Chapter 18
Biosensors: Modern Tools for Disease Diagnosis and Animal Health Monitoring

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Abstract Animals play a vital role in our lives as they provide milk, meat, and other by-products for daily consumption. At the same time, their health directly/indirectly affects human beings as we both share a common environment. The One Health concept makes it imperative that the animals should remain healthy as it will have an impact on our health as well as economy. Animal disease diagnosis, their health monitoring, prevention, and control of diseases are important from this aspect. Tools for rapid diagnosis of animal diseases are crucial for early diagnosis and imposing control measures. Molecular techniques such as, genome sequencing, restriction fragment length polymorphism (RFLP), DNA microarray, PCR, and real-time PCR are some of the rapid techniques, but they need expertise and sophisticated labs. Conventional methods such as isolation of the pathogen, serological techniques, are laborious and time taking process. Therefore, to overcome the said limitations of conventional and molecular tools, biosensors are the better alternatives as they are rapid and can be used as pen-side diagnostic tests. They can provide test results in a few minutes under field conditions. In this chapter, we give an elaborative description of various types of biosensors and their utility in animal disease diagnostics and health monitoring.

Keywords Biosensors · Animal disease diagnosis · Field-based tests · Rapid tests · Animal health monitoring

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18.1 Introduction

Globally, infectious diseases are the leading cause of mortality in man and animals. Infectious diseases of both domestic and wildlife pose a serious health risk to the human race as exhibited by the current ongoing COVID-19 pandemic. These infectious diseases can be of zoonotic origin affecting not only human health, but also, animal health and production. Such diseases pose a serious threat to a country’s growth, economy, and health perspectives. To prevent the spread of infectious diseases it is imperative to develop rapid and sensitive tests for early detection of the causative agents. It aids in adopting suitable preventive measures, compartmentalization of the infected zones, and designing strategies to prevent further spread of the disease. The viral agents can be detected in the clinical samples by gold standard virus isolation in cell culture system. This method has well-known merits and demerits such as economic investment, expert manpower, and time-consuming lengthy protocols (from days to weeks). On the other hand, molecular detection methods such as conventional Polymerase Chain Reaction (PCR), Real-time PCR, although they are both sensitive and specific, cannot be employed for pen-side diagnosis due to the involvement of sophisticated instruments.

In such a scenario, the development of a rapid, sensitive, and specific pen-side test is the need of the hour and is a challenge for the scientific community to combat the emerging and re-emerging diseases. With the limited infrastructure for the diagnosis of the emerging and re-emerging infection, biosensors can be an effective tool to perform field-based diagnosis and animal health monitoring. Easy handling, user-friendly, and minimum processing endows biosensor an effective tool for disease diagnosis.

In the past few decades, a lot of work has been done on this aspect for the detection of both viral and bacterial pathogens. Various molecules like nucleic acids, antibodies, antigens, and proteins of animal origin are detected in the clinical samples in these biosensing elements.

18.2 Biosensors Principles and Types

According to The National Research Council (part of the US National Academy of Science), a biosensor is defined as a detection device that incorporates a living organism or product derived from a living system as the biorecognition element (BRE) or a bioreceptor and a transducer (electrochemical, optical, or mass-based) to convert a biological reaction into a quantifiable signal. The BRE can be enzymes, nucleic acids, antibodies, aptamers, bacterial cells, bacteriophages. The first biosensor was found by Clark and Lyon in 1962 for glucose detection.

The boost up in the field of biosensor came from the pioneering work done by a group of scientists in the year 1962–1969 (Clark and Lyons 1962; Guilbault et al.
1962; Updike and Hicks 1967; Guilbault and Montalvo 1969). Later on, an electrochemical biosensor had been described using ferrocene to detect electroactive species (Di Gleria et al. 1986). This invention led to the commercialization of a glucose pen by the company Medisens. After these reports, there has been a large amount of work done in the domain of biosensors which has an extensive application for environmental sensing and biological monitoring (Luong et al. 2008).

The principle of a biosensor is based on the detection of a biomarker molecule in the clinical sample (Fig. 18.1). This biomarker can be any protein, antigen, or antibody of the infectious agent. The biomarker molecule is detected by the bioreceptor which is immobilized on a chip or another base. This bioreceptor could be DNA, RNA, monoclonal antibody, protein, or any cell. However, it is important to choose the bioreceptor carefully as it is the deciding factor for the sensitivity and specificity of a biosensor. Although there are several types of bioreceptors, the most common types used are nucleic acids, enzymes, and antibodies. The interaction between the biomarker and bioreceptor generates signals via transducer which are read and interpreted. The signals generated give information regarding the presence or absence of the pathogen in the sample.

Biosensors can be classified on the basis of biorecognition elements or transducer, more details are discussed below.

**Fig. 18.1** Principle of biosensor for disease/pathogen detection
18.2.1 Biorecognition Element (BRE)

The type of BRE to be used depends on the target molecule to be recognized. Each of the BRE has its own advantage and disadvantage, hence, its selection depends on the application. Various types of BREs used are as follows:

18.2.1.1 Antibodies

Antibodies are the most commonly used BRE and their use does not require antigen purification during sample addition (Chambers et al. 2008). Monoclonal, polyclonal, and recombinant antibodies can be used. Monoclonal antibodies recognize a single antigenic epitope hence detects the antigen with less sensitivity in comparison to the polyclonal antibodies. Polyclonal antibodies recognize several epitopes and are able to detect the antigen with more sensitivity than the monoclonal antibodies. However, polyclonal antibodies show batch-to-batch variation in titre and reactivity in comparison to monoclonal antibodies which show unique specificity in all batches (Nelson et al. 2000). The third generation of antibodies known as recombinant antibodies are less expensive and time-consuming in production (Sharon et al. 2005; Haurum 2006; Chambers et al. 2008).

