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

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 geographical specificity regarding their antigens. This study aimed to develop a high-performance, standardized in-house iELISA using highly purified lipopolysaccharide (LPS) from B. abortus, as the antigen. The LPS was extracted and purified using the cold butanol method and chromatographic purification, with nucleic acid and protein contamination levels as low as 0.023% and 1.04%, respectively. Optimal assay conditions were determined by checkerboard titration. The performance of the developed iELISA was evaluated using 364 bovine serum samples, yielding a sensitivity of 87% and a specificity of 98.94%, with an area under the curve (AUC) of 0.930. When compared with a commercial iELISA kit, the in-house version demonstrated strong agreement, with a Cohen's kappa coefficient of 0.868. In conclusion, the developed in-house iELISA kit offers high diagnostic efficiency and has the potential for further development and field-scale testing in the future.

Introduction

Bovine brucellosis is the zoonotic disease, causes livestock industry losses economically and poses a serious public health threat. While bacterial isolation remains the diagnostic gold standard, its practical and biosafety limitations are significant. Consequently, the World Organisation for Animal Health (WOAH) recommends the iELISA for diagnostics due to its high sensitivity and specificity.

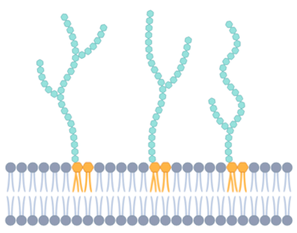
However, reliance on imported commercial iELISA kits is hindered by high costs, prolonged delivery times, and potential diagnostic inaccuracies arising from regional bacterial strain variations. To address these limitations, this study aimed to develop a high-performance, and standardized in-house iELISA, providing a locally optimized diagnostic tool for bovine brucellosis.

Objectives

- To optimize the assay conditions of the developed in-house iELISA for the diagnosis of bovine brucellosis.
- To compare the diagnostic performance of the developed in-house iELISA with an imported commercial kit for bovine brucellosis.

Methodology

- LPS Extraction and Purification



extraction of LPS from bacterial culture using the cold butanol method

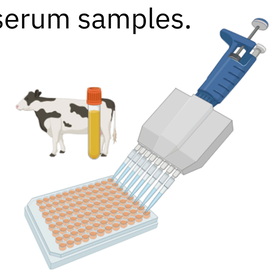
purification of crude LPS by the size exclusion chromatography (SEC)

Characterization of LPS
- SDS-PAGE
- Westren blot analysis
- HPLC

- iELISA test

Optimization of in-house iELISA by checkerboard titration

Comparison of the diagnostic performance of the developed in-house iELISA with an imported commercial kit using bovine serum samples.



Result and Discussion

- LPS Extraction and Purification

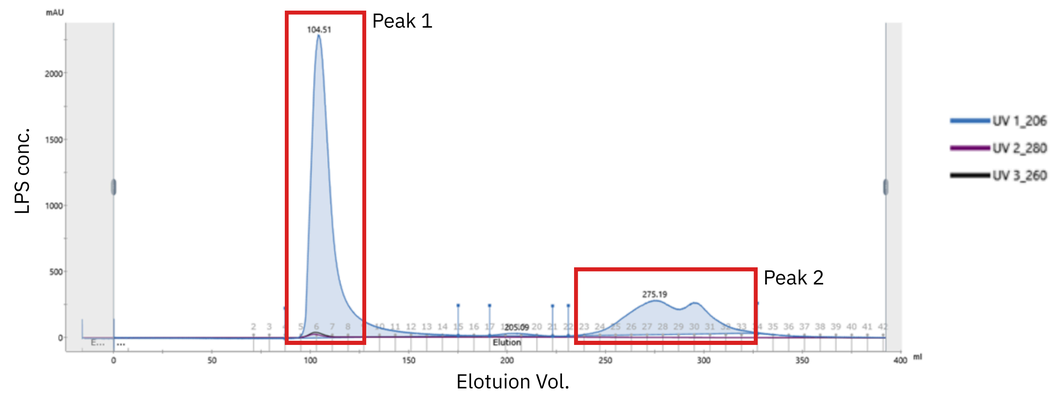


Figure 1. SEC Chromatogram of purified LPS showed two major peaks (1 and 2) at 206 nm absorbent.

