

Review Article

Antibody-Based Biosensors for Detection of Veterinary Viral Pathogens

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ABSTRACT

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Improved, cost-effective and rapid diagnostic are highly desirable for detection of veterinary pathogens. They are further desirable for veterinary viral pathogen detection as these pathogens generally cause major ailments in animals. Additionally, there are numerous emerging and reemerging viral pathogens with many of them being zoonotic, and having public health implications. However, the conventional methodologies for viral detection possess numerous lacunae and, subsequently, they fail to provide the indispensable and timely advantages desired for early diseases intervention. Biosensors offer a lucrative alternative to pathogen detection and their global market is rapidly increasing. Antibody-based biosensors are a class of biosensors with high specificity and have the potential of revolutionizing pathogen detection. They offer numerous advantages over the conventional or molecular methodologies, with the most significant being the option of "on-site" pathogen detection. As of yet, there are limited reports of the application of antibody-based biosensors in veterinary viral detection. However, we feel this technology holds a lot of potential, especially in wake of the recent developments in the areas of antibody-generation, nanotechnology and microfluidics along with the availability of improved antibody immobilization strategies. Consequently, it remains to be seen if biosensors can seize a part of the growing veterinary diagnostics market.

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INTRODUCTION

Infectious diseases of animal not only account for economic losses rendered by increased treatment costs, loss of production, morbidity and/or mortality but, additionally, many of these diseases pose public health risk as the pathogens involved harbor the potential of transmission to humans. The risks are further aggravated in case of viral infections which are comparatively difficult to detect and treat. The pathogens involved possess the ability to adapt themselves for survival, by mechanisms such as mutation, recombination, reassortment, infecting new hosts and acclimatizing to new environment.

Viral pathogens are responsible for some of the major infectious diseases of animals (Palmarini, 2007). A number of such diseases, with huge economic repercussions, e.g. classical swine fever (CSF), foot-and-mouth disease (FMD), infectious bursal disease (IBD), bovine virus diarrhea (BVD), canine distemper, swine influenza (SI), chicken infectious anemia (CIA), avian influenza (AI), rinderpest, bluetongue disease, peste-des-petits ruminants (PPR), Newcastle disease (ND), sheep and goat pox, infectious bovine rhinotracheitis (IBR), Marek's disease (MD), pseudorabies, porcine reproductive and respiratory syndrome (PRRS), etc. have been a thorn in the flesh of veterinarians for years now (Balamurugan and Kataria, 2006; Palmarini, 2007; Patel and Heldens, 2009). In addition, recent times have seen emergence of a number of emerging and re-emerging veterinary viral pathogens (Pépin and Tordo, 2010) and a high percentage of them have zoonotic implications (Heeney, 2006).

Rapid Diagnostics - Need of the Hour

Traditional methodologies for viral detection include isolation, in vitro culture, electron microscopy and immunoassays. These methods require specialized technical staff and equipment, and are strenuous, time-consuming, expensive and mostly insensitive (Villarreal, 2010; Dahlhausen, 2010). In addition, conventional assays lack the convenience of "on-site" testing and require a complex work flow starting from sample collection, sample labeling, sample storage and transport to appropriate facility, followed by further sample processing, after which the samples are assayed and the results are interpreted. In the meantime, there is risk of alleviating diseases conditions, spread on infectious disease affecting more population than the initial count and even death due to absence of appropriate disease intervention (Dahlhausen, 2010). Apart from the aforementioned disadvantages, possibilities of variations induced by the personnel, transportation of samples, processing and the testing conditions and lack of uniform analytical platforms further complicates the process making the data unreliable. Additionally, many of the emerging and reemerging viruses can only be handled at laboratories having recommended biosafety facilities (Poon et al., 2009) which are not very prevalent, even in developed countries. These high risk viruses also present a risk of accidental release while shipping or handling of samples causing further spread and, sometimes, serious public health issues (Poon et al., 2009). Subsequently, there is an impetus for rapid detection of viral pathogens to

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expedite inferences and, consequently, help in development of prompt measures to arrest the disease progression.