18.2.1.2 Nucleic Acids

Nucleic acids (NAs) can bind with the complementary sequence present in the sample through hybridization (Wetmur and Fresco 1991). One of the class of NAs that is used as BRE is Nucleic Acid Aptamers (NAAs) which are capable of binding two proteins (Ni et al. 2011). Such kind of biosensors which employ the use of NAAs are called as genosensors (Tosar et al. 2010; Martinkova et al. 2017). NAAs are high-affinity short stretch of oligonucleotides (Chambers et al. 2008). NAAs are easy to synthesize, are foldable, and easy to store, hence, they offer similar attributes as antibodies. Disadvantages of NAAs are degradation, cross-reactivity, and labeling costs (Chambers et al. 2008; Lakhin et al. 2013). Aptazymes are types of aptamers which have catalytic properties like enzymes (Chambers et al. 2008). Other types of aptamers are peptide nucleic acids (PNAs) which are synthetic DNA analogues having polyamide backbone (Chambers et al. 2008; Bonifazi et al. 2012; Briones and Moreno 2012; Gupta et al. 2017). Since, PNAs do not have any charge they have higher hybridization characteristics than NAs (Bonifazi et al. 2012; Briones and Moreno 2012).
18.2.1.3 Enzymes and Proteins

Enzymes are exclusively specific for their substrate hence are an excellent BRE for biosensors. Enzymes consist of protein components, cofactors, or prosthetic groups (Elnashar 2010). Enzymes are used extensively for biosensing purposes as they can measure a variety of reactions and the products generated thereof.

18.2.1.4 Cells

In certain cases, the immobilization of the enzymes may be difficult, or the isolation and purification of the enzymes may not be possible. In such a scenario, the whole cells offer a better alternative than enzymes as the enzymes inside the cell are stable for a long time. Also, the cells have multiple enzymes that can be used for several analytes in a single testing. The immobilized cells can also be genetically modified to express specific proteins to detect the analytes in the extracellular matrix (Fine et al. 2006; Jung et al. 2014; Vopálenská et al. 2015).

18.2.1.5 Biomimetics

In these kinds of biosensors, the non-biological receptors are used as BREs. These biosensors can mimic the original biological bioreceptor. The various forms of biomimetics are molecular imprinted polymers (MIP), membrane mimics, self-assembled monolayers (SAM), nanoparticles, nanostructured materials, and quantum dots (QDs). Molecular imprints aim to design artificial receptors like artificial antibodies. Nanoparticles and nanostructures along with other receptors can also act as biomimetics.

18.2.2 Classification of Biosensors on the Basis of Transducer

Transducers are the important components that determine the specificity of a biosensor as they are responsible to translate the biorecognition signals to measurable signals. Biosensors are mainly classified as Electrochemical, Mass-based, and Optical biosensors. These are further subclassified.

18.2.2.1 Electrochemical Biosensors

The electrochemical (EC) biosensors are the most common types of biosensors. They are inexpensive and have good bio-interaction. Due to the biochemical interaction on the sensor surface, the potential difference is measured as a recognizable signal. These
biosensors have been further classified into conductometric, impedimetric, potentiometric, and amperometric types which are based on the conductance, impedance, potential, and current generation, respectively (Lazcka et al. 2007; Setterington and Alocilja 2012).

The EC biosensors are the most successful biosensors which have been used commercially. However, the disadvantage associated with the EC biosensor is the random orientation and their immobilization without getting denatured (Jung et al. 2008). Potentiometric sensors measure the change in the potential at the electrode happening after the recognition of the analyte. However, this method comes with a limitation that for large molecules (bacteria) it cannot provide sensitive and specific signals. Amperometric sensors are the kind of sensors in which current is generated after oxidation or reduction due to analyte and bioreceptor interaction. The most common example of this type of biosensor is the measurement of glucose which is based on the amperometric sensing of glucose. These kinds of sensors impart good sensitivity but low specificity. Impedimetric sensors are the kind of biosensors which are good for detection of whole bacteria. The sensors are label-free, of low cost, high sensitivity, and are not affected by other analytes present in the sample matrix. Moreover, these can be miniaturized easily, hence, easily translated to the point of care systems. Conductometric biosensors measure the ability of an analyte to conduct current between electrodes or the reference electrodes. Several enzymatic reactions are capable of changing the ionic strength of the sample which can then be measured by a conductometric instrument.

With the recent advances in nanotechnology, the incorporation of nanoparticles has improved the diagnostic performance of the electrochemical biosensors by increasing the signal transduction. The signal transduction, which can be increased by using the nanomaterials along with the BREs can increase the signal significantly (Fig. 18.2). Nanoparticles provide increased surface area for more binding of the analyte and also have high conductance which results in increased signal intensity. The nanomaterials used are gold nanoparticles, iron oxide, graphene, and carbon nanotubes.

18.2.2.2 Optical Biosensors

Optical biosensors are useful in attending high specificity, sensitivity, and rapidity. It has been further divided into label-free and real-time detection. Based on the mechanism of the signal generation they have been further categorized as colorimetric sensors, surface plasmon resonance (SPR), fluorescence-based sensors, surface-enhanced Raman spectroscopy (SERS), and chemiluminescence-based sensors.
18.2.2.3 Mass-Based Biosensors

These biosensors are based on the principle of signal production which depend on the mass of chemicals interacting with a vibrating piezoelectric quartz crystal. Mass-based biosensors are label-free and offer increased sensitivity and specificity. The signal transduction is based on the minute change in the mass occurring on the sensor surface and is sensed by resonance phenomena (Turner and Zhang 2001). Various categories of mass-based biosensors are microcantilever-based (MCL) biosensors, surface acoustic wave (SAW) biosensors, and quartz crystal microbalances (QCM).
18.3 Applications of Biosensors in Animal Husbandry

Biosensors have a large application in the detection of pathogens, drug residues, food quality monitoring, animal location monitoring, and animal health monitoring (Fig. 18.3). Some of the details of the application of biosensors have been mentioned below.