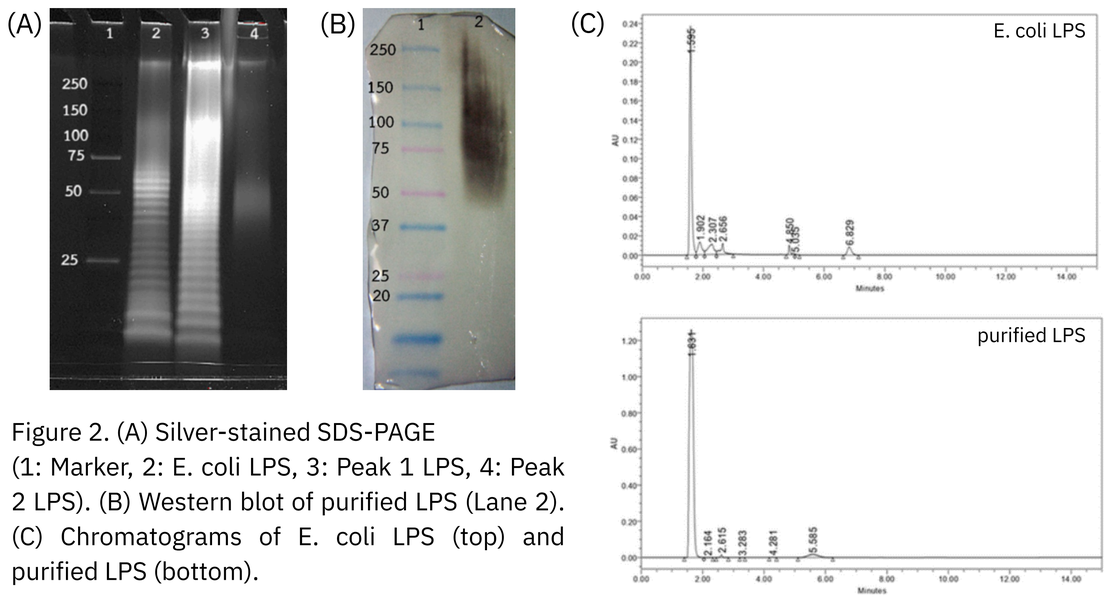


Figure 2. (A) Silver-stained SDS-PAGE (1: Marker, 2: E. coli LPS, 3: Peak 1 LPS, 4: Peak 2 LPS). (B) Western blot of purified LPS (Lane 2). (C) Chromatograms of E. coli LPS (top) and purified LPS (bottom).

- In-house iELISA opimization

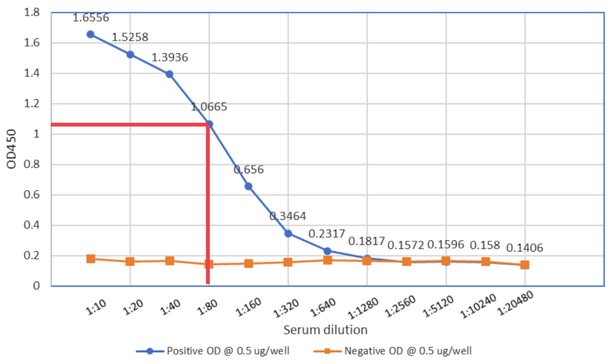


Figure 3. Checkerboard titration curve based iELISA optimization showed the optimal antigen concentration, a serum dilution and a HRP-rec-Protein G dilution are 0.5 µg/well, 1:80 and 1:8000 respectively, with a substrate incubation time of 1 min 20 s.

- Diagnostic performance of the in-house iELISA vs. commercial kit

iELISA performance parameters	Performance values	95% CI
Sensitivity	87.000%	78.796% to 92.893%
Specificity	98.936%	94.215% to 99.973%
AUC	0.930	0.884 to 0.961
Positive Likelihood Ratio	81.780	11.623 to 575.392
Negative Likelihood Ratio	0.131	0.079 to 0.218
Disease prevalence	10.000%	
Positive Predictive Value	90.086%	56.360% to 98.460%
Negative Predictive Value	98.561%	97.632% to 99.129%
Accuracy	97.743%	94.531% to 99.333%

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Observer A	IDVET
Observer B	ELISA

	IDVET		
ELISA	0	1	
0	79	13	92 (46.0%)
1	0	108	108 (54.0%)
	79 (39.5%)	121 (60.5%)	200

Kappa	0.86778
Standard error	0.035148
95% CI	0.79889 to 0.93667

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Figure 4. The developed in-house iELISA demonstrated high diagnostic efficacy, achieving a sensitivity of 87.00% and a specificity of 98.93% (AUC = 0.930) (A). Additionally, it showed strong agreement with the commercial iELISA kit (Cohen's kappa = 0.868) (B).

Conclusion

Highly purified LPS was extracted from B. abortus with minimal contamination and applied to the serological diagnosis of bovine brucellosis. The iELISA results indicated that the in-house iELISA exhibited outstanding diagnostic performance, with high sensitivity and specificity. Ultimately, the developed in-house iELISA kit offers excellent diagnostic efficiency holding the promising potential for further development and field-scale testing in the future.

References

1. Khurana, S. K., Sehrawat, A., Tiwari, R., Prasad, M., Gulati, B., Shabbir, M. Z., Chhabra, R., Karthik, K., Patel, S. K., & Pathak, M. (2021). Bovine brucellosis–a comprehensive review. Veterinary Quarterly, 41(1), 61–88.
2. Mittal, D., Grakh, K., Kumar, M., Jhandai, P., Dahiya, S., Gupta, R., Kumar, R., Prakash, A., Kumar, P., & Moudgil, P. (2025). Seroprevalence of Brucellosis in Haryana, India: A Study Using Rose Bengal Plate Test and Enzyme-Linked Immunosorbent Assay. Pathogens, 14(4), 373.