Molecular detection methodologies revolutionized the field of pathogen detection in last few years and have shown better sensitivities than antibody-based assays (Arora et al., 2006). However, these tests though rapid, have their drawbacks. Most of these methods require nucleic acid extraction, skilled personals and equipment, and are costly. The ever growing diagnostic market now emphasizes on generating sensitive and portable assays capable of rapid diagnosis. Modern technologies have made it possible to assemble complex analytical platforms into a single miniature device, biosensors, capable of multitasking from sample processing to detection within seconds to minutes, resulting in rapid turnaround time (TAT). Biosensors are easy to use and do not require trained personnel, laboratory equipment or reagents, thus, capable of testing and yielding results onsite, cutting short the lengthy process. **Biosensors**

A biosensor is a compact analytical device with a ligand-specific biorecognition element, e.g. antibody, enzyme, receptor, nucleic acid, aptamers, peptide/protein, lectin, cells, tissue or whole organisms, immobilized on a sensor surface integrated

directly or indirectly with a signal conversion unit called transducer. The physiological interaction between the ligand and the biorecognition element is translated, by the transducer, into a measurable electric signal, which is further deciphered by a computer-aided readout system for the user (Arora et al., 2010). Biosensors are chiefly classified based on the biorecognition element and the transducers. Antibody-based biosensors employ antibody as biorecognition element (Figure 1) and herein we will be focusing only on them as they are well suited for development of point-of-care-diagnostics and "onsite" diagnostics (Conroy et al., 2009). Consequently, a number of antibody-based biosensors are reported in literature. Table 1 presents some of the recent works on development of antibodybased biosensors for detection of viral pathogens of veterinary significance. Some of these biosensors were developed exclusively to aid in diagnosis of veterinary and zoonotic viruses (Stringer et al., 2008; Luo et al., 2010; Lum et al., 2012), a few were developed for detection of human viral pathogens but can be applied to veterinary diagnostics (Cavalcanti et al., 2012; Tran, 2012), while others were developed as surrogate to similar human viral pathogens (Connelly et al., 2012; Yakes et al., 2013).

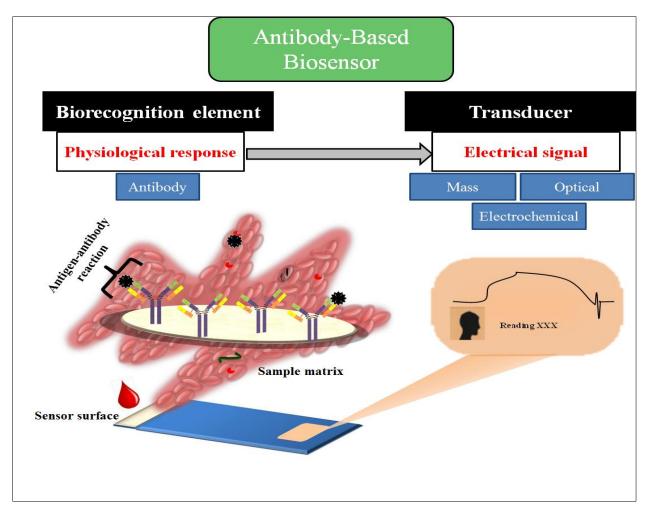


Figure 1: A schematic representation of an antibody–based biosensor; The specific physiological interaction between the antibodies coated on the sensor surface and their target analyte are analyzed in terms of change in the mass, electrochemical or optical characteristics, which are measured and translated in to electric signals *via* transducers. Generated signals are interpreted by a computer–aided recognition system that provides the final reading to the user in an easy to comprehend format.



