Research Article

Diagnostic Performance of Lateral Flow Immunoassay for Detecting Brucellosis Antibodies in Goats

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Abstract

Recently, lateral flow immunochromatographic assay (LFIA)-based brucellosis test kits have been imported into Thailand. These kits offer simplicity and rapid results, making them potentially valuable tools for facilitating goat trade while preventing disease transmission. However, their diagnostic performance has not yet been evaluated. This study assessed the sensitivity, specificity, and diagnostic agreement of a commercial LFIA-based test kit for brucellosis detection in goats by comparing the results with those obtained from validated laboratory diagnostic methods, which served as the reference standard. A total of 275 goat serum samples were analyzed using a commercial LFIA-based test kit. The results from the Rose Bengal test and microtiter plate agglutination test previously performed at the Kamphaeng Saen Veterinary Diagnostic Center, Faculty of Veterinary Medicine, Kasetsart University, served as references to evaluate the performance of the test kit. Based on the analysis of the commercial LFIA-based test kit, its sensitivity and specificity were 86.10% and 90.91%, respectively. In addition, its Cohen's kappa coefficient of 0.731 indicated a substantial level of agreement with the laboratory test results. Therefore, the commercial LFIA-based test kit could be recommended as a screening tool for brucellosis in goats, particularly in situations where immediate results are required.

Keywords: Brucellosis, Lateral flow immunochromatography, Antibody detection, Goat

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ประสิทธิภาพของชุดตรวจอิมมูโนโครมาโตกราฟีชนิดไหลในแนวราบในการตรวจหาแอนติบอดีต่อโรคบรูเซลโลซิสในแพะ

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บทคัดย่อ

ปัจจุบันมีผู้นำเข้าชุดตรวจโรคบรูเซลโลซิสที่อาศัยหลักการอิมมูโนโครมาโตกราฟีชนิดไหลในแนวราบมาในประเทศไทย ชุดทดสอบดังกล่าวมีขั้นตอนที่ไม่ยุ่งยากและทราบผลได้รวดเร็ว จึงอาจเป็นเครื่องมือที่เอื้อประโยชน์ในการซื้อ-ขายแพะ โดยไม่ก่อให้เกิดการแพร่กระจายโรค อย่างไรก็ตามชุดทดสอบดังกล่าวยังไม่ถูกประเมินประสิทธิภาพในการวินิจฉัย การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อประเมินค่าความไว ความจำเพาะ และความสอดคล้องในการวินิจฉัยของชุดทดสอบเชิงพาณิชย์ที่ใช้เทคนิคดังกล่าวในการตรวจวินิจฉัยโรคบรูเซลโลซิสในแพะ โดยเปรียบเทียบกับผลการตรวจจากห้องปฏิบัติการที่เป็นวิธีมาตรฐาน การศึกษานี้ได้ทำการวิเคราะห์ตัวอย่างซีรั่มของแพะจำนวน 275 ตัวอย่าง โดยใช้ชุดทดสอบอิมมูโนโครมาโตกราฟีชนิดไหลในแนวราบเชิงพาณิชย์ และใช้ผลการตรวจด้วยวิธี Rose Bengal test ร่วมกับ microtiter plate agglutination test จากศูนย์ชันสูตรโรคสัตว์กำแพงแสน คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ เป็นวิธีอ้างอิง เพื่อประเมินประสิทธิภาพของชุดทดสอบ ผลการวิเคราะห์พบว่าชุดทดสอบเชิงพาณิชย์ดังกล่าวมีค่าความไวและค่าความจำเพาะอยู่ที่ร้อยละ 86.10 และร้อยละ 90.91 ตามลำดับ ค่าสัมประสิทธิ์โคเฮนแคปปาอยู่ที่ 0.731 ซึ่งถือว่าชุดตรวจให้ผลสอดคล้องอย่างมากกับผลทดสอบทางห้องปฏิบัติการ ดังนั้นชุดทดสอบเชิงพาณิชย์นี้สามารถนำไปใช้เป็นเครื่องมือคัดกรองเบื้องต้นสำหรับการตรวจหาโรคบรูเซลโลซิสในแพะได้ โดยเฉพาะอย่างยิ่งในสถานการณ์ที่จำเป็นต้องทราบผลอย่างเร่งด่วน

คำสำคัญ: โรคบรูเซลโลซิส อิมมูโนโครมาโตกราฟีชนิดไหลในแนวราบ การตรวจหาแอนติบอดี แพะ

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Introduction

Brucellosis is a significant infectious disease in the livestock industry and a serious zoonotic illness caused by various species of the *Brucella* genus (Khan and Zahoor 2018). It affects a wide range of animal hosts, including goats, sheep, cattle, and dogs (Qureshi et al., 2023). In animals, brucellosis primarily impairs reproductive function, potentially resulting in abortion or reproductive failure. In humans, it manifests as a severe and debilitating illness (Pal et al., 2020). The disease contributes to significant impacts on the economy and on veterinary and human healthcare systems globally, particularly in developing countries (Pal et al., 2020; Khurana et al., 2021; Khoshnood et al., 2022). Therefore, investigating the epidemiological distribution of brucellosis is essential for developing evidence-based measures to control and prevent disease spread (Smits et al., 2003; Irmak et al., 2004; Zhang et al., 2018; Ghanbari et al., 2020; Dadar et al., 2021).

