the rapid detection of subtype H9 influenza viruses and receptor binding specificity in different types of samples. DAS-ELISA uses two monoclonal antibodies with 99.1% specificity and 93.1% sensitivity. Dual-function and blocking ELISAs are highly sensitive and 100% specific for a timely diagnosis of AIV infections. These tests represent valid alternatives to the haemagglutination inhibition (HI) test, mainly for the screening of a large number of samples, as these tests are as sensitive and specific as the HI test but are faster and easier to automate [24,25]. A magnetic immunofluorescence assay with an optical fibre spectrometer and a portable microfluidics chip was also reported for AIV detection [26].

A method was developed for the detection of avian influenza A/H7N9 using real-time PCR with a detection limit of 3.2×10^{-4} and 6.4×10^{-4} haemagglutination units (HAUs) for the *H7* and *N9* genes, respectively [27,28]. A microfluidics chip (fabricated using polydimethylsiloxane) with real-time fluorescence detection for the rapid identification of AIV H5 was suggested by Zhu et al. [29]. Protein and DNA microarrays are useful for the multiplexed detection and typing of AIVs that are currently classified as the H16 and N9 subtypes and provide new possibilities for screening applications, although their use in diagnostic laboratories is still limited due to economic and practical factors [30,31].

2.2. NP-based AIV biosensors

In the last decade, the number of studies reporting a diverse range of optical and electrochemical sensors for AIV detection has increased considerably. According to its well-established definition in the analytical chemistry lexicon, a “biosensor” is a detection system that relies on a biomolecule for molecular recognition and a transducer to produce an observable output [32]. With the recent advances in nanotechnology, nanomaterials have received great interest in the biosensor field due to their exquisite sensitivity in chemical and biological reactions. Various types of NPs, including metal NPs, oxide NPs, semiconductor NPs, and even nanodimensional conducting polymers, have been used in biosensors [33]. Nanomaterials have divergent roles in the biosensors used for AIV (Fig. 2); however, these roles are mainly classified as: 1) the immobilization of target biomolecules; 2) catalysis of electrochemical reactions; 3) enhancement of electron transfer; 4) labelling of biomolecules or 5) reactants. All these applications somehow result in improvements in speed, selectiveness and specificity, which are critical for disease control and monitoring [34].

Semiconductor quantum dots (QDs), *inter alia*, became very popular as novel fluorescent probes used for biological detection applications with respect to their high quantum yield and molar extinction coefficients, which are 10- to 100-fold larger than most organic dyes [35,36]. The application of metallic NPs, particularly noble metal (gold and silver)-based NPs, to virus detection has progressed. Another promising group of materials with considerably low detection limits for sensing AIV are fluorescent biosensors based on metal organic frameworks [37]. Magnetic beads or nanobeads are used to detect the amplified signals. Together with quartz crystal microbalance (QCM) aptasensors, the magnetic nanobead-amplified QCM immunosensors have been used to detect the H5N1 protein [38]. Magnetic particles, silver nanoparticles (AgNPs) and carbon-based materials are frequently used to assay and detect influenza viruses. Many of these particles are commercially available and/or were prepared using published procedures, with some modifications [39–42]. In particular, AuNPs are widely employed for developing biosensors due to their exceptional electrochemical properties [43]. Schemes using nanoparticle-based biosensors for the detection of AIV proteins are shown in Fig. 3. In addition to these NPs, some other nanomaterial-based techniques, including nanowell array electrode-based electrochemical quantitative systems [44] and fully integrated on-chip tubular nanomembrane sensors [45], have also been developed to efficiently detect viral DNA. The next sections provide a brief discussion of new trends and approaches in the field of AIV detection.

2.3. QD-based AIV biosensors

Several types of glycan-conjugated QDs decorated with 3-aminophenyl boronic acid (APBA) bound to the carboxyl groups on the surface have been used to develop specific and sensitive sensors for influenza virus. This highly luminescent QD-based reagent was introduced into another nanoprobe consisting of AuNPs modified with HA-specific antibodies [46]. Another fluorescence biosensor using the H5N1 antibody for the photoluminescence detection of the H5N1 virus was constructed by joining CdTe/CdS QDs, chromatophores and the β-subunit from the H5N1 antibody. A biotin-streptavidin-biotin bridge was utilized to connect the sensors. The bacteria *Rhodospirillum rubrum* served as the chromatophore source, and the chromatophores were isolated using an extraction process [47].

CdTe QDs prepared under a protective nitrogen atmosphere through the reaction of cadmium chloride, thioglycolic acid, trisodium citrate dihydrate, sodium tellurite and sodium borohydride are an important constituent of a sandwich FL-linked immunosorbent assay (sFLISA) based on QD-antibody conjugates. The reaction mixture for the CdTe QDs consisted of cadmium, thioglycolic acid and tellurium in 3:9:1 M ratio, which was heated for 6 h under reflux. The required QD-antibody conjugates were obtained by mixing the prepared CdTe QDs with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysulphosuccinimide in phosphate-buffered saline (PBS) for 30 min at room temperature [48]. CdTe QDs were applied to H9 AIV detection based on antibody-antigen reactions [49].

In our studies, we have explored the use of streptavidin-coated magnetic NPs conjugated with biotinylated glycans to isolate H5N1 HA. The isolation process was followed by HA labelling using CdS QDs [50]. The entire process was performed in a 3-D printed microfluidics chip with electrochemical detection; however, our QD-based approach also offers detection that utilizes fluorescence or simultaneous isolation and detection using magnetic QDs [51].