Table 1: Antibody-based biosensors for detection of viruses of veterinary significance

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Virus/Virus Subtype	Antibody Type	Transducer Type	Limit of Detection	Time	Reference
Electrochemical transducers					
Avian influenza virus (AIV) subtype H5Nl	Polyclonal antibody (pAb)	Impedimetric	$10^3 \mathrm{EID}_{50} \mathrm{mL}^{-1}$	Less than 2h	(Lum et al., 2012)
AIV subtype H5N1	pAb	Impedimetric	$10^3\mathrm{EID}_{50}\mathrm{mL}^{-1}$	Less than 2h	(Wang et al., 2009)
AIV subtype H5N2	Monoclonal antibodies (mAb)	Impedimetric	10 ^{1.2} EID ₅₀ mL ⁻¹	Less than lh	(Wang et al., 2011)
Avian metapneumovirus (aMPV)	Antiserum	Conductometric	10 ² TCID ₅₀ mL ⁻¹	2 h	(Bhattacharya et al., 2011)
AIV subtype H5	mAb	Conductometric	$0.0128~HA~unit/50~\mu L$	Not specified	(Ronghui Wang et al., 2010)
AIV subtype H5	pAb and mAb	Voltametric	1.4 μM recombinant H5 HA	10 min	(Kamikawa et al., 2010)
Bovine viral diarrhea virus (BVDV)	pAb and mAb	Conductometric	10^3 CCID mL ⁻¹	8 min	(Luo et al., 2010)
Dengue virus	mAb	Voltametric	0.33 ng mL ⁻¹ NS1 protein	Not specified	(Cavalcanti et al., 2012)
Dengue virus	mAb	Amperometric	12 ng mL ⁻¹ NS1 protein	Not specified	(Dias et al., 2013)
Japanese encephalitis virus (JEV)	Antiserum	Impedimetric	0.75 µg mL ⁻¹ JEV antigens	About 20 min	(Huy et al., 2011)
JEV	Antiserum	Conductometric	10 ng mL ⁻¹ JEV antigen	Not specified	(Tran et al, 2012)
Rabies virus	pAb	Impedimetric	0.5 μg mL ⁻¹	Not specified	(Hnaien et al., 2008)
Swine-origin influenza virus (S-OIV) subtype HIN1	Antiserum	Conductometric	180 TCID ₅₀ mL ⁻¹	Not specified	(Lee et al., 2011)
West Nile virus (WNV)	mAb	Voltametric	2 viral particles per 100 mL	30 min	(Nguyen et al., 2009)
Optical transducers					
AIV subtype H5N1	pAb	Fluorescence	3 ng μL ⁻¹ H5Nl	Not specified	(Nguyen et al, 2012)
AIV	Not specified	Resonance light scattering	0.15 ng mL ⁻¹ AIV antigen	Not specified	(Zou et al., 2012)
BVDV	mAb	Light scattering	10 TCID ₅₀ mL ⁻¹	Less than 5 min	(Heinze et al., 2009)
Duck hepatitis virus serotypel (DHVI)	pAb	Ellipsometry	$8 \times 10^{-9.5} LD_{50} mL^{-1}$	30 min	(Huang et al., 2011)
Feline calicivirus (FCV)	pAb	Surface plasmon resonance (SPR)	10 ⁴ TCID ₅₀ mL ⁻¹	Less than 15 min	(Yakes et al., 2013)
FCV	pAb and mAb	Fluorescence	$1.6 \times 10^{5} \text{PFU mL}^{-1}$	Not specified	(Connelly et al., 2012)
Foot-and-mouth disease virus (FMDV)	mAb	Optical	Simple yes/no readout	Not specified	(Bhatta et al., 2012)
Infectious bursal disease virus (IBDV)	mAb	SPR	2.5 ng mL ⁻¹	Not specified	(Hu et al., 2012)
Porcine Rotavirus	pAb	Reflectance	-36 FFU	30 min	(Pineda et al., 2009)
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)	mAb	Fluorescence	3 particles µL ⁻¹	Not specified	(Stringer et al., 2008)
PRRSV	pAb	Ellipsometry	$2.4 \times 10^{3} \text{CCID}_{50} \text{mL}^{-1}$	20 min	(Chen et al., 2013)
Rabies virus	mAb	SPR	~ 70 pg mL ⁻¹ nucleoprotein and glycoprotein	Not specified	(Xu et al., 2012)
S-OIV subtype H1N1	pAb	SPR coupled with fluorescence	8.25×10^4 copies mL ⁻¹	Not specified	(Chang et al., 2010)
S-OIV subtype H1N1	pAb	SPR	30 PFU mL ⁻¹	Less than 20 min	(Su et al., 2012)
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Virus/Virus Subtype	Antibody Type	Transducer Type	Limit of Detection	Detection Time	Reference			
Mass-based transducers								
AIV subtype H5Nl	pAb	Bulk acoustic waves (BAW)	0.0128 HA unit	Less than 2 h	(Li et al., 2011)			
Coxsackie virus B4	mAb	Surface acoustic waves (SAW)	Not specified	Not specified	(Bisoffi et al., 2008)			
Influenza A and B viruses	mAb	BAW	10 ³ PFU mL ⁻¹	Not specified	(Peduru Hewa et al., 2009)			
Sin Nombre virus (SNV)	scFv	SAW	7000 viral particles	Not specified	(Bisoffi et al., 2008)			
Miscellaneous detection								
Bluetongue virus	pAb and mAb	PCR and real-time fluorescence PCR	10 ⁻² fg mL ⁻¹ recombinant VP7	Not specified	(Yin et al., 2012)			

