A Comparative Study on the Diagnostic Performance of a Locally Produced iELISA Kit Versus a Commercially Available Foreign Kit for Bovine Brucellosis

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Abstract

Background: Bovine brucellosis, caused by *Brucella abortus* (*B. abortus*), is a significant zoonotic disease that leads to substantial economic losses in the livestock industry. Although indirect ELISA (iELISA) is highly effective for diagnosis, current imported commercial kits are expensive, involve long delivery times, and lack of geographical specificity of *B. abortus* antigens.

Objectives: This study aimed to develop a highly efficient in-house iELISA for bovine brucellosis diagnosis using highly purified lipopolysaccharide (LPS) derived from local *B. abortus*, as the antigen, and to comparatively evaluate its diagnostic performance against commercially available ELISA kits in terms of sensitivity, specificity, accuracy, and agreement (Cohen's kappa) using field serum samples.

Methods: The LPS was extracted and purified using the cold butanol method, enzymatic treatments and size exclusion chromatographic (SEC). Then, it was characterized by monitoring of the obtained weight, the size, the morphological structure, the purity, the physiochemical properties, the specificity using of SDS-PAGE, Western blot, fluorescent-based Qubit test, the limulus amoebocyte lysate (LAL) including HPLC. To determine the optimal condition of in-house iELISA and a cut off value of the test, a checkerboard titration method with reference positive bovine serum and fetal bovine serum (FBS) were used to perform for obtaining the proper LPS, serum and conjugate concentration in the reaction. The efficiency of developed in-house iELISA such as sensitivity, specificity and accuracy, were evaluated using 215 positive and 94 negative bovine composite reference serum (CRS) samples obtained from commercial iELISA and Rose Bengal test (RBT). The agreement of the in-house and commercial iELISA was tested using randomized field bovine serum (n = 200) and reported in Cohen's kappa coefficient value.

Results: The highly, purified LPS of multiplex PCR-confirmed *B. abortus* was obtained from the 1st peak of SEC chromatogram contained 1.46% (w/w) of dried weight (DW) of *B. abortus*. Under the SDS-PAGE stained by silver nitrate, LPS showed the ladder pattern with the molecular weights ranged from >25-100 kDa similar to *E. coli* LPS. Low nucleic acid and protein contamination as 0.023% (w/w) and 1.04% (w/w) were observed. Western blot method illustrated the immunological specific reaction between monoclonal antibody (mAb) against *B. abortus* LPS and obtained purified LPS at a band position of 75-100 kDa. Retention time (Rt) of purified *B. abortus* LPS was 1.63 minutes closely to 1.59 minutes of purified *E. coli* LPS after examining by HPLC. LAL test displayed that the obtained LPS (1 mg/ml) had low endotoxin biological activity equal to 9.45 EU/ml. The checkerboard titration test indicated the optimal concentration of purified LPS, serum dilution and conjugate concentration used in in-house iELISA were 0.5 µg/100 µL, 1:80 and 1:8,000 with substrate incubation time as 1.20 minutes. The cut off value of the test was 0.191. The in-house iELISA had the sensitivity, specificity as 87% of 98.94%, respectively with an area under the curve (AUC) of 0.930. Compared with a commercial iELISA test, the in-house version demonstrated a strong agreement, with a Cohen's kappa coefficient value as 0.868.

Conclusion: The developed in-house iELISA for bovine brucellosis diagnosis test contributed the high diagnostic efficiency and strong agreement result to the commercial iELISA test that it has the promisingly potential for further development and use as a low-cost field-scale testing in the future.

Keywords: Bovine brucellosis, *Brucella abortus*, Indirect ELISA, Lipopolysaccharide antigen, Diagnostic performance

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