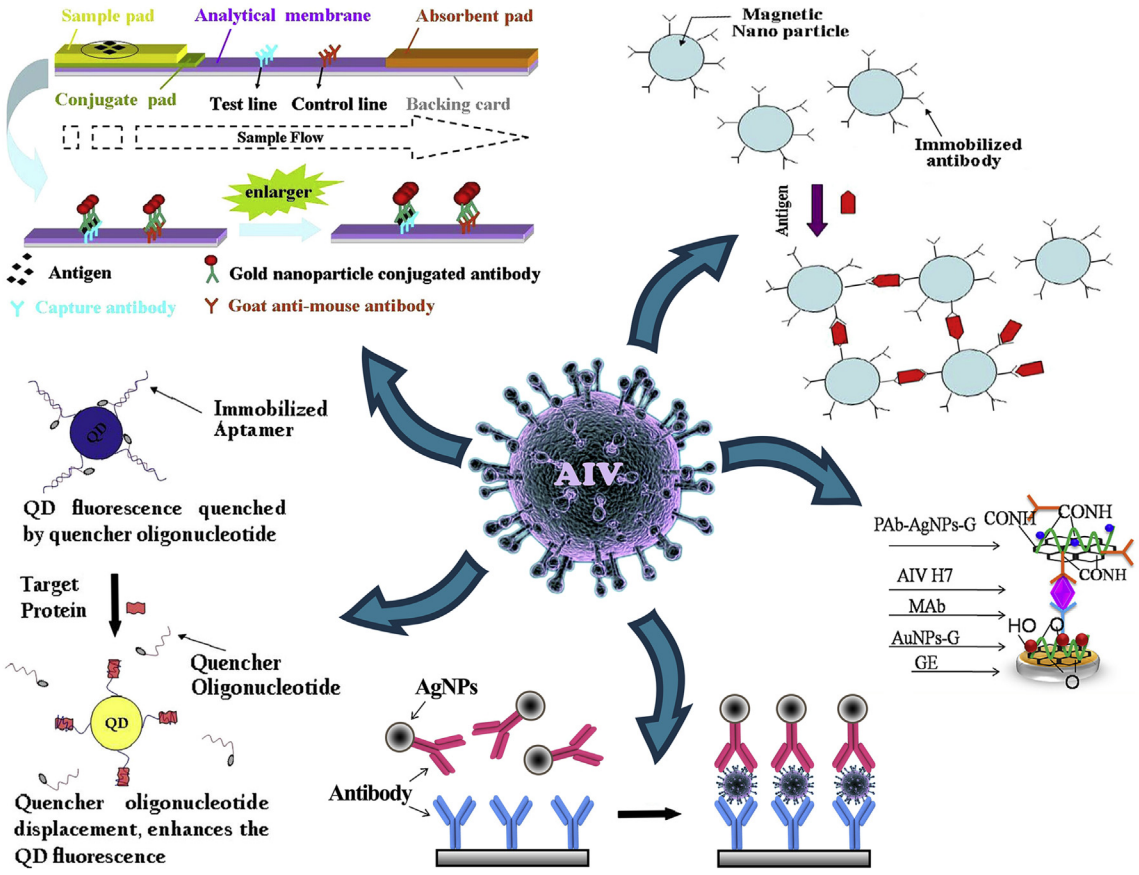

Fig. 2. Schematic representation of AIV detection methods using different nanomaterials. The schemes were adopted with permission from Li et al., Shojaei et al. and Huang et al. [24,39,84]. PAb - polyclonal antibody, AgNPs-G - silver nanoparticle-graphene nanocomposites, MAb - monoclonal antibody, AuNPs-G - gold nanoparticle-graphene nanocomposites, and GE - gold electrode.

Streptavidin-modified QDs were recently employed in the gene-based detection of H7N9 [52], whereas amine-modified QDs were employed in the antibody-based detection of H7N9 virions [53].

Nucleic acids are one of the most important and promising targets in clinical diagnosis; therefore, many research groups are focusing on NP-based hybridization biosensors (Fig. 4). Electrochemical biosensing applications represent fast, compact, easy to operate, and relatively low cost methods to specifically detect nucleic acids. The crucial step is the fabrication of an electrode surface that enables a significant increase in the amount of immobilized recognition probes and promotes high sensitivity and selectivity towards the target molecules. In accordance with this requirement, glassy carbon electrodes (GCEs) are decorated with CdSe hollow microspheres together with a single-stranded DNA (ssDNA) probe. This reagent exhibits high sensitivity and selectivity in detecting avian influenza RNA segments [54]. In our laboratory, we have also designed a hybridization probe consisting of paramagnetic particles and QDs with a targeted DNA for the detection of AIV (H5N1). The optical properties of the QDs were beneficial, but we mainly focused on the electroactivity of the metal part of QDs and the oligonucleotides themselves (Fig. 4a) [55].

Both QDs and other Cd-based spherical nanostructures, such as CdSe hollow microspheres, exhibit promising features for influenza virus detection. The different morphologies and sizes (starting from approximately 0.8 μm) of these CdSe microspheres are controlled by the composition of the mixed reaction solution (ethanolamine and glucose). The different reducing properties of different ratios of the constituents in solution play key roles in determining the characters of the prepared microspheres. CdSe microspheres have been used to construct a DNA biosensor (ssDNA/CdSe) on a GCE electrode surface [56]. In a separate study, CdSe/ZnS core/shell QDs conjugated with aptamers were used as fluorescent reporters in a hydrogel system to detect the virus [57].