CCID: cell culture infective dose; EID: embryo infectious dose; FFU: focus-forming units; HA: hemagglutinin; LD: lethal dose; NSI: non-structural 1 protein; PFU: plaque-forming unit; TCID: tissue culture infectious dose; VP7: viral protein 7.

Antibodies as Biorecognition Elements

Recent times have witnessed a sudden surge in antibodies applications (discussed by Ayyar et al., 2012), contributed by advances in molecular biological techniques. Due to the simplification of protein expression, peptide synthesis and purification processes, target antigen can be generated in large amounts for antibody production and characterization. Further development of molecular platforms and recombinant DNA technology has aided in selection of high affinity antibodies and tailoring it to desired characteristics. Availability of improved antibody purification methodologies has also contributed to antibody's increased utilization (Ayyar et al., 2012).

Biorecognition is the key aspect in a biosensor design so it is very essential to choose the biorecognition probe carefully. Antibodies are undoubtedly the most popular class of biorecognition probes owing to their high binding affinity (10^{-7} to 10^{-11} M12 Kd) (Connelly and Baeumner, 2012) and explicit target specificity. These characteristics contribute to the sensitivity and specificity of the biosensor to detect the target in complex sample matrices.

Antibodies or immunoglobulins are Y-shaped proteins produced by B-cell as host's immune response to counter antigen. Antibodies bind the target antigen and destroy it in order to protect the host. A typical antibody (immunoglobulin G, IgG) (Figure 2) is composed of a fragment antigen binding (Fab) region and a fragment crystallizable (Fc) region, made up of four polypeptide chains: two heavy chains (50 kDa) and two light chains (25 kDa). These chains are further divided into variable and constant domains based on the sequence variation. A combination of variable heavy (V_H) and variable light (V_L) chain binds the antigen. The constant domain activates a cascade of events in the immune mechanism to inactivate the antigen. For biosensor application, a suitable host is immunized with the antigen of interest along with the adjuvant to achieve a specific antibody response against the target. Antibodies used as probes can be polyclonal, monoclonal or recombinant (Ayyar

Polyclonal antibodies (pAbs) are a mixture of antibodies produced against highly immunogenic regions of the antigen with different epitope specificities and clonal affinities, produced by the B cell population of the host in response to an antigen. Large animals such as rabbit, goat and sheep are preferred for pAb generation due to the volume of blood that can be drawn leading to the increased concentration of pAbs harvested from the animal serum. PAb generation is less time consuming and offers the advantages of being stable. PAbs are very useful in detecting similar antigens, contributed by multitude of antibodies obtained against various epitopes of the

