



Evaluation of smooth lipopolysaccharide based latex agglutination test in serodiagnosis of brucellosis

Ankush Kiran Niranjan¹, Ankita Jain², Rajesh Rathore³

^{1,2}Division of Bacteriology & Mycology, IVRI, Bareilly, India.

³CADRAD, IVRI, Bareilly, India

Abstract

In this study, sLPS antigen coated latex bead was prepared and their evaluation was done along with RBPT, commonly employed for the serodiagnosis of brucellosis. sLPS was extracted from Brucella abortus S99 and coated on polystyrene latex beads in a phosphate buffer saline coating buffer. A total of 200 samples were collected from unorganized dairy farm and tested with RBPT and sLPS based latex agglutination test. The accuracy, relative sensitivity and specificity of sLPS based LAT were found 99%, 93.55% and 100% respectively. This study conclude that sLPS based LAT can be used as an alternative screening test for bovine brucellosis.

Keywords- sLPS, RBPT, brucellosis, bovine, serodiagnosis

I. INTRODUCTION

Bovine brucellosis, also called as “Mediterranean fever” or “Bang’s Disease” or “Contagious Abortion”, is highly zoonotic bacterial infection, mostly transmitted by direct or indirect contact from infected animals or their products. It affects all age groups of both sexes in animals and human beings (Jagapur et al., 2013). It is found worldwide however, it has been eradicated from many countries and mainly caused by biovars of *Brucella abortus*. In southern Europe and western Asian countries the infection can also be caused by *B. melitensis*, where cattle are kept in close association with sheep or goats.

The gold standard test for diagnosing animal brucellosis is the isolation of *Brucella* spp. (OIE, 2008). However, it is a time consuming and laborious process. It also puts the laboratory personnel under great risk for infection. So the serological test are generally preferred. The serological diagnosis of brucellosis was begun more than 100 years ago with Wright and Smith (1897) describing the first serological test, STAT for its diagnosis, and since then a considerable number of serological tests have been developed and modified to detect antibodies against the *Brucella organism* for diagnosing the disease, viz., RBPT, MRT, CFT, ELISA and RIA etc.

Rose Bengal Plate Test (RBPT) was commonly used for the screening of brucellosis. It is the 8% suspension of coloured whole cell killed bacteria and the killed bacterium has lot of antigens on its surface that may cross react with other bacterium like *Yersinia enterocolotica* O:9 (Nielsen et al., 2004) and shows some false positive reactions. In brucellosis majority of antibodies produced against the sLPS and outer membrane proteins of *B. abortus*. Here we prepared the purified sLPS based latex agglutination test (LAT) and compared with RBPT to evaluate the diagnostic potential of this purified sLPS antigen based LAT. The LAT also has the field applicability and results can be obtained within 2 minutes.

II. MATERIALS AND METHODS

2.1 Materials

A. Bacterial Culture

Cultures of *Brucella abortus* S99 was maintained in the CADRAD, IVRI, Izatnagar which is already characterized by cultural, biochemical and molecular methods.

B. RBPT

RBPT was procured from Division of Biological Products, IVRI, Izatnagar.

C. sLPS coated latex beads

The latex beads (2.6 % suspension) was procured from Polysciences, Inc. (USA).

D. Serum samples

Serum samples were collected from the unorganized dairy farms where vaccination against *Brucella* was not practiced.

2.2 Methods

A. Extraction of sLPS

Extraction of sLPS from *Brucella abortus* S99 was done by as per the protocol given in OIE, 2004 with slight modification. sLPS was run on SDS-PAGE and stained with silver stain and Coomassie brilliant blue stain separately. The carbohydrate content of sLPS was determined by phenol-sulphuric acid assay (Dubois et al., 1956).

B. Coating of sLPS on latex beads

Two hundred fifty μ l latex beads (2.6% suspension) were taken in microcentrifuge tubes and then centrifuged at 8000 rpm for 5 min. The beads used for sLPS antigen coating was treated with phosphate buffer saline (PBS, pH 7.4). The pellet resuspended in PBS and again centrifuged at 8000 rpm for 5 min and supernatant was discarded. This step was repeated again. After latex beads were washed twice in PBS, 650 μ l varying concentration of sLPS (20, 40, 60, 80 and 100 μ g/ml in PBS) were added to make 1% latex suspension and then incubated at 37°C for 8 hours with constant shaking. The sensitized beads were centrifuged at 8000 rpm for 5 min and supernatant discarded. The pellets resuspended in blocking buffer (PBS with 0.5% bovine serum albumin) and incubated at 37°C for 30 minutes with constant shaking. The blocked beads were centrifuged at 8000 rpm for 5 min and supernatant discarded. The pellets were finally resuspended in 650 μ l PBS containing 0.1% sodium azide (for prevention of fungal contamination) and kept at 4°C for further use.

C. Rose Bengal plate Test

The RBPT was performed according to the protocol described by Alton *et al.* (1975). Before starting the test, both serum and RBPT antigen were brought to room temperature. Briefly, 30 μ l each of serum and antigen were placed on a grease free clean glass plate. With continuous swirling, the plates were observed for appearance of agglutination. Appearance of agglutination within 4 min of mixing was considered as positive while the absence of agglutination was considered as negative.