Changes in QD characteristics will allow researchers to develop multiplex detection assays and will play a major role in the development of assays. Currently, QD-based detection of H5N1, H7N9, and H9 has been the primary focus. However, both genotyping and immunotyping of various AIV subtypes will be possible using multiplex assays employing specific QDs for each strain.

2.4. Use of magnetic NPs to sense AIV

Several rapid and sensitive methods using magnetic NPs have been reported for the detection of different subtypes of AIV [58–61]. A novel electrochemical magneto-immunosensor based on the connection between immunomagnetic separation and catalytic enzyme-based amplification in combination with a biotin-streptavidin system rapidly and ultrasensitively detects H9N2 AIV [59]. Moreover, immunogenic nanobeads, which were modified with a monoclonal HA-specific antibody in combination with QDs, were used to detect the virus without pretreating the sample through the high-affinity biotin-streptavidin system in an immune sandwich mode [60]. Another approach demonstrating highly sensitive AIV antigen detection takes advantage of resonance light scattering (RLS). In the presence of AIV antigens, an increase in RLS signal intensity is observed at 545 nm. Changes in the signal intensity are caused by the formation of an immunocomplex between the AIV antigen and magnetic silica NPs modified with covalently

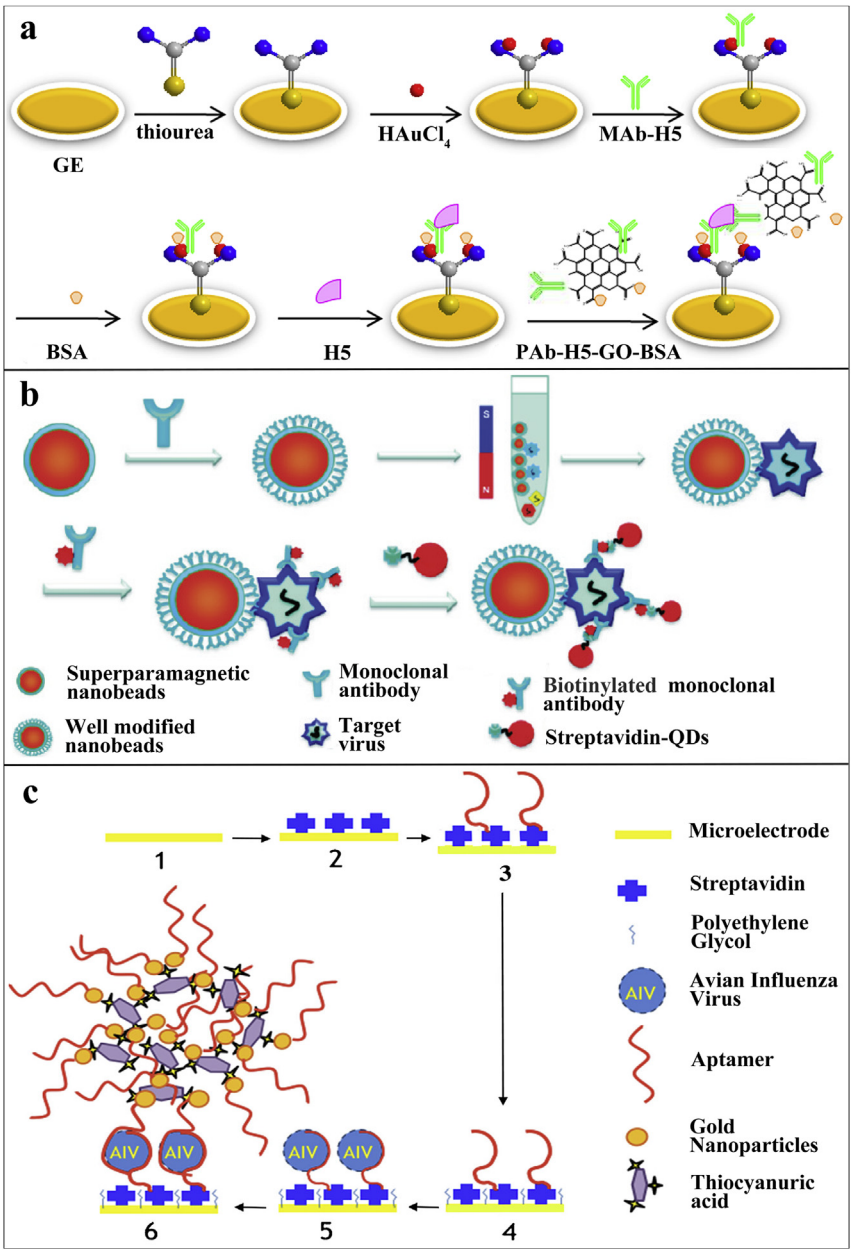

Fig. 3. Schemes for nanoparticle-based biosensors used to detect AIV proteins. Schemes a, b and c were adapted with permission from Xie et al., Zhao et al. and Karash et al., respectively [60,69,87]. GE - gold electrode, GO - graphene oxide, BSA - bovine serum albumin, PAb - H5-polyclonal antibody, and MAb - H5-monoclonal antibody.

