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REVIEW ARTICLE

Biosensor as an alternative diagnostic method for rabies virus detection: A literature review

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Abstract

Rabies virus as a neurotropic agent causes rabies in humans and animals. Rabies virus transmission usually occurs through direct contact with saliva of rabid animals. However, serological and molecular tests commonly are used in diagnosing rabies but all the detection methods of rabies have some limitations. It is necessary to develop a rapid, effective, and low-cost biosensor as an alternative tool to detect rabies virus. In this review, we studied related biosensor researches to rabies virus detection for comparing it with other detection test including serological and molecular methods. Given that very limited studies have been conducted in this field, biosensors as quick, effective, and high sensitivity tools can be used in diagnostic of rabies as an alternative tool instead of other detection methods. According to the important role of rapid detection of rabies in the control of infection and public health measures, development of a biosensor as a quick tool can be very significant in the diagnosis of rabies.

KEYWORDS
biosensor, diagnostic, rabies

1 | INTRODUCTION

Rabies is one of the most feared and deadly infectious diseases that is transmitted by the bite of a rabid animal, which is invariably fatal due to encephalomyelitis. This disease is caused by rabies virus that belongs to *Lyssavirus* genus of the *Rhabdoviridae* family in *Mononegavirales* order.[1] Despite the global vast attempt and

Abbreviations: CSF, cerebrospinal fluid; dFAT, direct fluorescent antibody test; ELISA, enzyme-linked immunosorbent assay; EM, electron microscopy; FAVN, fluorescent antibody virus neutralization; MIT, mouse inoculation test; PCR, polymerase chain reaction; RFFIT, rapid fluorescent focus inhibition test; SPR, surface plasmon resonance; WHO, World Health Organization

TABLE 1 Common tests for the detection of rabies virus

Diagnostic for assays rabies	Tests	References
Virus isolation	• Mouse inoculation test • Rapid tissue culture infection test (RTCT)	8
Antibody detection-based assays	• direct rapid immunohistochemical test (dRIT) • Fluorescent antibody technique (FAT) • Rapid rabies enzyme immunodiagnosis (RREID)	8,37,38
Immunochromatographic techniques	• Rapid immunodiagnostic test (RIDT)	39
Antibody detection-based assays	• Mouse neutralization test (MNT) • Rapid fluorescent focus inhibition test (RFFIT) • Fluorescence antibody virus neutralization test (FAVN)	40–42
Nucleic acid detection–based assays	• TaqMan RT-PCR • Nucleic acid sequence-based amplification (NASBA) reverse-transcriptase polymerase chain reaction (RT-PCR) • Loop-mediated isothermal amplification (LAMP)s • Microarray detection of lyssaviruses	9

implementation of extensive control programs, public health awareness schemes, and development of postexposure prophylaxis, rabies kills an estimated 60,000 humans every year in more than 150 countries and territories, many of whom are children, and over 95% of the mortality happens in Asia and Africa, following China and India.[2] In fact, rabies is mostly fatal with no specific antiviral treatment globally.[3] In most underdeveloped countries, mortality in humans is high due to poor laboratory facilities to rabies diagnosis, whereas in many developing countries, mortality is low because of underreporting, inadequate clinical trials, and poor knowledge on the transmission and prevention of rabies disease.[4,5] Several test methods, recommended by the World Health Organization (WHO), are available for the diagnosis of rabies. Due to the high mortality rate associated with rabies infection, early detection techniques can control and prevent the disease.

Recently, biosensors, as an analytical device and valuable technique, are used for the detection of infectious diseases and analysis of a variety of microorganisms in biomedicine, environmental, food quality control, agricultural, and pharmaceutical industry.[6,7] The aim of this review is to introduce a cheap, rapid, and simple detection method for rabies disease that can be used in both developed and underdeveloped countries.

2 | RABIES DETECTION METHODS

The main current methods for the diagnosis of rabies[8,9] (Table 1) are direct fluorescent antibody test (dFAT), polymerase chain reaction (PCR), mouse inoculation test (MIT), enzyme-linked immunosorbent assay (ELISA), and virus neutralization test such as fluorescent antibody virus neutralization (FAVN) test and rapid fluorescent focus inhibition test (RFFIT).[10–13] dFAT is the most sensitive, specific, reliable, and cost-effective routine diagnosis method of rabies antigens in brain tissue, but it has some drawbacks: (1) it is only efficient for postmortem samples, (2) as an accessory to dFAT, mouse inoculation is carried out in developing countries, (3) it requires several days to get the result.[14–16] PCR seems to be a reliable and sensitive diagnostic tool for antemortem diagnosis of this virus nucleic acid in the clinical samples such as cerebrospinal fluid (CSF), saliva, skin biopsy, and corneal impression smear but relies on expensive equipment; therefore, it is not suitable for detecting rabies in humans and animals in underdeveloped countries.[3,14] MIT has performance and accuracy and also is a cheap and fast method but it has higher risks regarding biosafety and mental health of the technicians.[17] ELISA is considered as a quick method, easy to perform, and also there is no need of live rabies virus or cell-culture facilities; however, they vary in sensitivity and specificity.[1,18] Although virus neutralization tests, FAVN test and RFFIT, are known as the most reliable tests, they are expensive, time consuming, and require live rabies virus.[1,19]

