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Short Communication

Amperometric immunosensor of *Brucella abortus* CE-protein antigen shows post-zone phenomena

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ABSTRACT

Brucella abortus is an intracellular pathogen of zoonotic nature and causes brucellosis in humans, means it can be transferred from cattle to a human and remains pathogenic. In this study, attempts were made for detection of *B. abortus* cell envelop protein (CE-protein) antigen by using amperometric immunosensors. Screen printed electrodes were characterized electrochemically with 1-naphthol and used in sandwich enzyme linked immunosorbent assay system to detect CE protein antigen. Linear range was found in the range of 1.56 μg/mL to100 μg/mL with a regression equation of I (nA) = 43.12 + 1.235 (antigen (μg/mL)) (R^2 = 0.991). Relative standard deviation was found 5.5%. At high concentrations post-zone like phenomenon was observed. At very low concentrations the response was higher compared to that of at higher concentrations. However, no false results were observed. The detection limit was found to be 0.1 μg/mL. By conventional plate ELISA it is possible to detect 50 μg/mL only. Experiments were conducted using suspected human patient serum samples and results were compared with ELISA and RBPT test. The immunosensor was found to be highly sensitive compared to ELISA and RBPT. In order to confirm that whether these results obtained from electrochemical technique (amperometric technique) was true positive or false positive, we further diluted the patient serum samples and then tested. We found that even after dilution of patient serum samples results obtained were same as before dilution of patient serum samples. Hence, it is concluded that electrochemical technique does not give false result. Thus electrochemical immunosensors can be used for diagnosis of brucellosis in patient serum sample. Shelf life of immunosensor was found 18 days.

1. Introduction

Microorganisms belonging to the genus *Brucella* are gram negative, facultative intracellular pathogen and causes brucellosis in humans. The disease is zoonotic in nature, means it can be transferred from cattle to a human and remains pathogenic. Brucellosis has become an emerging disease in many parts of the world. Brucellosis is widely distributed in both animals and humans, especially in developing countries. Exposure to infected animals and animal products cause brucellosis in humans. The bacteria are transmitted by contact with infected animal, ingestion of infected milk, milk products and inhalation of aerosols. So, this disease is primarily spread through extreme exposure to cattle and also by use of unpasteurized milk. This disease is also transmitted from mother to child in case of human but rarely. Brucellosis is considered by FAO, WHO and O I E as the most widespread zoonosis in the world. The prevalence of this disease is very high in India [1].

The lipopolysaccharide (LPS) of smooth *Brucella* species is the strongest antigen compared to other antigenic molecules and has been considered as the most important antigen as it elicits strong and long lasting immune response in brucellosis [2,3]. Cell Envelop (CE-protein) protein is an outer membrane protein of *Brucella abortus* and the molecular weight of immunogenic CE-protein antigen varies from 11.8 kDa to 110.8 kDa [4].

There are several standard tests used in the diagnosis of brucellosis. These are standard plate agglutination test, standard tube agglutination test, acidified plate antigen test, rivanol precipitation plate antigen test, serial dilution milk ring test, complement fixation test, standard buffered brucella antigen card test, mercaptoethanol agglutination test and ELISA [5]. These tests are mainly based on the detection of antibodies directed against the lipopolysaccharide portion of the cell membranes. Tests are also available

Abbreviations: SPE, screen printed electrode; CE-protein antigen, cell envelope protein antigen; ELISA, enzyme linked immunosorbent assay; RBPT, rose bengal plat test; PCR, polymerase chain reaction; CAb, capturing antibody; RAb, revealing antibody; ALP, alkaline phosphatase; PBS, phosphate buffer saline; DEA, diethanolamine; LPS, lipopolysaccharide; CV, cyclic voltammetry.

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for the detection of *Brucella* antigen. Al-Shamahy et al. reported enzyme linked immunosorbent assay for *Brucella* antigen detection in human sera [6]. Al-Farwachi et al. also reported modified ELISA test for detection of *Brucella* antigen in the aborted ovine fetal stomach content [7]. Immuno-histochemical technique and polymerase chain reaction (PCR) were also carried out for detection of *Brucella* infection [8,9].

Several methods were reported in the literature for detection of brucellosis in recent years. However, these methods are either time-consuming and/or of low in sensitivity. Some of the methods require highly qualified personnel (e.g., PCR) [10] or sophisticated instrumentation (PCR, florescence microscopy, and flow cytometry) [11,12]. The requirement of highly qualified manpower and sophisticated instrumentation techniques make them less useful in the diagnosis.