18.3.1 Biosensors for Detection of Bacterial Infection

18.3.1.1 Detection of *Escherichia coli*

*Escherichia coli* causes disease related to gastroenteritis, urinary tract infection, peritonitis, and septicemia in cattle, sheep, goats, pigs, and poultry. *E. coli* is a gram-negative rod-shaped bacterium. For example, in poultry, it causes colibacillosis followed by the localization of this pathogen in the respiratory and urinary tract of the birds. The virulent strains causing colibacillosis are O1: K1, O78: K80, and O2: K1. In poultry, *E. coli* infection is characterized by reduced growth rate, decreases egg production, and high mortality subsequently causing heavy economic loss to the stakeholders. *E. coli* causes colibacillosis in other farm animals also like cattle, goats, and pigs (Bélanger et al. 2011). In newborn calves it causes septicemia and in milking cattle causes mastitis. Moreover, due to the excessive use of antibiotics, there is an increased incidence of antibiotic resistance in *E. coli*. Thus, it becomes
imperative to detect the virulent strains of *E. coli* and to differentiate between them from the non-pathogenic strains.

The conventional and molecular methods used for the detection of *E. coli* infection are isolation of bacteria from clinical samples, ELISA, and PCR. However, these methods involve high cost, time, and manpower (Hobson et al. 1996; Fournier-Wirth et al. 2006). To overcome these hurdles advanced methods involving the use of biosensor have been explored. For detection of *E. coli*, the novel biosensors such as surface plasmon resonance (Yazgan et al. 2014), quartz crystal microbalance system (Jiang et al. 2011), chemiluminescence (Zhang et al. 2014), and electrochemistry (Kim et al. 2015a; Guo et al. 2016; Idil et al. 2017) have been explored. The *E. coli* cells are captured by specific antibodies directed against *E. coli* antigens like flagella. The signals generated are subsequently amplified by using suitable detectable labels such as fluorophores, enzymes, and biofunctionalized nanoparticles. The biosensors based on sandwich format have been developed for the rapid detection of *E. coli*. In an electrochemical biosensor (Jaffrezic-Renault et al. 2007) the *E. coli* cells are captured by anti-LPS antibodies coupled with magnetic nanoparticles which are conducted on the graphite ink electrode. The use of immunomagnetic beads aid in the detection of analyte present in the test sample.

The conductometric method can detect *E. coli* from the culture from 1 to $10^3$ CFU mL$^{-1}$. The immunosensors based on conductometric method have been used successfully for detection of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from 10 to $10^3$ CFU mL$^{-1}$. These pathogens were found to be undetectable using immunoblot methods. This test was also found to be specific as the gram-positive bacteria *Staphylococcus epidermidis* was found to be undetectable (El Ichi et al. 2014).

A point of care test using stacked paper membranes has also been proposed (Eltzov and Marks 2016, 2017) which can quantify *E. coli* in less than five minutes. When the infected clinical sample is added to the bottom layer of the membrane, the bacterial cells are pushed up to the top layer during which they bind with anti-*E. coli* antibodies conjugated with horseradish peroxidase (HRPO) which allows measurement of generated signals. In the case of the negative sample, the anti-*E. coli* antibodies are prevented to move to the topmost membrane due to their binding with the immobilized bacterial cells on the capture layer. HRPO specific substrate produces signals only with *E. coli* cells bound with HRPO labeled antibodies. This test is 1000 times more sensitive than ELISA and was able to quantify up to 100 cells mL$^{-1}$. Other added advantages were its rapidity and portability.

### 18.3.1.2 Detection of *Clostridium Perfringens*

*C. perfringens* is an important enteric pathogen and all domestic animals and poultry are infected by this pathogen. It is a gram-positive spore-forming bacteria with 17 different toxins (Gibert et al. 1997; Petit et al. 1999) and is also an important agent of foodborne illness. Cell-based biosensors are one of the emerging technologies which
have mammalian cells as sensing elements to detect the pathogens or toxins in the clinical and food samples (Yoo and Lee 2016).

For this purpose, the mammalian cells fixed on 96 well plates and modified electrodes have been used (Banerjee and Bhunia 2010) to detect \textit{C. perfringens} and their toxins in the samples, as each type of \textit{C. perfringens} carries a unique sequence of virulence genes. In a microarray system, oligo probes against six toxin-producing sequences of \textit{C. perfringens} have been designed and used successfully (Sergeev et al. 2004). The fluorescently labeled PCR amplicon is hybridized with the oligoprobes which is subsequently read to detect the presence of \textit{C. perfringens} in the test sample. This platform enables the simultaneous detection of several bacterial strains and their toxins detected simultaneously (Volokhov et al. 2011).

Toxins of \textit{C. perfringens} can also be detected by using specific antibodies in an immunosensor. It has been used successfully for the detection of epsilon toxin from \textit{C. perfringens} (Palaniappan et al. 2013). The monoclonal antibody specific to the epsilon toxin was fixed on the carbon nanotube and was able to detect toxins with comparable sensitivity to ELISA.