Generally, some tests for the detection of rabies virus include virus isolation, EM, histologic examination, and immunohistochemistry; these are suitable tools to study the structure, molecular typing, histopathology, and virulence of rabies viruses.[20] Nucleic acid detection-based assays such as PCR are more sensitive and generate good

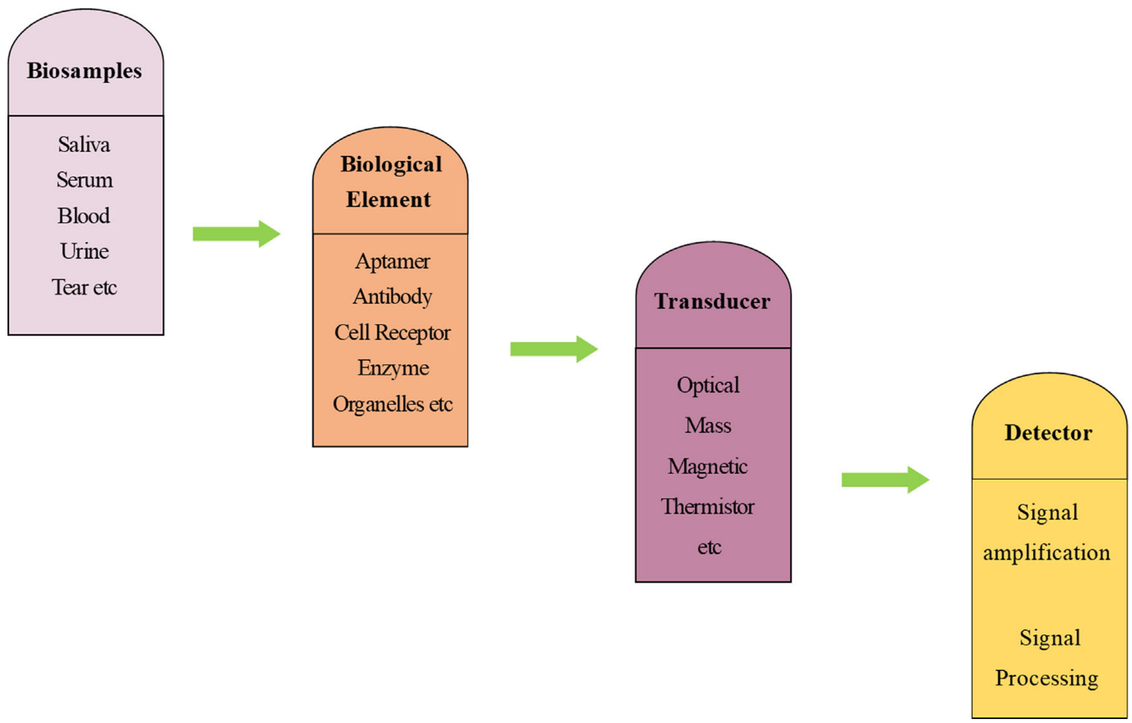

FIGURE 1 Schematic of different components of a biosensor

results to detect rabies virus antigen in saliva, CSF, and brain but they are expensive.[21] The development of the biosensors as novel tools to diagnose infectious disease makes considerable major attention in the field of the biomedicine. These sensors present high sensitivity to detect many biochemical species.[22]

3 | LIMITATION OF RABIES ROUTINE DETECTION METHODS

All the detection methods are routinely used by WHO reference laboratories, but there are some limitations including the need of expensive equipment and designing primers for PCR test, efficiency only for postmortem samples, and time consuming to get the result for dFAT, higher risks regarding biosafety and mental health of the technicians for MIT, variation in sensitivity and specificity in ELISA, and also requirement for live rabies virus in virus neutralization test. On the other hand, some of these detection techniques are not suitable for detecting rabies in humans and animals in underdeveloped countries, for instance, as an accessory to dFAT, mouse inoculation is carried out in developing countries.[1,3,14–19]

Therefore, there is a great need for a new method that would be reliable, affordable, and sensitive for using in both developed and underdeveloped countries.