bound AIV antibodies [61]. Magnetic nanobeads with a 30 nm diameter decorated with immobilized anti-H5 antibodies were used to more efficiently capture H5N1 viruses and in subsequent detection processes using QCM. The immunosensors, which use polyclonal antibodies against the HA surface antigen on H5N1 AIV, were bound to gold atoms located at the QCM crystal surface via a self-assembled single layer of 16-mercaptohexadecanoic acid, which causes a change in frequency [62]. A lower detection limit and greater specificity for H5N1 screening were achieved due to the amplification of the impedance signal when chicken red blood cells were used for biolabelling [63]. In a subsequent study, immunomagnetic nanoparticles were used for virus separation in combination with a microfluidics chip and an interdigitated microelectrode for impedance measurements. Modification of the NPs with H5N1 aptamers facilitated the capture of whole H5N1 virions. Subsequent binding to concanavalin A/glucose oxidase-AuNP bionanocomposites resulted in the formation of a complex, which was detected using enzyme catalysis after treatment with glucose [64]. Off-line magnetic bioseparation of H5N2 virions from tracheal and cloacal swabs from infected chickens and the subsequent utilization of a microfluidics impedance biosensor achieved comparable sensitivity and selectivity to real-time RT-PCR with a detection time of less than 1 h. Biotin-labelled anti-H5 antibodies conjugated to streptavidin-coated magnetic beads were used for virion separation [65]. An electrochemical magneto-immunoassay coupled to biometallization and anodic stripping voltammetry is the basic principle of a highly sensitive electrochemical immunosensor for H7N9 AIV. This approach allowed the accumulation of product generated by the enzymatic reaction on the surface of a magneto electrode through silver deposition [66]. Paramagnetic

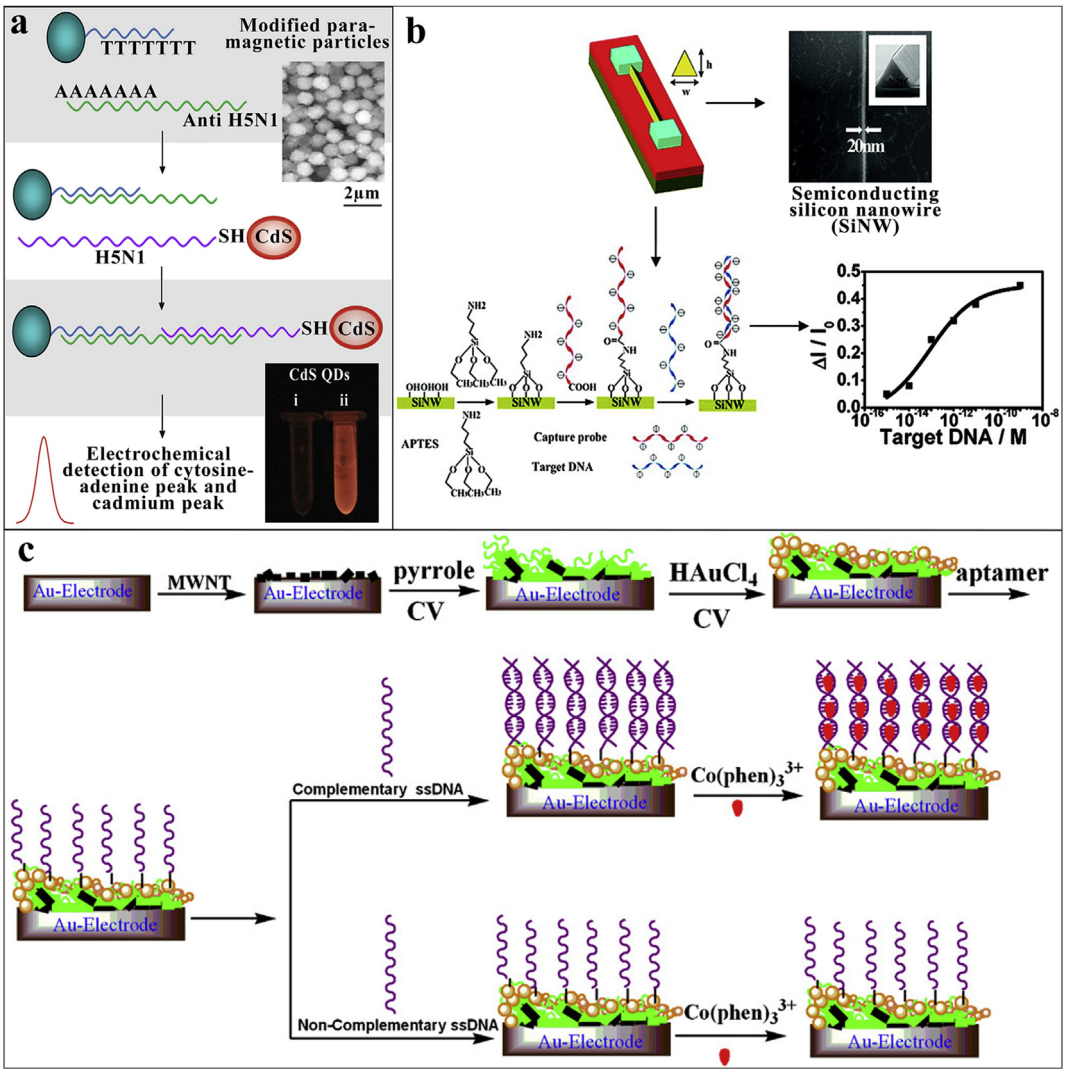

Fig. 4. Schemes for nanoparticle-based biosensors used to detect AIV nucleic acids. Scheme a was adapted from our previous study [55]. Schemes b and c were adopted with permission from Gao et al. and Liu et al., respectively [89, 90]. CV- cyclic voltammetry and MWNT – multi-wall carbon nanotubes.

particles with a nanomaghemite core (γ-Fe_2O_3) functionalized with tetraethyl orthosilicate and 3-aminopropyl triethoxysilane on the NP surface exhibited the ability to bind H7N7 influenza virions. The immobilized virions were directly and sequentially analysed using ion-exchange chromatography and visible spectrophotometry (VIS) [67].