antigen. However, there are issues of batch variation, lack of high affinity and singular epitope specificity (Ayyar et al., 2012), which can be essential in some of the biosensor applications. Monoclonal antibodies (mAbs) are obtained by fusion of antibody producing B-cells with the immortal myeloma cells, which leads to the generation of hybrid cells called 'hybridoma'. Hybridomas retain the antibody-producing feature of B-cells and the immortality processed by the myeloma cells. Each Bcell produces antibody against a single epitope of the antigen, which after rigorous selection and cloning-out procedure are isolated and antibodies are produced from hybridoma cells with single clonal composition. Murine hosts are commonly used for mAb generation, however, there are reports of using rats and rabbits for this purpose. MAbs offers the advantages of being reproducible, large quantity generation, homogeneous composition with high epitope specificity and binding affinity. However, mAb production is expensive and time consuming compared to pAbs and its development requires considerable skills. Recombinant antibodies (rAbs) are antibodies or antibody fragments generated in vitro using molecular techniques. Recombinant antibodies are made by combining the inherent property of immune system along with random recombination of V_H and V_L, to generate of a vast library of antibodies, which is further screened for specific binders (e.g. Ayyar et al., 2010; Welbeck et al., 2011). RAb generation and isolation is usually time consuming, however, development of large diverse libraries using synthetic and semisynthetic approaches has made it possible to isolate rAbs against various target without going through the tedious immunization and library construction procedures, thus saving time and resources. They are advantageous compared to pAbs and mAbs due to the fact that by employing combinatorial recombinant technologies large library of antibodies can be generated. Antigen specific-selection is done by using molecular display platforms (phage display, ribosome display and yeast display) and further screening based on binding kinetics can be carried out through high-throughput methods (Saerens et al., 2008). Rabs are amenable to engineering for improving its characteristics such as binding, stability, purification and specificity along with the feasibility of format optimization. Discovery of single chain variable fragments (scFv), camelids (VhH) and shark V-NAR, has apprehended much attention in last few years (Saerens et al., 2008; Conroy et al., 2009). These single chain fragments exhibit high stability, expression and purification properties. As biosensor probes they may serve as a better alternative to full length antibodies due to their small size that can help in high density immobilization and reduced



non-specific binding due to the absence of Fc fragment (Saerens et al., 2008).

Transducers

A transducer converts the biorecognition event into a measurable signal. Many different types of transducers have been described, however, most of them can be classified as

electrochemical, optical, mass-based and calorimetric with the first three being the commonly employed ones for pathogen detection (Luong et al., 2008; Velusamy et al., 2010; Monošík et al., 2012).

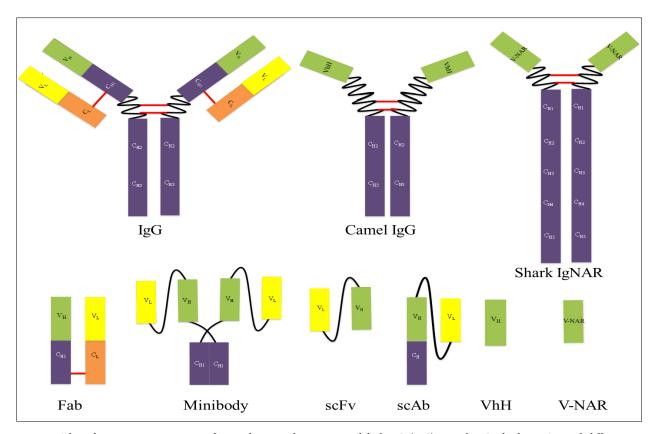


Figure 2: The schematic representation of typical mammalian immunoglobulin G (IgG), camel IgG, shark IgNAR and different recombinantly generated antibody fragments. The molecular weights of the presented antibodies range from 12 kDa – 150 kDa; The V_H and V_L domain of antibody / fragments combine together to bind their cognate antigen, however, VhHs and V–NARs bind the antigen by their variable heavy domain only. The number of antigen binding sites determine the valency of the antibody; Depending on the purpose of antibody application, multivalent antibodies with single or multiple specificities can be constructed; For biosensor application mostly mammalian IgG have been exploited till date; However, nowadays there is increased interest in use of recombinant antibody formats like scFvs, VhHs and V–NARs.