Effective control of brucellosis requires a well-defined strategy in which reliable diagnostic methods constitute a fundamental component (Khurana et al., 2021; Qureshi et al., 2023). Although bacterial culture remains the gold standard for diagnosis due to its high accuracy, it is labor-intensive, time-consuming, and costly (Abdoel et al., 2008; Poester et al., 2010; Quintero et al., 2018; Qureshi et al., 2023). Serological tests, including the Rose Bengal test (RBT) and indirect enzyme-linked immunosorbent assay (iELISA), are extensively used due to their convenience, reliability, and high sensitivity in detecting brucellosis (Poester et al., 2010; Šerić-Haraćić et al., 2022). These methods detect antibodies produced in response to *Brucella* spp. infection. Following infection, animals produce antibodies against the pathogen within 5–14 days (Poester et al., 2010). In the absence of a vaccination program against this disease for goats in Thailand, antibody detection serves as a practical indicator of natural exposure or ongoing infection. However, all of these serological methods require well-equipped laboratory facilities and skilled personnel. In addition, some are also time-consuming, which may limit their feasibility under actual field conditions and impede timely disease control efforts.

Recently, lateral flow immunochromatographic assay (LFIA)-based test kits have been developed and used as an animal-side test to detect various animal diseases (Di Nardo et al., 2021), including brucellosis. LFIA platforms rely on capillary flow to drive interactions between sample constituents and assay reagents (Koczula and Gallotta 2016). As brucellosis diagnosis is predominantly serological, these assays are typically designed for antibody detection. A positive signal is generated when the anti-*Brucella* antibodies in the sample form an antibody–antigen complex that is subsequently captured by the immobilized components on the test strip. This method is a convenient and rapid diagnostic tool that does not require specialized technicians or laboratory facilities (Poester et al., 2010). Therefore, it can serve as an additional option for brucellosis screening and support control strategies.

However, user confidence in selecting these tests may be undermined by the limited number and variability of study results on the sensitivity, specificity, and accuracy of brucellosis antibody LFIA-based test kits (Abdoel et al., 2008; Ahmed et al., 2016; Gusi et al., 2019). A study with a larger sample size would provide more reliable results. Therefore, the objective of the present study was to evaluate a commercial LFIA-based kit from Shenzhen Lvshiyuan Biotechnology Co., Ltd. (China) for detecting *Brucella* antibodies in goats. This particular kit was selected based on its availability in Thailand, preliminary validation results, and suitability for use under field conditions, without any commercial bias. The study aimed to assess the sensitivity, specificity, and accuracy of the LFIA-based test kit in goats, compared with laboratory test results.

Materials and methods

Sample size determination

The required sample size was calculated based on the estimated sensitivity and specificity of 85.7% and 94%, respectively, from a preliminary study of 25 samples with known positive and negative status confirmed by RBT and microtiter plate agglutination test (MPAT) (unpublished data). Using a formula for estimating proportions with 95% confidence and 5% precision, a total of 275 samples were included, comprising 187 positive and 88 negative samples for this study. The experimental protocol was approved by the Animal Care and Use for Scientific Research Committee, Kasetsart University (ACKU65-VET-079).

Sample characterization and reference testing

Goat serum samples were selected from those submitted since 2017 for brucellosis testing at the Kamphaeng Saen Veterinary Diagnostic Centre, Faculty of Veterinary Medicine, Kasetsart University, Thailand. RBT (IDEXX Laboratories; Pourquier Rose Bengal Ag; France) was used as the initial screening method in the laboratory. Serum was mixed with an equal volume of antigen on a glass slide and gently stirred for 4 minutes at room temperature. A positive result was determined by the visual presence of agglutination (WOAH 2016). If the result was negative, the sample was considered negative for brucellosis based on the high sensitivity of RBT. However, if the result was positive, confirmation was performed using the MPAT. Serum was serially diluted two-fold from 1:10 to 1:1280. Then, *Brucella abortus* antigen was added to each well and incubated at 37 °C for 48 hours. Positivity was defined by the presence of agglutination at MPAT titers ≥ 1:40 (Bunleupongphan et al., 2014). Positive and negative controls were performed whenever a new batch of antigen was introduced.