D. Latex agglutination test

The LAT was performed on glass slides by placing equal volumes (20 μ l) of serum and latex beads sensitized with sLPS. The slide was rotated briefly for mixing the sensitized beads and the serum samples. The result was read within 2 min. The test score was positive if agglutination occurred, indicated by the formation of fine granular particles, which tend to

settle at the edge of the droplet. If the suspension remained homogenous, the test was scored negative.

E. Evaluation of RBPT and LAT

The relative sensitivity, specificity and accuracy of LAT for serodiagnosis of brucellosis were evaluated in comparison to rose Bengal plate agglutination test (RBPT) as described below,

Sensitivity = $a/(a + c) \times 100$, where 'a' is the number of sera positive by LAT and RBPT while 'c' is the number of sera positive by RBPT but negative by LAT.

Specificity = $d/(b + d) \times 100$ where 'd' is the number of sera negative by LAT and RBPT while 'b' is the number of sera negative by RBPT but positive by LAT.

Accuracy = $(a + d)/(a + b + c + d) \times 100$.

III. RESULTS AND DISCUSSION

The detection of antibodies in sera is a useful method for the diagnosis of brucellosis in bovines. This study was aimed to detect the diagnostic ability of purified sLPS antigen alone. A comparative analysis of LAT to the routinely used RBPT was done.

3.1 Extraction of sLPS

Extraction of sLPS from *B. abortus* S99 was done. The purity of extracted sLPS was analyzed by SDS-PAGE and then separately stained with silver stain and Coomassie brilliant blue stain. In silver staining yellowish-brown bands appeared while in Coomassie brilliant blue there were no bands (Fig. 1), it showed that sLPS was free from protein contamination. The carbohydrate concentration of sLPS was found 125 µg/ml and the total yield from 5 g wet weight was 1.5 mg.

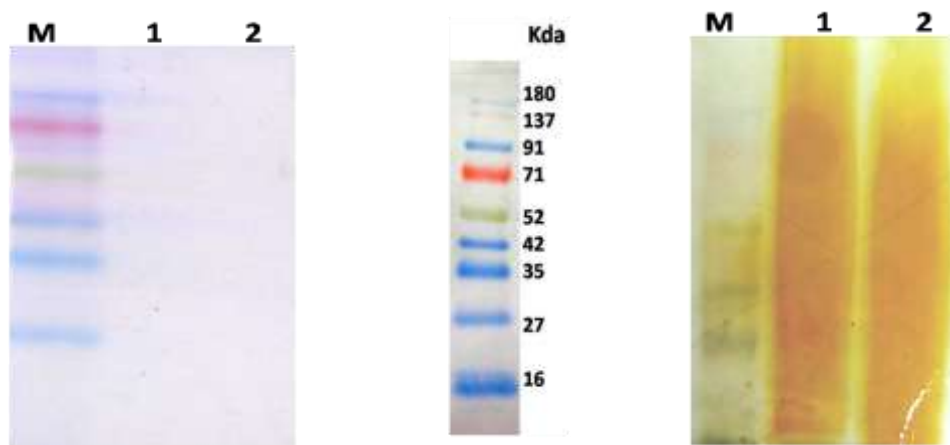


Fig 1: SDS-PAGE analysis of sLPS by Coomassie Brilliant Blue Stain (a) and silver stain (b)

Lane M : Protein molecular weight marker

Lane 1& 2 : sLPS

3.2 Coating of sLPS on latex beads

Latex beads were made of polystyrene and the sLPS antigen was coated on these beads by physical adsorption phenomenon. 50 µg/ml concentration was found optimum for the sLPS coating. The unoccupied sites were blocked by 5% BSA so as to prevent the autoagglutination and sodium azide was added as an anti-microbial agent.

3.3 RBPT and sLPS based LAT

RBPT is known to be a rapid, simple and sensitive but has low specificity, but the most common test used and more sensitivity was recorded by many workers (Nasir et al., 2004; Barbuddhe et al., 2004; Brahmabhatt et al., 2009; Kaushik et al., 2006; Prithiviraj, 2010) in prevalence studies of brucellosis. sLPS coated beads bind with the antibodies present in the serum against the brucellosis and forms agglutination. Both test were applied to the panel of 200 serum samples. Out of 200 serum samples 29 and 31 were found positive by RBPT and sLPS based LAT, respectively (Table 1).

Table 1: Evaluation of diagnostic values of sLPS antigen-based LAT in comparison to Rose Bengal plate agglutination test (RBPT)

	RBPT +	RBPT -	Total
LAT +	29 (a)	0 (b)	29
LAT -	2 (c)	169 (d)	171
Total	31	169	200

Sensitivity: $29/31 \times 100 = 93.55\%$; Specificity: $169/169 \times 100 = 100\%$; Accuracy: $198/200 = 99\%$,

IV. CONCLUSION

In the present study, it can be concluded that sLPS based latex agglutination test had good sensitivity and comparable specificity as compared to RBPT. As purified antigen detects brucellosis more specifically, it can be used along with routinely diagnostic test RBPT. LAT is also a pen-side test and need not higher technical expertise, it is of field applicability.

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