18.3.1.3 Detection of \textit{Campylobacter}

\textit{Campylobacter} causes disease in wild animals, pets, domestic livestock, and human beings. \textit{C. jejuni} and \textit{C. coli} mainly infect cattle sheep, dogs, cats, pigs, and turkeys. \textit{C. fetus} mainly infects cattle, goats, and sheep. \textit{Campylobacter} causes enteritis with diarrhea, fever, vomiting, abortion, and infertility. To detect \textit{Campylobacter}, various bioreceptor molecules like proteins, aptamers, nucleic acids, antibodies, and cells have been used. Out of these, DNA-based biosensors are very useful due to their low cost, specificity, and less time required to detect the pathogen.

An organic light-emitting diode (OLED) biosensor has been used for the detection of \textit{Campylobacter} in meat samples (poultry) through capturing \textit{Campylobacter} DNA via a labeled DNA probe on a glass slide. This captured DNA is detected by a secondary DNA probe labeled with a fluorophore. It allows detecting \textit{Campylobacter} in the sample with a sensitivity of 0.37 ng\,\mu\text{L}^{-1} DNA and 1.5 \times 10^1 \text{CFU}\,\text{g}^{-1} (Manzano et al. 2015).

A biosensor based on surface plasmon resonance (SPR) has also been proposed for the detection of \textit{C. jejuni} (Wei et al. 2007); which can detect \textit{C. jejuni} up to 10^5 \text{CFU} \text{mL}^{-1}. An optical biosensor based on SPR and diffraction optic technology has also been developed (Gnanaprakasa et al. 2011) targeting the hippuricase gene of \textit{C. jejuni}. The complementary probe was thiolated and subsequently fixed on the gold surface of the chip. After hybridization of probe and hippuricase gene, it led to the transduction of an optical signal which could quantify \textit{C. jejuni} up to 5 \text{nM}. In another study (Wadl et al. 2009) a lateral flow device was developed to detect \textit{C. jejuni} or \textit{C. coli} which can detect pathogen up to 7.3 log of \text{CFU} \text{g}^{-1}.

In recent study, functionalized magnetic particles with tripled silica layer and coated with anti-\textit{C. jejuni} antibodies have been found to detect \textit{C. jejuni} in water with a detection limit of 10^2 \text{CFU} \text{mL}^{-1} (Zhang et al. 2015).
18.3.1.4 Detection of Salmonella

Salmonella is a gram-negative rod-shaped bacterium which is associated with diarrhea and food poisoning in man and animals. An important reservoir of Salmonella is poultry. Several serotypes of Salmonella are present which can infect both man and animals (Alcaine et al. 2006). Infection arises through contaminated food and drinking water. Several workers have tried to develop different forms of biosensors for the detection of Salmonella. A wireless and mass sensitive biosensor for the detection of S. typhimurium has also been developed which was able to detect $1.6 \times 10^2$ CFU mL$^{-1}$ (Chai et al. 2012). A lateral flow system developed by DuPont can detect Salmonella in 10 min. In this test, the samples are initially pre-enriched before sample addition to the strip which contains precoated antibodies against Salmonella.

A lateral flow immunochromatographic strip using the invA gene of the Salmonella as a target has also been developed (Bulut 2014). An aptamer-based lateral flow biosensor has been developed to detect Salmonella (Fang et al. 2014). It includes an amplification step which forms a single-stranded DNA followed by its deposition on a sensor membrane. This test has been found to have a sensitivity of $10^1$ CFU mL$^{-1}$.

Further improvement in Salmonella detection has also been made by using microfluidic devices where efficient binding of S. typhimurium bacterial cells has been achieved by using anti-Salmonella antibodies. By using quantum dots conjugated to antibodies a portable fluorometer was also developed which could detect Salmonella cells up to $10^3$ CFU mL$^{-1}$ in chicken meat extract (Kim et al. 2015b).

18.3.2 Biosensors for Detection of Viral Infection

18.3.2.1 Detection of Avian Influenza (AI) Virus

Avian influenza virus (AIV) poses a serious threat to mankind and has been divided into highly pathogenic AIV and low pathogenic AIV. Highly pathogenic AIV tends to rapidly spread which may cause high mortality. Infection by low pathogenic AIV results in mild gastrointestinal and respiratory symptoms but may turn into a highly pathogenic type after multiple infections (Morse et al. 2012). A variety of tests which include haemagglutination inhibition test, haemagglutination test, virus neutralization test, ELISA, virus isolation in cell culture, virus propagation in embryonated chicken eggs are available to detect the virus infection. However, these processes require large cost input, specific maneuvers, and infrastructure are required for their conduction.

Moreover, certain strains of Avian influenza-like H5N1 kills the embryos very quickly, thus, the amplification obtained in eggs is not enough to proceed further. Similarly, molecular methods require RNA extraction and the requirement of high Biosecurity Levels equipment’s. In such conditions, pen-side tests for detection provide a window for an early and rapid on-site detection (Chang et al. 2009; Chiou et al. 2010; Tseng et al. 2014). Some serological tests are also available to detect the
serological response against AI. However, they are endowed with low sensitivity and false-negative results due to which it limits the application of serological tests.

The site of the binding of the influenza virus is different for both humans and birds. In the case of humans, the virus binds to the $\alpha$-2, 6 sialic acid glycan of the upper respiratory tract. In the case of birds, it binds to the $\alpha$-2, 3 sialic acid expressed in the intestine. Hence, based on this differentiating feature, biosensors have been developed to detect and differentiate between the human and avian influenza viruses.