2.5. AuNP-based AIV biosensors

AuNPs have become very important in the detection of AIV. For example, Diba and colleagues developed an amperometric system with an AuNP-modified electrode to detect H5N1 viral proteins [43]. AuNPs were electrodeposited onto working carbon electrodes using a solution containing $HAuCl_4$ and the surface was functionalized with 3-mercaptopropionic acid before an H5N1 aptamer was spread over the chip surface and the entire chip was washed with PBS [43]. AIV-specific aptamers were also conjugated with AuNPs to detect whole viruses [68]. AuNPs are used to dramatically amplify the detected signals [69]. Several studies have been performed to develop AuNP-based immunosensors to detect AIV HA [70,71]. An immunosensor for the detection of peptides derived from AIV H5 HA was described by Jarocka et al. The gold electrode was modified with 1,6-hexanedithiol, AuNPs and then with anti-HA H5 monoclonal antibody MAb 6-9-1 antibody-binding fragments (Fab') via S-Au covalent bonds. The interactions between the Fab' fragments and HA were detected by electrochemical impedance spectroscopy (EIS) in the presence of an $[Fe(CN)_6]^{3-/4-}$ marker [71]. In another study, streptavidin-coated AuNPs were used to identify influenza H1N1, H3N2, and H5N1 strains. The purified streptavidin was mixed with an AuNP solution and prepared using the citrate method [72].

A biosensor for the rapid and sensitive detection of H7 subtypes based on luminescence resonance energy transfer (LRET) from $BaGdF_5$:Yb/Er NP upconversion to AuNPs was developed. Amino oligonucleotides were covalently bonded to poly(ethylenimine)-modified $BaGdF_5$:Yb/Er NPs, whereas the thiol-modified oligonucleotides with H7 HA gene sequences were conjugated to AuNPs [73]. Different colourimetric biosensors have recently been developed for the detection of different AIV subtypes [53,74–76]. A facile colourimetric assay was developed to assess the interactions between viral HA proteins and host glycan receptors using glycan-functionalized AuNPs (gAuNPs). The gAuNPs were prepared from citrate AuNPs by the addition of disulphide-modified sialic acid receptors in methanol with stirring at room temperature for 24 h

[74]. Another colourimetric assay based on enzyme-induced metallization was proposed for the detection of alkaline phosphatase (ALP); it was further applied to the highly sensitive detection of AIV particles coupled with immunomagnetic separation. In this case, glutathione-capped 6 nm AuNPs were used [75]. The colourimetric detection of influenza virus using an immunosensor is also possible without enzymatic reactions for the fast and simple detection of viruses. AuNPs modified with monoclonal anti-HA antibodies are used in this type of immunosensor. The virus has multiple recognition sites for the AuNP-antibody probes on its surface, which causes the probes to be specifically arranged on the surface of the virus. This aggregation of the probes causes plasmon coupling between adjacent AuNPs, which in turn results in a red shift in the absorption spectrum. A change in colour from red to purple is detected very easily with the naked eye [76].

A microarray preparation for H5N1 detection through interfacial structure-directing forces arising from self-assembled monolayers (SAMs) of gold substrates and hybrid $Ni(en)_3Ag_2I_4$ crystals provides a template-free reference method for the fabrication of highly oriented hybrid arrays [77]. A plasmonic bioassay was reported for the detection of human influenza virus based on the functionalization of AuNPs with an α-2,6-thio-linked sialic acid derivative. The glycoNPs consisted of the sialic acid derivative and a thiolated polyethylene glycol that self-assembled onto the gold surface. These glycoNPs were able to distinguish between human (α-2,6 binding) and avian (α-2,3 binding) RG14 (H5N1) influenza viruses [78]. Gold immunochromatographic strips can enhance the detection sensitivity by inducing AuNP clustering around immunogold particles immobilized on nitrocellulose strips. AuNPs additionally provide an intense signal that is visible with the naked eye [24]. Silver-stained AuNPs carrying neuroaminidase HA and matrix gene oligonucleotides hybridized to influenza were successful in discriminating the H5N1 AIV viral RNA from the H1N1 and H3N2 subtypes in a multiplexed, nanoparticle-based genomic microarray assay [79].

An optical analytical system was reported to be developed by integrating a microarray and fabricating AuNPs onto a glass chip. The gold surface was covered with the gold-binding polypeptide (GBP)-fusion protein, which was expressed in recombinant *Escherichia coli* cells. The GBP-fusion method allows the proteins to be immobilized in their bioactive forms, facilitating the study of antigen-antibody interactions [80]. Western blotting (or immunoblotting) is often used to detect specific proteins in a mixture of other proteins, such as in a tissue homogenate or other biological samples, for the diagnosis of infectious diseases in humans and animals. An improved technique was also reported where AuNPs were used to avoid the diffusion of the conserved peptide from avian influenza nonstructural protein from the membrane [81]. AuNPs were employed to coat an immobilin-polyvinylidene difluoride membrane. AuNPs bind to the sulphhydryl and amine groups of amino acids, but small molecules are retained on the metal membrane. The diffusion of the desired peptides and small protein molecules to the metal-coated membrane is avoided during Western blotting. This technique has become useful for detecting small proteins or peptides. In a separate study, a nanoporous gold film was used in a bio-nanogate-controlled enzymatic reaction system to detect the virus [82].