Electrochemical biosensors measure the change in electrical properties following biorecognition, as a result of production or consumption of the ions or electrons (Mohanty and Kougianos, 2006). Electrochemical biosensors are further classified into amperometric, potentiometric and impedimetric conductometric (Velusamy et al., 2010). (i) Amperometric biosensors measure the generated current at a constant potential and are the most commonly used class of electrochemical biosensors (Velusamy et al., 2010). A variant of amperometric biosensors are voltametric biosensors that measure electrical current during controlled variations of the potential. (ii) Potentiometric biosensors measure difference in potential (Velusamy et al., 2010). (iii) Impedimetric / conductometric biosensors function by measuring the change in electrical resistance / conductance of the solution (Mohanty and Kougianos, 2006).

Optical biosensors measure changes in intensity of light. Detection elements in such biosensors are frequently based on luminescence, fluorescence, phosphorescence, colorimetry, reflectance, light polarization and rotation, interference, spectroscopy, ellipsometry and surface plasmon resonance (SPR) (Luong et al., 2008; Velusamy et al., 2010; Huang et al., 2011). However, fluorescence and SPR-based biosensors are most common (Velusamy et al., 2010).

Mass-based biosensors detect a change in mass that occurs following the interaction between the biorecognition element and the target analyte (Mohanty and Kougianos, 2006; Holford et al., 2012). Such sensors generally use piezoelectric materials that change their resonant frequency, following the change in mass, generating acoustic waves. The traveling wave either propagates along the surface of the substrate (surface acoustic wave, SAW) or through the surface of the substrate (bulk acoustic wave, BAW) (Rocha–Gaso et al., 2009). The most commonly used piezoelectric biosensors employ Quartz Crystal Microbalance (QCM) (Holford et al., 2012) and is based on bulk acoustic wave propagation.



Immobilization of Antibodies to the Sensor Surface

Apart from selecting suitable antibodies for probing, a critical factor that influences biosensing mechanism is the immobilization of the antibodies to the sensor surface, which in turn depends on the properties of the sensor interface. Various types of sensor surfaces have been studied (gold, silver, glass, platinum, silica) for this purpose. Ideally sensor surface should be stable, providing large surface area for high density probe immobilization, possessing excellent electrical and thermal conductivity, low diffusion rates, and less signal-to-noise ratio due to matrix effects (Holford et al., 2012). It is hard to get all the desired characteristics on one surface and still keep it small for the device to be portable. Advent of high performance matrices such as carbon nanotubes, fabricated nanoparticles, self-assembled monolayers (SAMs) and quantum dots has led to the development of new generation sensor platforms compatible with the aforementioned sensor needs (Holford et al., 2012). The antibodies can be coupled to the sensor surface by various methods. Passive absorption, covalent coupling, matrix entrapment, encapsulation and affinity tags are the most commonly used methods with their own pros and cons.

Passive Absorption

Physical absorption is a simple process in which the antibodies attach to the sensor surface by Van der Waals, hydrogen or hydrophobic interactions or a combination of all. This process requires minimal antibody manipulation, leaving the antibody unmodified, achieving a high immobilization level. However, as the antibodies are attached by weak interactions, such surfaces have issues of antibody leakage, random antibody orientation, uneven distribution and no tolerance to surface regeneration (Saerens et al., 2008).

Covalent Coupling

Covalent coupling is the most commonly employed method for antibody immobilization to the sensor surfaces. These surfaces are available commercially or can be chemically activated by using glutaraldehyde, periodate, carbodiimide and maleinimides succinimide esters to crosslink the antibodies. Covalent coupling provides a uniform and a stable surface immune to issues like aggregation or antibody leaching over time or regeneration procedures. Conversely, the immobilization requires the manipulation of antibodies, which may render it non-functional or may even degrade it in the process. This can be avoided by protecting the antigen binding region in the antibody before modification (Yoon et al., 2011). General covalent coupling procedures do not ensure proper orientation of the antibodies, which is important for antigen binding and in turn biosensor sensitivity. There are many ways to overcome this issue for e.g. identifying free thiol groups, farther from the active site, or engineering one in the antibodies can help in directional orientation of the antibodies on the gold surface either directly or through a linker or using an intermediate layer such as protein A or G which can be covalently coupled to the surface (Makaraviciute and Ramanaviciene, 2013).