A biosensor based on glycan-immobilized field-effect transistor has been demonstrated to differentiate between the avian (H5) and human (H1) influenza viruses with a sensitivity of up to $10^{-18}$ mol L$^{-1}$ (Hideshima et al. 2013). Sialic acid bound to gold nanoparticles can bind with the HA of the virus, thus producing the signals for virus detection (Lee et al. 2013). The glycans have been printed in the glass slides to form a microarray (Dinh et al. 2014) which can detect various strains of influenza virus up to ten plaque-forming units. Portable lateral flow tests have also been developed for the diagnosis of influenza virus (Sajid et al. 2015). In these kinds of lateral flow assay, the sample to be tested is diluted in the detergent solution followed by its addition into a sample pad. Thus, the components in the sample start moving in the pad. If the sample is positive for the influenza virus, the virus particles bind with the anti-influenza antibodies conjugated with gold nanoparticles which are pre-embedded in the conjugate pad. The complex of virus-antibodies-GNP reaches the test lines where anti-influenza A and anti-influenza B antibodies are pre-coated. These antibodies are directed against the HA protein of influenza virus. In the case of positive samples, a visible red line is formed. The unbound GNPs reach the control line and bind with the secondary antibodies which subsequently form a red line. If the virus particles are absent in the test sample, only the GNP labeled antibodies will move and bind to the control line. However, these tests could not differentiate between the various subtypes of LPAIV and HPAIV.

This shortcoming can be overcome by using aptamers against AIV or RNA/DNA probes on the test lines, which enables the differentiation of subtypes as well. These lateral flow assays are simple and easy to use, thus, it makes them indispensable. To amplify the signal generated silver nanoparticles have been used successfully and amplification of 1000 times has been achieved (Huang et al. 2015). By using quantum dots a limit of detection of 0.09 ng mL$^{-1}$ was achieved for detection of AIV (Li et al. 2012) and a sensitivity of 100 times than ELISA was achieved.

For the quantitative detection of influenza A virus (subtypes H5 and H9) a quantum-dot-based lateral flow test has been developed (Wu et al. 2016). An electrochemical sensor employing the anti-M1 antibody has been found to detect all serotypes of influenza A virus (Nidzworski et al. 2014). After conjugation of the anti-M1 monoclonal antibodies with GNPs in a quartz crystal microbalance assay, a limit of detection up to $1 \times 10^3$ PFU mL$^{-1}$ has been achieved (Hewa et al. 2009).

An electrochemical immunosensor that detects and quantifies PB1-F2 protein of the influenza virus has been found to be sensitive for detection of influenza virus (Miodek et al. 2014a, b) with a limit of detection of 0.42 nM. A surface plasma wave biosensor has also been developed for the detection of Influenza A virus (Su et al. 2012). Although
this method is sensitive, accurate, and fast, however, it comes with a limitation that it can be used only for laboratory purposes.

Aptamers which are artificial nucleic acids with known 3D structures can differentiate between various serotypes of the influenza virus. These aptamers are synthesized by using systematic evolution of ligands by exponential enrichment (SELEX) method. Aptamers-based biosensors open newer insights; because they do not require any animal host for synthesis, are less expensive than antibodies, and consume less time. Moreover, they bind the target molecules in the quantity of picomolar which offers more sensitivity in the detection of the target molecule. Therefore, based on this concept aptasensors are developed for the detection of AI virus. These aptamer-based biosensors have been found to have target specificity to detect both the Avian influenza virus as well as its subtypes (H5N1 and H1N1). Due to the binding of aptamers with HA, it opens new opportunities for its use as an antiviral (Sung et al. 2004; Cheng et al. 2008).

For the multiplexed detection of influenza virus multiplexed RT-PCR microarray coupled with DNA microarray has been used for subtyping the influenza virus (Li et al. 2001). A microarray for rapid detection of various subtypes of influenza virus (H1N1, H3N2 in man and H5N1 from poultry) has also been developed (Townsend et al. 2006).

### 18.3.2.2 Detection of Bluetongue and Epizootic Hemorrhagic Disease Viruses

Bluetongue (BT) is a non-contagious infectious disease of domestic and wild ruminants caused by Bluetongue virus (BTV). It infects sheep, cattle, and deer. It is transmitted by biting midges belonging to the family Ceratopogonidae. The cattle may be subclinically infected and may act as reservoirs of the virus. Economic losses are caused due to reduced milk and meat production. Epizootic hemorrhagic disease is another disease that mainly affects sheep. It is caused by Epizootic hemorrhagic disease virus of family Reoviridae and genus Orbivirus. This virus is also transferred through the same arthropod that transfers BTV. However, this disease is exhibited by high mortality in contrast to BT which has low mortality, both having similar clinical signs and symptoms.

There is a wide array of tests like real-time PCR, DNA microarray, microsphere bead-based tests, and genotyping methods to detect the blue tongue-virus (Weis et al. 2015; Wilson et al. 2015). A lateral flow assay to detect the BTV antibodies has been employed and commercialized (Hanon et al. 2016). Magnetic modulation biosensing has also been developed using FRET assay which allows fluorescent detection of the virus using fluorescently labeled probes tagged to magnetic microspheres (Danielli et al. 2009).
18.3.2.3 Detection of Foot-and-Mouth Disease Virus

Foot-and-mouth disease (FMD) is caused by foot-and-mouth disease virus (FMDV) and is an economically important disease. It is a highly contagious disease of domestic and wild ruminants. The FMDV can be detected virus isolation, enzyme-linked immunosorbent assay (ELISA), and reverse-transcriptase polymerase chain reaction (RT-PCR) (King et al. 2006).