2.6. AgNP-based AIV biosensors

AgNPs have also been used to detect the influenza virus. AgNPs are mainly used to develop immunosensors; for example, a versatile and ultrasensitive immunosensor was reported for the detection of influenza virus that combined AgNP-labelled antibodies with indirect fluorescence. The strong affinity of silver to sulphur was applied for antibody labelling. Influenza A (H1N1) virus was applied as the target antigen using sandwich-type immunoreactions on high binding ELISA plates. The antibody-labelled AgNPs were then released by an acid solution to produce silver ions, which catalyse *o*-phenylenediamine oxidation and promote fluorescence detection [83]. In a separate study, an ultrasensitive sandwich-type immunoassay was developed to detect AIV using antibody (Ab)-AgNP-graphene bioconjugates and a gold electrode fabricated with Ab-AuNP-graphene nanocomposites [84]. As shown in the study by Jazayeri and co-workers, AgNPs with a positive surface charge encapsulate expression vectors that are used to develop a systemically administered safe and effective nonviral gene delivery system for AIV research [85].

2.7. Carbon-based NPs and biopolymers and their applications in detecting AIV

Graphene oxide (GO) based electrochemical immunosensor can be developed for the accurate detection of influenza viruses [86]. In this immunosensor, 1-naphthol is used as an electroactive substance, and Pt/CeO_2/GO composites are employed as a catalytic amplifier. Another type of electrochemical immunosensor was developed by Xie et al. for the sensitive detection of AIV. In this case, the virus was captured by a GO-H5-polyclonal antibody-bovine serum albumin (GO-PAb-BSA) nanocomposite that was employed as a signal amplification material. The immunosensor increased the detection sensitivity by 256-fold compared with that of an immunosensor without GO-PAb-BSA [87]. Graphene was also reported as an enhanced biosensor for detecting AIV after the formation of a composite with zinc oxide [88]. The electrode, which was modified with multi-walled carbon nanotubes (MWCNTs) and AuNPs, offers a porous structure for immobilizing aptamers against an H5N1-specific sequence [89]. Various types of NPs have been used to fabricate comprehensive arrays, as was reported for silicon nanowires (SiNWs) and their utilization in a field-effect transistor (FET) label-free biosensor [90]. The reported biosensor enables multiplexing because of the presence of 16 individually aligned SiNWs with high specificity for discriminating single-base mismatches in avian influenza nucleic acids.

Another promising nanomaterial is the 2-D flower-like VS_2-graphene-AuNP composite, whose immobilization on a GCE surface provides a number of binding regions for aptamers specific to H5N1 RNA sequences [91]. Substantial advantages of this electrode modification are the perfect stability and acceptable reproducibility, which are very important when developing biosensors for use in clinical practice.

Yeo and co-workers developed a coumarin-derived dendrimer-based fluorescent immunochromatographic strip test (FICT) assay as a quantitative diagnostic tool. In this study, the coumarin-derived dendrimers were conjugated with latex beads to obtain fluorescent emissions covering broad output spectral ranges. This assay provides the benefit of the easy discrimination of the fluorescent emission from the latex beads along with the simple insertion of a long-pass optical filter at a wavelength distinct from the excitation wavelength [92].

According to Ross et al., polyanhydride NPs enhance the efficacy of subunit vaccines by providing the dual advantages of adjuvanticity and sustained delivery, resulting in improved immunogenicity and protein stability. In their experiment, a recombinant H5 (H5(3)) trimer was encapsulated and subsequently released from polyanhydride NPs composed of sebacic anhydride and 1,6-bis(*p*-carboxyphenoxy)hexane [93].

The intranasal administration of inactivated viruses with NPs composed of poly-γ-glutamic acid (γ-PGA) and chitosan are able to target the mucosal membrane as a mucosal adjuvant. This process

is safe and induces a high degree of protective mucosal immunity in the respiratory tract. Intranasal immunization with this mixture of recombinant influenza HA (rHA) antigen or inactivated virus and γ-PGA/chitosan NPs (PC NPs) was shown to induce high anti-HA immunoglobulin A response in lung tissue and an IgG response in serum, including anti-HA neutralizing antibodies. It also induced an influenza virus-specific cell-mediated immune response [94].

2.8. Advantages of NP-based AIV sensors

Various diagnostic approaches have been employed to detect influenza viruses. Conventional methods are generally sensitive and accurate; however, they also have some drawbacks. Strict laboratory conditions, a well-trained staff, and expensive instrumentation are required to detect AIV using PCR, ELISA and ELLA. NP-based biosensors for AIV detection have recently attracted a large amount of interest among scientists, mainly due to their valuable properties, such as electron transfer abilities, mechanical strength, chemical stability, and thermal and electrical conductivity. A number of AIV detection methods have been proposed using different QDs, NPs, and carbon-based nanomaterials. The easy interactions between nanomaterials and AIV have helped researchers develop simple and effective virus detection methods. Selected examples of the application of nanoscaled materials in biosensors are shown in Table 1.