Matrix Entrapment

Matrix entrapment relies on trapping the antibody into a polymeric gel matrix and then immobilizing to the sensor surface. The matrices are thin and porous to allow antigenantibody interaction. Most commonly used matrices are starch, cellulose, alginate, polyacrylamide, polycarbonate, polyurethane and silica gel. This is a simple and a reliable method of antibody immobilization generating a stable surface, however, it is necessary to ensure that the used matrix is compatible with the sensor surface and will not interfere with antibody interaction (Gupta and Chaudhury, 2007; Monošík et al., 2012).

Affinity Tags

Mostly recombinant antibodies are expressed by conjugating it genetically with peptides / proteins, which acts as an affinity tag, which facilitates its purification and detection. This immobilization technique is based on covalent immobilization of a specific binding partner to the affinity tag on to the sensor surface, creating a stable surface. The chimeric antibody, to be used as biorecognition probe, is passed over the sensor surface causing a directional orientation of the antibody due to affinity tag. Such immobilization procedure requires less/no antibody modification, mild regeneration conditions and the amount of the probe immobilized on the surface can be controlled. This method keeps the binding regions of the probe free and very beneficial in the regard that crude lysate containing antibody can be used, as only specific antibody will be immobilized (Ayyar et al., 2010).

Conclusions and Future Perspectives

It has been more than 50 years since the inception of biosensors (Clark and Lyons, 1962). However, to date only a few biosensors have been commercialized (reviewed by Luong et al., 2008). The main drawbacks that have traditionally prevented the biosensors, in general, from reaching market have been lack of sensitivity, lack of stability and lack of applicability to unprocessed samples (Luong et al., 2008; Monošík et al., 2012). Antibody-based sensors exhibit high specificity owing to the specific interaction, and high affinity and avidity of an antibody with its respective antigen (Saerens et al., 2008; Conroy et al., 2009) and thus, serve as good candidates for use with unprocessed samples. However, in some cases matrix effects may necessitate certain remedial measures (Johnsson et al., 2002; Rodriguez-Mozaz et al., 2006). Most of the reported antibody-based biosensors use polyclonal or monoclonal antibodies (Conroy et al., 2009; Arora et al., 2010) that have stability issues associated to them. In addition, these full antibodies might lose their activity following immobilization on to the sensor surfaces due to disorientation or stearic hindrances (Saerens et al., 2008). However, the availability of chicken antibodies, VhH, V-NAR and different recombinant antibody formats (Figure 2) along with improved immobilization chemistries have helped overcome many of these concerns (Schade et al., 2005; Saerens et al., 2008).

Advances in nanotechnology and microfluidics have allowed miniaturization and subsequently, development of point-of-care and lab-on-a-chip diagnostics (Luong et al., 2008; Wang, 2013). This has revolutionized the field of biosensing and thus, there is increased interest in biosensors. Consequently, there is market expansion in the biosensor market which is expected to be worth by US\$16.8 billion by 2018 (http://www.marketresearch.com/Industry-Experts-v3766/Biosensors-Global-Overview-6846583/). However, veterinary pathogen detection is still to harness this technology to its benefit with biosensing currently finding use chiefly in mastitis detection (Viguier et al., 2009).

Viral veterinary diagnostics can greatly benefit from biosensors allowing rapid, robust cheap and simple alternatives to conventional viral detection methodologies. In addition, biosensors allow "on–site" testing, and can be performed and interpreted, within a matter of seconds or minutes, by farmers or veterinarians. This is a lucrative preposition compared to collection and shipment of samples followed by waiting for weeks to get results. Consequently, biosensors can allow veterinarians to provide specific and timely treatment to animals and thus, reducing the resulting morbidity and mortality. Additionally, it allows checking the spread of contagious pathogens to another animals and humans (in case of zoonotic pathogens).



There have been a few studies on development of biosensors for detection of veterinary viral pathogens (Table 1). However, none of the biosensors for veterinary virus detection has made to market yet and it can be rightly concluded that biosensing for veterinary pathogens is still in its infancy. It needs to be seen if the global technology push coupled with the inevitability of cheap, rapid and "on–site" diagnostics in veterinary sector will instigate an upsurge of interest in biosensor development for veterinary pathogen detection.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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