All samples had been stored at -20 °C. Samples were classified as hemolyzed or non-hemolyzed based on the visual comparison of serum color to a reference chart; those exhibiting pink-to-red discoloration were classified as hemolyzed, while clear-to-pale-yellow samples were classified as non-hemolyzed.

Lateral flow immunochromatographic assay

The selected serum samples were used in a blind test using a commercial brucellosis LFIA-based test kit (Shenzhen Lvshiyuan Biotechnology Co., Ltd.; China), strictly following the manufacturer's instructions. Two independent readers performed the visual assessment under adequate lighting conditions within 15 minutes of running the test. Positive results were further classified as either strong or weak positives based on the intensity of the red strip on the test line (T-line), as determined by visual observation. A bright red line was defined as a strong positive, whereas a faint or lighter line, still distinguishable from a negative result, was considered a weak positive. In cases of disagreement, a third, senior reader adjudicated the final result.

Statistical analysis

The commercial brucellosis test kit was compared to the laboratory results based on calculations of sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV). Cohen's kappa coefficient was computed to assess the reliability of the two tests. The Cohen's kappa coefficient was interpreted using the criteria proposed by Landis and Koch (1977). The association between hemolysis and the occurrence of false-positive and false-negative results was analyzed using Fisher's exact test. The relationship between the intensity of positive samples and their status compared to the laboratory results was also evaluated using the same method. All statistical analyses were performed using Stata (version 14; StataCorp LP; College Station, TX, USA) with a 95% confidence level.

Results

The test kit yielded false-positive and false-negative results in 8 and 26 samples, respectively, when compared to the reference laboratory methods. Of the 27 weakly positive samples, 22 were positive based on laboratory results, while 5 were laboratory-negative (Table 1). The performance of the test kit, compared to the laboratory RBT and MPAT results, is shown in Table 2. The Cohen's kappa coefficient of 0.731 (95% CI [0.647, 0.814]) indicated substantial agreement with the laboratory test results (Landis and Koch 1977).

There were 53 hemolyzed samples out of the 275 total samples (Table 1). Fisher's exact test revealed that hemolysis was not significantly associated with either false-positive results ($p = 0.185$) or false-negative results ($p = 0.795$). However, weak positive results were significantly associated with the occurrence of false positives ($p = 0.003$).

Discussion

The LFIA-based brucellosis test kit provided positive results in eight laboratory-negative samples. These false-positive results may be associated with bacterial infections, particularly those caused by bacteria containing O-polysaccharides such as *Escherichia coli* and *Yersinia* spp., (Weynants et al., 1996; Chenais et al., 2012; Ahmed et al., 2016; Bodenham et al., 2021; Yin et al., 2021). Cross-reactions may occur between antibodies from these infections and the cell wall of *Brucella* spp., particularly when lipopolysaccharides are used as antigens in the test kit. Therefore, it is essential to carefully rule out other Gram-negative bacteria when a positive result is obtained. These findings underscore the need for the development of more specific antigens that can differentiate *Brucella* spp. from other Gram-negative bacteria, thereby reducing cross-reactivity and improving diagnostic accuracy.

Inappropriate samples, such as hemolyzed serum, may result in false-positive outcomes (Gusi et al., 2019). In the present study, three hemolyzed samples gave false-positive results. However, the test kit manual did not mention excluding

Table 1. Diagnostic results from the lateral flow immunoassay (LFIA)-based brucellosis test kit and laboratory tests.

Type of sample	Commercial LFIA test kit	Laboratory test (sample)		Total (sample)
		Positive	Negative	
Non-hemolyzed	**Positive**	**129**	**5**	**134**
	- Strong	109	1	110
	- Weak	20	4	24
	Negative	**22**	**66**	**88**
Hemolyzed	**Positive**	**32**	**3**	**35**
	- Strong	30	2	32
	- Weak	2	1	3
	Negative	**4**	**14**	**18**
	Total	**187**	**88**	**275**

hemolyzed samples. This aligns with our findings, as Fisher's exact test showed no significant association between hemolysis and false positives. However, to minimize hemolysis during blood collection, it is crucial to avoid using needles that are too small and to withdraw blood gently. Collection tubes containing blood samples require gentle handling. Additionally, prompt sample processing is essential, as prolonged time at room temperature significantly increases red blood cell breakdown (Heireman et al., 2017).

Interestingly, most false-positive samples (5 out of 8) showed weak positive results. The statistical analysis revealed a significant association between false-positive results and weakly positive reactions. Therefore, samples yielding ambiguous positive outcomes should be classified as inconclusive or suspect, necessitating re-testing. Ideally, confirmatory testing with a highly specific method should be performed. To address the inherent subjectivity in visual reading, establishing a rigorous color intensity cutoff or a reader device is essential for consistent and reproducible reading of faint lines (Di Nardo et al., 2021). However, the practical application of such a standard remains inherently challenging.