A pen-side test for detection of FMDV antigen has been developed (Reid et al. 2001; Ferris et al. 2010). In a GNP-IPCR (Gold nanoparticle improved immune PCR) the immune complex is formed by capturing the virus particles by polyclonal antibody followed by addition of gold nanoparticle conjugated oligonucleotides and FMDV specific monoclonal antibodies. The signal DNA is released by heating followed by an analysis by PCR/real-time PCR (Ding et al. 2011). This assay was found to detect 10 fg mL$^{-1}$ of purified FMD particles with high sensitivity in clinical samples. A GNP biosensor using thiol-linked oligonucleotides has been designed for detection of FMDV (Hamdy et al. 2018). Recently, a GNP-based dot-blot assay has been developed for the detection of anti-FMDV antibodies (Jain et al. 2018).

18.3.2.4 Detection of Bovine Respiratory Syncytial Virus

Bovine respiratory syncytial virus (BRSV) is one of the major respiratory diseases of cattle and is present worldwide. It is a single-stranded negative-sense RNA of the family Paramyxoviridae. An electrochemical biosensor for the detection of mRNA of RSV has been developed (Cai et al. 2013). This kind of sensor has been developed by using molecular beacons which are oligonucleotides hybridization probes.

In another study enzymatic substrate reaction using HRPO and TMB have been used for the detection of RSV antigens (Rochelet et al. 2012). It was an electrochemical assay in which the RSV specific antibodies were immobilized on the polystyrene slide, followed by the addition of the antigen and then enzyme-labeled antibody. The signal generated by HRPO-TMB breakdown was transduced through an electrode. This test was found to be rapid which can be conducted within 25 min. An immuno-PCR has also been developed to detect the RSV particles which could detect virus particles up to 4.1 PFU mL$^{-1}$ and showed a $10^3$ higher limit of detection than ELISA and RT-PCR (Perez et al. 2011).

18.3.2.5 Detection of Bovine Viral Diarrhea Virus (BVDV)

An integrated nanowire-based immunosensor has been developed for the detection of BVDV in serum (Montrose et al. 2015). For the detection of anti-BVDV antibodies, an electrospun biosensor based on the principle of capillary separation and conductometric immunoassay has been developed (Luo et al. 2010). This biosensor has been found to have a detection limit of eight minutes and $10^3$ CCID mL$^{-1}$ (Luo et al. 2010). A microparticle immunoagglutination assay on a microfluidic chip has been used to detect BVDV particles (Heinze et al. 2009).
18.3.2.6 Detection of Porcine Reproductive and Respiratory Syndrome (PRRS) Virus

Several immunodetection-based biosensors have been developed for the detection of PRRSV. Infrared electrochemical luminescence biosensor (Shao et al. 2017), platinum nanotube-based fluorescent immunoassay (Chen et al. 2015), enzyme-linked aptamer antibody sandwich (Lee et al. 2013), Fluorescence Resonance Energy Transfer (FRET) based optical biosensor (Stringer et al. 2008) using gold nanoparticles and quantum dots have been developed for detection of PRRSV.

18.3.3 Biosensors for Detection of Mastitis Pathogens

Mastitis is the inflammation of the mammary gland and most of the time it is infectious in origin. Milk from mastitic animals is not suitable for purpose of human consumption because of the altered chemical composition, organoleptic properties (Seegers et al. 2003; Adkins and Middleton 2018; Ashraf and Imran 2018), and shelf life of the dairy products (Hogeveen et al. 2010). Mastitis can be classified as clinical or subclinical. The clinical form is manifested by visible features like the presence of clots, flakes, change in colour, swelling of the mammary gland. Subclinical mastitis does not manifest any clinical sign, but the milk quality and production are declined. Moreover, the excessive use of antibiotics to treat mastitis gives rise to the problem of antimicrobial resistance (Pol and Ruegg 2007). Therefore, it becomes imperative to detect mastitis at an early stage so that preventive measures can be applied as soon as possible (More 2009).

In this regard, there is an increasing demand for pen-side tests to confirm the infectious origin of mastitis (Adkins and Middleton 2018). Due to the persistence of the pathogen in the mammary gland, and the immunological response is generated. Therefore, for the detection of subclinical mastitis, several approaches have been suggested which are based on the testing for immunological modulators and chemical properties of milk (Viguier et al. 2009; Adkins and Middleton 2018; Ashraf and Imran 2018). Out of these increased somatic cell count (SCC), N-acetyl-b-D-glucosaminidase (NAGase) and lactate dehydrogenase (LDH) are the most commonly used markers (Viguier et al. 2009; Addis et al. 2016; Adkins and Middleton 2018; Ashraf and Imran 2018).

Both colorimetric and fluorometric assays have been developed for the detection of LDH and NAGase activity. A fluorometric assay has been developed for the estimation of the catalytic activity of NAGase on the substrate 4-MUAG (Hovinen et al. 2016). A portable spectrophotometer has also been developed to estimate the LDH activity (Hiss et al. 2007).

Electroconductivity (EC) metres for the measurement of increased EC of mastitic milk (due to increased sodium and chloride ions) has also been developed (Norberg et al. 2004; Lam et al. 2009; Viguier et al. 2009). However, they are found to vary between animals.
The pathogens associated with the causative agent of mastitis also need to be identified. Timely identification is essential to reduce the excessive use of administration. Most of the pathogens causing udder infection are derived from the environment. The most common pathogens associated with mastitis are *Streptococcus agalactiae, Staphylococcus aureus, Escherichia coli, Streptococcus dysgalactiae, Streptococcus uberis, Corynebacterium sp., Pseudomonas aeruginosa, Mycoplasma sp., and Klebsiella*. An electrochemical sensor based on a screen-printed carbon electrode has been devised to detect the NAGase enzyme in the mastitic milk with a limit of detection of 10 mU mL$^{-1}$ (Pemberton et al. 2001a).