4 | BIOSENSORS

Biosensors have been extensively studied and developed as a potential tool in medical diagnostics. A biosensor consists of three parts. The first component of the biosensor is the biological element that detects the analyte and produces a response signal; then, transducer as second part of the biosensor converts the produced signal into a measurable response.[23] Detector as the third component of the biosensor intensifies and processes the signals by using an electronic display system before displaying it[24] (Figure 1).

Base on the type of biological element or the mode of physicochemical transduction, biosensors can be categorized. According to the transducer, biosensors can be classified as optical, piezoelectric, thermal, and electrochemical biosensors.[25]

The principle of optical biosensors method is based on light absorbed or emitted as a result of a biochemical reaction. Optical biosensors are based on different optical methods such as absorption, luminescence, fluorescence, and surface plasmon resonance (SPR).[26]

Piezoelectric (quartz crystal microbalance-based) biosensors involve measurement of mass change happening as a consequence of biomolecular interaction. Piezoelectric crystals are used to measure the mass change by associating with the change in the piezo crystal's oscillation frequency.[27]

TABLE 2 Comparison of reported biosensor for detection of rabies

Sensor platform	Method	Limit of detection (LOD)	Target	References
Optical	SPR	70 pg/ml	N and G Antigens	[34]
Electrochemical	Gold microelectrode	0.5 g/ml.	Antigen	[21]
Fluorescent and Colorimetric as dual-mode	Y-shaped DNA	Fluorescent: 15 pM Colorimetric: 60 pM	DNA	[36]

Principle of thermal biosensors is that according to the measurement of the thermal changes occurring on biochemical recognition, changes in enthalpy are important in most of the biochemical reactions and sensitive thermistors can measure the heat changes.[28] The most widely explored area in biosensors is the electrochemical biosensors; some of its advantages include specificity, low detection limit, and simplicity of construction.[29]

5 | BIOSENSORS BENEFITS AND LIMITATIONS

Today, biosensors play an important role in detection of large number of infectious diseases. They are considered to be powerful tools to detect infectious diseases because biosensors have multiple potentials such as specificity, high sensitivity, portability, fast and reproducible response, and also inexpensive platform.[30] Though biosensors are critical tools for analytical purposes but usage of biosensors is limited because of various problems that include increasing the signal-to-noise ratio, sample preparation methods, short lifetime of biological substance, and usage of many electronic devices for data analysis and collection.[31–33]

6 | BIOSENSING RESEARCHES FOR RABIES DETECTION

Construction of biosensor platform of rabies virus can be categorized in three key steps including: (1) detection of different biorecognition elements such as RNA or proteins of rabies, human microRNA (miRNA), and human immunoglobulins; (2) use of an immobilized bioreceptor including DNA probe, antibody/antigen, enzyme, ligand, and aptamer on a transducer; and (3) hybridization detection methods including electrochemical, piezoelectric, colorimetric, fluorescent, magnetic, and acoustic technologies.[6]

Jing et al.[34] fabricated SPR-based biosensor to detect rabies virus. SPR biosensing as a label-free optical can measure refractive index changes related to real time without labeling the interacting molecules in a sample for detection molecules immobilized on the SPR sensor.[35] In their work, the limit of detection was approximately 70 pg/ml to detect nucleoprotein and glycoprotein of rabies virus from brain tissue of guinea pigs.[34]

In another study, Mouna et al. introduced an immunosensor based on functionalized gold electrode to detect rabies antigen. They used immobilized specific antirabies polyclonal antibodies on a functionalized gold microelectrode for the development of an immunosensor to detect rabies viral antigen; detection limit of this biosensor was 0.5 g/ml[21] (Table 2).

Zhou et al.[36] developed a novel fluorescent and colorimetric dual-mode approach to detect oligonucleotide of rabies virus via Y-shaped DNA assembly. Their work showed high sensitivity and selectivity to detect rabies virus oligonucleotide. The operation of developed dual-mode sensor was easy and also showed high detection performance.

7 | CONCLUSION

Recently, biosensing technologies as innovative diagnostic tools have been used to detect viruses. Biosensors as high-sensitive tools take shorter time to obtain the result in comparison with the traditional methods. According to the important role of rapid detection of rabies in the control of infection and public health measures, development of a biosensor as a quick tool can be very significant in the diagnosis of rabies; however, more studies are needed in this field. In this review, the current developments of biosensors were widely overviewed for rabies virus detection. Optical, electrochemical, and also fluorescent and colorimetric as dual-mode were used for transducing, and the antibody and specific nucleic acids are the usual choices for biological elements to be immobilized. These biosensor platforms in comparison with the conventional assays present more promising applications to increase human health for developing countries and rural areas of cities.

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