Antibodies against *Brucella spp.* can be degraded through stratification, dehydration, and proteolysis when samples are frozen at -20 °C. This degradation may impair the ability to detect antibodies in serological tests (Hussein and Ammar 2004). Some serum samples in the present study were stored at -20 °C for up to 5 years, while a few may have been exposed to repeated freeze-thaw conditions. Additionally, bacterial contamination in serum can accelerate antibody degradation (Hussein and Ammar 2004). The degradation of antibodies in stored serum samples may partly explain the false-negative results observed in the present study, thereby reducing the overall sensitivity. To avoid preservation-related issues, samples should be sterilized using filtration, aliquoted into multiple tubes, and stored in a temperature-controlled freezer to prevent repeated freezing and thawing (Hussein and Ammar 2004). However, the use of fresh samples may reduce the risk of antibody degradation and contamination associated with frozen samples and repeated freeze–thaw cycles during prolonged storage, providing a more accurate reflection of the evaluated LFIA's performance. Human error may contribute to false readings (Kim and Park 2004; Wang et al., 2020; Colombo et al., 2023). For example, weak positive results may be misread when interpreted visually, particularly under poor lighting conditions. To reduce this risk, test results should be examined in a well-lit environment.

With a PPV of 95.27% and a specificity of 90.91%, a positive test result is highly reliable for confirming an infection, supporting the immediate implementation of disease control measures. The high proportion of positive samples in this study (187/275, 68%) likely contributed to the elevated PPV observed. PPV reflects the probability that a positive test result correctly identifies an individual who is infected. Generally, as the proportion of positive cases in a population increases, the PPV also increases, assuming constant test sensitivity and specificity. Therefore, it is essential to consider the specific population when interpreting test results.

On the contrary, a negative result may not be entirely reliable due to the NPV of 75% and the sensitivity of 86%. This indicates that some infected animals may test negative. Specifically, among animals with a negative test result, approximately 75% are truly not infected. Conversely, the remaining are infected, representing false negative cases. These missed diagnoses pose a major risk of continued disease transmission, threatening the health of both the animals and humans. Therefore, when this kit is used for screening, additional testing is recommended for negative results to improve overall diagnostic accuracy, particularly in animals with a high-risk history. Such supplementary testing is essential for preventing false negatives, which is crucial for effective disease control within the herd. Routine monitoring of the herd is also advised.

Table 2. Performance of lateral flow immunoassay-based brucellosis test kits.

Diagnostic test	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)	Cohen's kappa coefficient	Reference method
LFIA (Present study) with 95% CI	86.10 (81.14, 91.06)	90.91 (84.90, 96.92)	87.64 (83.15, 91.28)	95.27 (92.07, 98.47)	75.47 (67.28, 83.66)	0.731 (0.647, 0.814)	RBT+MPAT
LFIA (Shome et al., 2018)	80.77	98.53	96.97	84.00	98.17	0.80	RBT

Note: PPV = Positive predictive value; NPV = Negative predictive value; RBT = Rose Bengal test; MPAT = Microtiter plate agglutination test.

A comparison of the performance of another commercial LFIA-based brucellosis test kit, reported by Shome et al. (2018), revealed that the test kit used in the present study had higher sensitivity but lower specificity and a lower Cohen's kappa coefficient (Table 2). However, drawing direct comparisons is limited by considerable differences in the reference standards between the studies. The previous study used only RBT, whereas the present study applied a combination of RBT and MPAT as reference methods, with positive RBT results confirmed using MPAT. This change resulted in different sensitivity and specificity values for the reference methods themselves. Furthermore, the two studies used different commercial kits and the type of antigen used in the test kits can significantly influence their performance and contribute to the observed discrepancies (Souza et al., 2024).

The test kit used in the present study is applicable to both serum and whole blood samples. For on-site screening, whole blood is more convenient. However, it was not possible to use whole blood samples in the present study. Therefore, further research may be necessary to evaluate the efficacy of the test kit with whole blood samples. Although the cost of the test kit is higher than that of laboratory tests, it is more suitable for field use, where equipment, specialized personnel, and laboratory facilities are not available. Additionally, using the test kit on-site eliminates the need to send samples to a laboratory, thereby reducing delays caused by transportation time. Furthermore, the test kit is easy to use and provides results more quickly than laboratory tests, making it a practical option for disease screening. Early detection enables rapid decisions to stop and control the spread of the disease.

Conclusion

The sensitivity and specificity of the commercial test kit were 86.10% and 90.91%, respectively. Cohen's kappa coefficient of 0.731 indicated substantial agreement with the laboratory test results, based on RBT and MPAT. Therefore, the test kit could be used as an effective screening tool for brucellosis in goats in conjunction with other confirmatory diagnostic methods.

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