For the detection of *Mycoplasma bovis*, a single-stranded DNA aptamer has shown high specificity and binding affinity towards P48 protein of *M. bovis* (Fu et al. 2014). A biochip for the DNA amplification of genes specific for mastitis-causing pathogens could detect six pathogens and *M. bovis* with a limit of detection up to $10^3$ CFU mL$^{-1}$ (Lee et al. 2008). An electrochemical sensor that is based on electrochemical spectroscopy has been developed for the detection of pathogenic *Staphylococcus aureus* (Braiek et al. 2012), with a limit of detection of 10 CFU mL$^{-1}$. The assay was found to be specific as it did not detect *E. coli* and *S. epidermidis*. In another recent study, the electrochemical paper-based analytical device (EPAD) was developed. In this paper-based genosensors they used graphene nanodots (GNDs) and zeolite (Zeo) with the *S. aureus* specific DNA probe to detect the target (Mathur et al. 2018). This limit of detection (LOD) of this sensor was found to be 0.1 nM of ss-DNA in test samples.

### 18.3.4 Biosensors for Detection of Drug Residues in Meat and Dairy Products

The drug residues in food can give rise to antimicrobial resistance (Du and Liu 2012; Leibovici et al. 2016). There is a chance that via food (chicken, milk, egg, fish, meat, and honey) these antimicrobial-resistant strains may be transferred to human beings.

A label-free amperometric immunosensor was developed for the detection of kanamycin which showed a limit of detection of up to 5.74 pg mL$^{-1}$ and was used to test kanamycin residues in animal-derived food samples (Wei et al. 2012). A label-free immunosensor designed using silver hybridized ferroferric oxide nanoparticles was found to detect kanamycin with a detection limit up to 15 pg mL$^{-1}$ in pork meat samples (Yu et al. 2013).

Aptamer-based biosensors (Jiang and Yu 2008; Derbyshire et al. 2012; Citartan et al. 2012; Bai et al. 2014; Niu et al. 2014), have also been developed for the detection of kanamycin residues in food samples. For detection of chloramphenicol electrochemical sensors have been developed which are based on multiwalled carbon nanotubes (Yang and Zhao 2015). Similarly, an electrochemical aptasensor has also been developed for chloramphenicol detection which showed a detection limit of 0.059 ng mL$^{-1}$ (Zhang et al. 2011).
An electrochemical sensor using three-dimensional reduced graphene oxide architectures has also been developed (Zhang et al. 2016) which was able to detect chloramphenicol up to 48.45 ng mL$^{-1}$. A molecularly imprinted polymer (MIP) has been synthesized for the electrochemical detection of streptomycin (Que et al. 2013). To detect Streptomycin, magnetic molecularly imprinted polymer nanospheres (mMIP) have been used (Liu et al. 2013) by assembling AUC$l_4$ ions on the magnetic bead surface. This was followed by the o-phenylenediamine polymerization on the surface of magnetic beads.

For the detection of Streptomycin an electrochemical aptasensor based on gold nanoparticle-functionalized magnetic multiwalled carbon nanotubes and nanoporous PtTi alloy has been developed (Yin et al. 2016). An electrochemical arc-shaped aptasensor has also been developed for the quantification of Streptomycin in milk (Danesh et al. 2016).

### 18.3.5 Biosensors for Measuring Physiological, Metabolic, and Biochemical Parameters of Livestock

Apart from disease diagnosis in livestock and poultry, biosensors have also been used to monitor other physiological, metabolic, and biochemical parameters like animal jaw movement, breath analysis, analysis of metabolites in perspiration, analyzing tears for glucose monitoring, analysis of stress in fish, and detection of ovulation. Jaw movements of animals are required to know the grazing behaviour of cattle. The rumination behaviour has been recorded by using a pressure sensor as a noseband along with a data logger which records the jaw movements via pressure (Braun et al. 2013). Acoustic analysis is used to estimate the biting and chewing movements for the analysis of food intake by the cattle (Laca and WallisDeVries 2000). Microphones have been used to record the jaw movement sounds of grazing animals (Ungar and Rutter 2006). Machine learning algorithms are used to analyze the signal pattern for the estimation of the intervals between the jaw movements, intensity, and duration (Navon et al. 2013). Accelerometer sensors have also been used to estimate the jaw movement and grazing behaviour of animals (Tani et al. 2013; Mattachini et al. 2016; Giovanetti et al. 2017). These sensors can convert the acceleration generated from movement or gravity into a suitable voltage output.

Some of the biosensors have also been developed to analyze the metabolites in sweat. These are the electrochemical sensors used for the measurement of sodium and lactate levels in sweat. They are also used to measure sweat as a measure of stress. Biosensors have also been used to analyze the infection by $M. bovis$ by estimating the volatile organic compounds in breath (Ellis et al. 2014). A nanobiosensor and a strip-based lateral flow biosensor has been developed to detect $M. bovis$ and $M. avium$ subspecies paratuberculosis, respectively. An amperometric biosensor using immobilized glucose oxidase has also been used to detect glucose in the tear (Iguchi et al. 2016).
 Implanted biosensors inside the sclera of fish have also been used to monitor the stress biomarker as an indicator of the health of fish as well as the pollution status of the water (Hibi et al. 2012; Wu et al. 2015). Biosensors have also been implicated in determining ovulation in cattle. An amperometric progesterone biosensor has been developed to determine the ovulation in cattle. It consists of an anti-progesterone monoclonal antibody immobilized on a screen-printed carbon electrode (Pemberton et al. 2001b). Biosensors have also been developed to detect the progesterone in milk with software which lists the insemination time (Mazeris 2010) with more advancement integration of novel aptamer with a Surface Plasmon Resonance (SPR) imaging sensor (Zeidan et al. 2016). Intravaginal probes that can detect the conductivity and temperature inside the vagina have also been used to predict the ovulation of the cattle (Andersson et al. 2015, 2016).