3. Future trends

In the past decade, the control of AIV has experienced many difficulties, which have caused many agricultural and economic problems in many countries. However, these problems might provide an opportunity for AIV to acquire efficient human-to-human transmission, causing morbidity and mortality in human populations. Thus, the development of a rapid and expansive global influenza surveillance and response network and strategies to effectively decrease the global transmission of this disease is important. The key to solve this issue is the development of new technologies and improvements in current technologies for the detection and treatment of this disease. The development of existing and new diagnostic techniques will provide researchers the opportunity to rapidly detect influenza viruses. For example, one-step RT-PCR enables the rapid and accurate identification of

Table 1
Nanoparticle-based biosensors for the detection of AIV.

Detection method	Target	NPs	NP role	LOD	Ref.
DPV	Nucleic acid	CdSe QDs	Recognition of immobilized probe	1.0×10^{-12} M	[54]
DPV	Nucleic acid	MWCNTs/AuNPs	Recognition of immobilized probe	4.3×10^{-13} M	[89]
FET	Nucleic acid	SiNW	Array platform, signal amplification	1.0×10^{-15} M	[90]
SWV	Nucleic acid	$Ni(en)_3Ag_2I_4$	Array platform, signal amplification	5.0×10^{-12} M	[77]
DPV	Nucleic acid	VS_2-graphene, AuNPs	Recognition of immobilized probe	5.2×10^{-14} M	[91]
Impedance	HA	Streptavidin-coated magnetic NPs	Magnetic immunoseparation	*n. a.*	[65]
Impedance	HA	Streptavidin-coated magnetic NPs	Magnetic immunoseparation	10^3 EID_{50}/mL	[63]
Impedance	H5N1 AIV	Streptavidin-coated magnetic NPs/AuNPs	Immobilization/signal amplification	8.0×10^{-4} HAUs/200 μL	[64]
FRET	HA	Gly-QDs/AuNPs	Specific interaction/detection	38 pM	[46]
LSV	H7N9 AIV	Magnetic NPs-antiHA/ALP	Immobilization/detection	6.8×10^{-12} M	[95]
DPV	H5N1 AIV	AuNPs	Recognition of the immobilized probe	0.1×10^{-12} M	[43]
DPV	HA	Streptavidin-coated magnetic NPs/CdS QDs	Specific isolation/detection	*n. a.*	[50]
PFP	H9 AIV	CdTe QDs	Detection of proton flux	*n. a.*	[49]
IA	H5N1 AIV	Ab-coated magnetic NPs	Magnetic immunoseparation	*n. a.*	[96]
FC	Nucleic acid	magnetic NPs/QDs	Specific isolation/detection	25-mer DNA sequence	[97]
IA	H5N2 AIV	magnetic NPs/AuNPs	Specific isolation/detection	10 ng/mL	[98]
IA	H5N1 AIV	CdTe QDs	Recognition of immobilized biomolecules	1.5×10^{-4} μg/mL	[48]
IA	HA	Magnetic nanobeads	Recognition of the immobilized probe	0.128 HAUs	[62]
IA	AIV peptide	AuNPs	Reaction with the peptide	*n. a.*	[81]
IA	H5N1 AIV	Ab-coated, electrically active, magnetic NPs	Act as the transducer in an electrochemical biosensor	*n. a.*	[99]
RLS	AIV antigen	Magnetic silica NPs	Recognition of the immobilized probe	0.15 ng/mL	[61]
OA	NM	Silica NPs	Specific binding/detection	1 pg/mL	[80]
PD	H5N1 AIV	GlycoNPs	Binding/colour change	*n. a.*	[78]
IA	AIV	Ab-conjugated AuNPs	Specific binding/signal amplification	*n. a.*	[24]
DPV	Nucleic acid	CdS QDs	Specific binding/detection	15 ng/mL	[55]
Impedance	AIV	AuNPs	Specific binding/detection	*n. a.*	[100]
IA	Nucleic acid	Streptavidin-coated AuNPs	Specific binding/detection	10^2 copies of RNA	[72]
IA	HA	AuNPs	Immobilization of Ab-binding fragments	2.2 pg/mL	[71]
LRFT	Nucleic acid	Oligonucleotide-conjugated AuNPs	Specific binding/detection	7 pM	[73]
IA	H1N1 AIV	Ab-conjugated AgNPs	Specific binding/detection	1.0×10^{-13} g/mL	[83]
IA	HA	GO	Specific binding/detection	2^{-15} HAUs/50 μL H5	[87]
IA	H9N2 AIV	AuNPs	Detected by a colour change	17.5 pg/mL	[75]
Impedance	H5N1 AIV	AuNPs	Signal amplification	0.25 HAUs	[69]
Impedance	HA	AuNPs	Specific binding/detection	0.6 pg/mL	[70]
Amperometry	H1N1, H5N1 & H7N9	ZnO nanorods	Specific binding/detection	1 pg/mL	[101]
IA	H7 AIV	AgNPs-G	Specific binding/detection	1.6 pg/mL	[84]
IA	H7N9 AIV	AuNPs	Signal amplification	1.25 pg/mL	[53]
Amperometry	Nucleic acid	G-ZnO nanocomposite	Specific binding/detection	7.4357 μM	[88]
SERS	H3N2 AIV	Fe_3O_4/Au NPs	Support and capture substrates	10^2 $TCID_{50}$/mL	[102]