Over the past decade, the interest in the development of a simple, inexpensive and disposable biosensor for the detection of pathogens under field conditions has increased. Recently, extensive work has been done on the use of electrochemical immunosensors for environmental [13] and clinical diagnosis [14,15]. Electrochemical immunosensors combine the high specificity of traditional immunochemical methods with the low detection limit of a modern electrochemical system. The electrochemical techniques employed for immunoassays are mostly based on amperometric methods [14–16] and voltammetric methods [17]. For immunosensors based on electrochemical methods, it is also possible to miniaturize the electrode for field applications [16]. Various types of electrodes such as the carbon paste electrode (CPE) [15,18], glassy carbon electrode [17], platinum electrode [19] and gold electrode [20] have been used for electrochemical immunosensors. Recently, several immunosensor devices have been developed using disposable screen-printed electrodes (SPEs) [13,21]. SPEs are inexpensive and can be mass produced using screen printing technology [20,22–25]. Carbon inks are particularly attractive because they are relatively inexpensive and lead to low background currents and broad potential windows [15].

In this present study, we used anti-*B. abortus* CE-protein antibody raised in mice (as capturing antibodies) and anti-*B. abortus* CE-protein antibody raised in rabbit and conjugated to alkaline phosphatase (ALP) (as revealing antibody) for the detection of *B. abortus* CE-protein (antigen) based on sandwich ELISA system. The capturing antibodies were adsorbed (physical adsorption) on SPEs, which were subjected to sequential incubation in CE-protein (antigen) and revealing antibody. In the detection of *B. abortus* CE-protein antigen, we observed post-zone like phenomenon. Due to this post-zone phenomenon, we observed high amperometric response at low concentration of *B. abortus* antigen and low amperometric response at high concentration of *B. abortus* antigen. However, no false negative results were observed. In order to confirm that whether these results obtained from electrochemical technique (amperometric technique) was true positive or false positive, we further diluted the patient serum samples and then tested. We found that even after dilution of patient serum samples, results obtained were same as before dilution of patient serum samples. Hence, it is concluded that electrochemical technique does not give false result. Since, in the detection of *B. abortus* (CE-protein antigen) no false negative results were observed. Hence, there is no need to dilute the samples further. We also measured shelf life of immunosensor and was found 18 days. To the best of our knowledge, this is first report concerning *B. abortus* CE-protein detection by an amperometric immunosensor using SPE. In addition to this, we compared the *B. abortus* CE-protein amperometric immunosensors with conventional methos used in serodiagnosis of brucellosis like plate ELISA [26] and RPPT [27]. By using electrochemical methods, cost-effective and field-deployable diagnostic devices can be made. The adsorption of antibodies by these SPEs was proved by optical density measurements (Fig. was not shown).

1.1. Post-zone phenomenon

For the detection of antigen–antibody complex, the ratio between antigen and antibody is the influencing factor. When antigens and antibodies are in optimum ratio, lead to cross linkage and hence agglutination appears, resulting in positive reaction. When antigen concentration become in excess (post-zone phenomenon), agglutination is hidden by mass of unagglutinated antigens and this resulted in false negative reaction. This phenomenon is known as post-zone phenomenon (Fig. 1). Yorgancigil et al. diluted the specimens to avoid post-zone effect in detection of *Brucella* infection [28]. These false-negative reactions can be detected by higher dilutions of antigen sample, which reduces the antigen concentration into the range that produces visible agglutination. Hence, it is necessary to dilute the sample to confirm whether it is negative or not. In this present study, we observed post-zone like effect in electrochemical immunosensor. However, no false negative result was observed. Also the sensitivity of immunosensor was much higher than ELISA.

2. Materials and methods

2.1. Apparatus

Cyclic Voltammetry (CV) experiments and chrono amperometric experiments were performed with CH Instrument, 440A. The

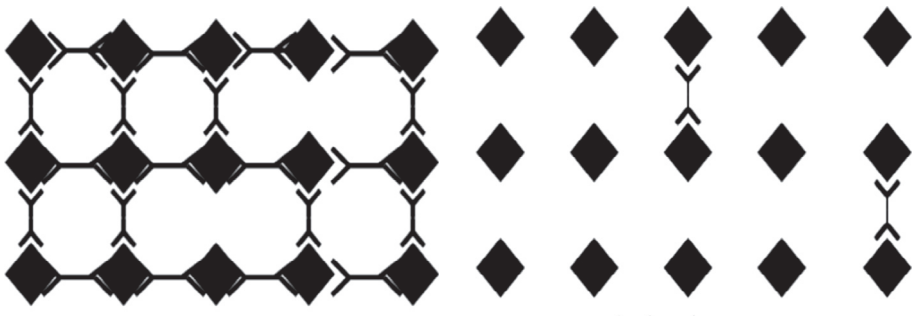

Fig. 1. (a) Showing agglutination. (b) Agglutination hidden by mass of unagglutinated antigen.