Testing of saliva for various metabolites is an extremely useful non-invasive method (Bandodkar and Wang 2014) and is useful for disease diagnosis. The biomarkers found in saliva are useful in the early detection and diagnosis of diseases (Malon et al. 2014). Detection of corticosteroid hormones in saliva as a marker of stress has been used successfully. A cortisol immunosensor has been developed for the quantitative analysis of salivary cortisol (Yamaguchi et al. 2013). Similarly, α-amylase in the saliva is an indicator of psychosocial stress. An α-amylase biosensor has also been developed which is based on the hydrolysis of starch by α-amylase (Wu et al. 2007). The resonance of the starch gel immobilized sensor increases on the hydrolysis of starch by α-amylase. Salivary glucose biosensors have also been developed to measure glucose on the saliva of domestic pets and human beings (Stein and Greco 2002; Reusch et al. 2006). Sensors have also been developed for monitoring the physiological status of livestock. A livestock monitoring system integrated with a wireless sensor network for the collection of data on breathing rate, heart rate, and cattle movement has been developed (Park and Ha 2015).

Bioacoustics based system has been developed to detect the sex (Pereira et al. 2015), routine activities of poultry (Fontana et al. 2016), disease diagnosis like necrotic enteritis (Sadeghi et al. 2015). A sound detection system has been developed to differentiate between New Castle Disease, Infectious Bronchitis, and Avian Influenza (Banakar et al. 2016).

### 18.4 Conclusion

The Biosensors have a wide application in the field of veterinary sciences. Starting from the disease diagnosis biosensors have been used successfully in monitoring the metabolism and physiological parameters of animals and poultry. Animals’ health plays an important role in any country’s economy as directly and indirectly it affects the country’s economy as well as human health. In the past few decades, there has been excessive use of antibiotics, the emergence of viral diseases, the emergence
of new antibiotic-resistant bacterial strains, antibiotic residues in milk and other food commodities derived from animal sources. This imbalance is the outcome of the indiscriminate use of the available resources like antibiotics in feed/treatment of animal diseases, human activities like deforestation, mutation in viruses, and overlapping of human niche with that of wildlife. Excessive antibiotic use leads to antibiotic bacterial strains. Moreover, the infectious diseases in animals cause heavy economic losses and most of them are important from a zoonotic point of view. Therefore, with an increasing demand for rapid diagnosis of the viral/bacterial diseases, antibiotic residues, and monitoring the health status of animals, rapid pen-side tests need to be developed. The conventional diagnostic techniques take time, demands labour and sophisticated instruments for their interpretation. Biosensors can be the rapid pen-side tests that deliver the test results in a few minutes for early diagnosis and preventive measures required for disease control. Moreover, there is a requirement of multitargeting biosensors in the field to detect the target analyte with more confidence to overcome the impediment of false positive and false negative test outcomes. The nucleic acid-based biosensors are one of the biosensors for the sensitive detection of analytes. Biosensors powered with nanotechnologies and next-generation sequencing can offer more robust tools for the detection of pathogens and animal surveillance.

The layers on which the bioreceptors are immobilized also play an important role in the electrochemical signals and non-specific binding which depends on their thickness and surface charge. Moreover, choosing novel bioreceptors like bacteriophages, single-chain antibodies, non-antibody-binding proteins, and half antibodies can offer high specificity. Antibodies are the most widely used bioreceptors, however, their production and purification is costly. Moreover, the stability of antibodies on the sensor surface is not long-lasting and the binding capacity of these antibodies decreases with due time course. Hence, to counter these difficulties, some advancements in bioengineering have been implicated like peptoid nanosheets (Olivier et al. 2013). Such types of implications comprise thick sheets of 3–5 mm thickness formed by self-assembly of antibody mimetic peptoids. These thick sheets have surface loops expressing antigen-binding sites. The whole aggregation is stable and easy to produce. Some other alternatives for stable antibodies are, DARPins (Stumpp and Amstutz 2007), camel-derived heavy variable chain (VHH) (Hassanzadeh-Ghassabeh et al. 2013; Muyldermans 2013), single-chain antibodies expressed via yeast surface display (Richman et al. 2009), single-chain variable fragments (ScFv) (Ahmad et al. 2012), and adhirons (Tiede et al. 2014). These alternatives can be synthesized easily, are small, and can be produced in bacterial expression systems. Another important aspect is the regeneration of the sensor surface. Regeneration of the biosensor surface is cost-effective. Biosensor surface can be regenerated successfully after using the above bioreceptor molecules. These bioreceptors can withstand harsh buffers without affecting their binding capacity.

Nanomaterial-based electrochemical biosensors are also good biosensors due to their low cost, good selectivity, sensitivity. They need a precise choice of the transducer surface. The incorporation of the nanomaterials accelerates the sensitivity and specificity of the detection of the analyte. Electrochemical biosensors offer very
low detection of analytes like as observed while detection of antibiotic residues of kanamycin, streptomycin, and tetracycline. Moreover, the portability and reusability of electrochemical biosensors add to its other features of rapid on-site detection. The sensitivity of the electrochemical biosensors can be increased further by replacing the enzyme-based labels by the nano labels.

Antibody-based biosensors can also offer high sensitivity for the target molecule depending on the selective binding of the target molecules with the immobilized antibodies and the stability of the antibody molecules on the sensor surface. There is also a need for international recognition and widespread use throughout the world these biosensors must be validated via intra-lab and inter-lab validation.

From future perspective, development, and validation of nanobiosensors can open new insights in animal disease diagnostics. With the recent advancements in nanotechnology, nanobiosensors will be an excellent platform with increased sensitivity for analyte detection.

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