LOD - limit of detection, DPV - differential pulse voltammetry, SWV - square wave voltammetry, EID - effective interaction depth, HAUs - haemagglutination units, FRET - Förster resonance energy transfer, LSV - linear sweep voltammetry, PFP – proton flux pumping, IA - immunoassay, FC - flow cytometry, PPNWs - polypyrrole nanowires, RLS - resonance light scattering, OA - optical analysis, NM - neuraminidase, PD - plasmonic detection, LRFT - luminescence resonance energy transfer, G - graphene, SERS - surface enhanced Raman scattering spectroscopy, $TCID_{50}$ - tissue culture infection dose at the 50% end point, and *n. a.* - not available.

potential zoonosis subtypes, i.e., H5, H7, and H9 [103]. Moreover, linear-after-exponential PCR represents a sensitive amplification assay that includes increased levels of multiplexing and rapid DNA sequencing of the amplicons without purification. Light-upon-extension RT-PCR provides a simple detection method that uses two primers, one of which is labelled with a fluorophore molecule, and generates a melting curve for analysis upon completion of the amplification reactions [104]. Microarrays and DNA chips represent techniques for the detection of large numbers of genetic elements after hybridization with target probes based on high density arrays. Some new technologies that enable the easy and rapid detection of proteins or nucleic acids and improved detection of the influenza virus include liquid-phase microarrays using luminex technology combined with flow cytometry and fluorescent microspheres. The proximity ligation assay labels proteins via nucleic acid amplification, which increases the potential utility of this method in detecting influenza viruses. This method is based on antibody-mediated recognition of viral or bacterial surface proteins that are coupled with DNA [105].

Next generation sequencing (NGS) techniques provide many possibilities regarding the genomic surveillance of avian influenza [106]. NGS tools are currently providing researchers unprecedented new data on genetic materials from avian influenza hosts. The identification of host genetic variants (i.e., SNPs) [107] have provided researchers new opportunities to understand the evolutionary boundaries of host/pathogen resistance. NGS also provides valuable information on the expression profile of the host during infection [108], bringing us closer to an understanding of pathogen-host interactions. The critical capacity of influenza virus for human-to-human transfer is not well-understood but has been documented on several occasions. This critical point will be easily overcome in the near future, likely leading to an increase in cross-species transfer to the spread of influenza viruses [109]. We believe that the genetic compatibility between species will play a major role in cross-species influenza transfer. Therefore, the collection of genetic information from hosts and pathogens will provide insights towards a better understanding of disease transfer in the future.

The application of antiviral drugs that target host components required for virus replication and propagation at the molecular level represents a significant approach for AIV control. Current attention is specifically focused on HA, matrix protein 2 and NA inhibitors, which directly inhibit host factors through single molecules or complex mechanisms (intracellular signalling cascades) [110].

The application of nanotechnology in the near future represents a new trend in the detection and treatment of AIV, particularly the use of QDs, localized surface plasmon resonance with metallic NPs, enhanced fluorescence and dye-immobilized NPs. The effective management of the influenza virus relies on early detection and warning, which are enabled by the use of various nanomaterials. Due to their properties (chemical, physical and electronic), nanomaterials should provide excellent tools for the early detection, early warning and effective treatment of disease threats. The modification of electro-analytical analyser with various nanomaterials will ensure large effective surface areas, high catalytic capabilities and high conductivities using transducers as effective mediators. This strategy will enable the transfer of electrons between the receptor active site and the electrode and rapid AIV detection. The modification of various fluorescent QDs with virus-specific antibodies or oligonucleotides will enable the highly sensitive and rapid detection of antigens (present in samples) using fluorescent readout signals, which can be detected using immunoassays, PCR methods or high resolution microscopy [111]. The simple chemical synthesis of AuNPs and possibilities for surface modification with different molecules, such as peptides, proteins, DNA and antibodies, should provide researchers additional opportunities to improve virus detection in the future. Due to their surface plasmon resonance properties, AuNPs aggregate, which leads to colour changes in solution the potential for the rapid and simple detection of influenza viruses [112]. Moreover, aptamers are considered future materials due to their unique structures and ability to be synthesized for specific molecules, which will promote improvements in detection methods. The modification of aptamers with various types of fluorescence molecules represents an easy method to detect AIVs using surface plasmon resonance. This procedure also allows researchers to monitor and specifically and simultaneously select the target-bound aptamers.

4. Conclusions

Although substantial progress in the development of new techniques for AIV control has been reported in the last few decades, more sensitive, simple and rapid techniques for controlling virus pathogenicity are still urgently required. Biosensors based on nanomaterials are attracting significant interest in detecting influenza viruses due to their electron transfer properties, mechanical strength, chemical stability, catalytic activity in electrochemical reactions and thermal and electrical conductivity properties. The application of different QD-, NP-, and carbon-based nanomaterials promotes easy interactions between these materials and viruses, enabling researchers to construct portable biosensing analyser that should effectively detect influenza viruses. Modification of the surface of magnetic NPs with aptamers, antibodies or oligonucleotides specific for viruses, even at low concentrations, enable the isolation of AIVs from blood, serum and other biological fluids using external magnetic fields; the isolated AIVs are then rapidly detected using labelled antibodies or other target-specific reagents. Taken together, the current state-of-the-art in AIV biosensors will soon provide analysers with selectivity and sensitivity that are comparable to or even better than conventional methods, such as ELISA or PCR. Their possible *in situ* applications will be very important for controlling future epidemic and pandemic situations that will require the fusion of several technologies.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2017.06.045.

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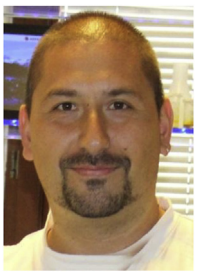

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