A Comparison of Brucella IgG and IgM ELISA Assays With Agglutination Methodology

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Despite brucellosis having a low incidence rate in developed nations, it still remains the leading zoonotic disease in the world. Culturing of *Brucella* spp. provides good specificity but in cases where the fever is intermittent, sensitivity is problematic. This has led to the development of serological methods of detection. Brucella agglutination methods have been considered the serological gold-standard since their inception, although commercial Brucella IgG and IgM enzyme-linked immunosorbent

assays are available to potentially aid in the diagnosis of the disease. In our study, anti-*Brucella* IgG and IgM assays were compared with agglutination. Individually the IgG assay tested had an accuracy of 56% and the IgM assay had an accuracy of 77%. These poor accuracies reinforce Centers for Disease Control's conclusion that nonagglutination tests should not be used to confirm brucellosis. J. Clin. Lab. Anal. 24:160–162, 2010. © 2010 Wiley-Liss, Inc.

Key words: Brucella; serology; agglutination; ELISA

INTRODUCTION

Brucellosis is caused by bacteria of the *Brucella* genus and is transmitted to humans from animals containing specific species, including *B. abortus* (cattle), *B. melitensis* (goats and sheep), *B. suis* (swine), and *B. canis* (canines) (1). Although brucellosis is rare in most developed nations, it is still the leading cause of zoonotic disease in the world with over 500,000 new cases reported annually (2). Because symptoms are nonspecific and may include undulating fever, malaise, and generalized body aches, a detailed patient history is important, including travel locations, animal contact, and ingestion of unpasteurized milk and cheese products (1,3). In industrial countries treatment may often be delayed due to the rarity of the disease and the indiscriminant presentation. This has led to the need for rapid testing that is both sensitive and specific.

A definitive diagnosis of brucellosis requires identification of *Brucella* cultured from a clinical specimen; however, this may not be practical due to the highly infectious nature of the organisms or sensitive in cases where the fever is intermittent (4). Serological

techniques, including the serum agglutination test (SAT) and enzyme-linked immunosorbent assays (ELISAs) have been developed as a sensitive alternative to blood culture. Although ELISA assays may differentiate between IgG and IgM antibodies, offer a reduction in cost, have shorter run times and require less training to interpret compared with agglutination methodologies, evidence exists that ELISA assays may not have sufficient specificity to be used as a diagnostic tool (4,5).

In this study, separate commercially available ELISA assays for the detection of anti-*Brucella* IgG and IgM antibodies were compared with the agglutination methodology.

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MATERIALS AND METHODS

Human Sera

This study was approved by the Institutional Review Board of the University of Utah, IRB 7275. Thirty-seven serum samples, originally sent for *Brucella* serology testing to ARUP Laboratories between 2008 and 2009, were collected, de-identified, and stored at 2–8°C.

Brucella Serum Agglutination Testing

All 37 serum samples were tested for anti-*Brucella* antibodies by the SAT based on the method first described by Renoux (6). Briefly, patient serum was serially diluted in a sodium chloride solution and mixed with a small volume of *B. abortus* antigen suspension (Remel, Lenexa, KS). According to the manufacturer, the antigen preparation used reacts with *B. abortus*, *B. melitensis*, and *B. suis*. After an incubation at 37°C for 24 hr, each sample was analyzed for agglutination. A clinically significant specimen is considered to have a titer of 1:80 or greater.

ELISA Testing

All 37 serum samples were tested for anti-*Brucella* IgG and IgM antibodies on the Calbiotech Brucella IgG and IgM ELISA Kits (Spring Valley, CA) according to the manufacturer's instructions. Briefly, patient sera and positive and negative control sera provided by the manufacturer were diluted by adding 10 µl of patient or control sera in 200 µl of dilution buffer and added to wells of a microtiter plate coated with *B. abortus* outer membrane, which is shared by *B. abortus*, *B. melitensis*, and *B. suis*. Following a 20-min incubation at room temperature (RT), each well was washed and either IgG or IgM enzyme conjugate was added. After an additional 20-min incubation at RT the wells were washed and substrate was added. A final 10-min incubation at RT was stopped by adding a stop solution. All wells were read on a spectrophotometer at 450 nm. Samples were compared with a calibrator sample that was present on each run and an Absorbance (Ab) Index value was calculated for each sample. An Ab Index greater than 1.1 was considered positive, samples with an Ab Index between 0.9 and 1.1 were considered equivocal, and all other samples were considered negative.

Statistical Analysis

To determine the overall agreement, clinical sensitivity, clinical specificity, and the 95% confidence intervals (CI) for sensitivity and specificity, two-by-two contingency

table analysis was used (7). Equivocal results were excluded for the purposes of calculating agreement, sensitivity, and specificity. Results of the Calbiotech assays were compared with the agglutination assay and any samples that disagreed were repeated on the all discrepant assays to ensure accurate results.

RESULTS

When the IgG ELISA results were compared with the agglutination results, overall agreement, sensitivity, and specificity were calculated to be 56.3%, 41.7% (95% CI, 22.0–62.1%), and 65.0% (95% CI, 53.2–77.3%), respectively (Table 1). There were seven false positive and seven false negative results on the IgG ELISA. When the IgM ELISA results were compared with the agglutination results, overall agreement, sensitivity, and specificity were calculated to be 77.4%, 83.3% (95% CI, 61.7–94.8%), and 73.7% (95% CI, 60.0–80.9%), respectively (Table 1). There were five false positive results and two false negative results on the IgM ELISA.

To determine whether the ELISA method compared better with the agglutination method if both the IgG and IgM results were considered together, combined ELISA results were generated. A combined ELISA result was considered positive if either the IgG or IgM ELISA values were positive, and equivocal if either of the IgG or IgM ELISA results were equivocal with neither result

TABLE 1. Summary of Results Comparing the ELISA Results With the Agglutination Results

	Agglutination ^a	
	Positive	Negative
Calbiotech IgG ^b		
Positive	5	7
Negative	7	13
Equivocal	2	3
Calbiotech IgM ^c		
Positive	10	5
Negative	2	14
Equivocal	2	4
Combined IgG and IgM ^d		
Positive	12	9
Negative	1	11
Equivocal	1	3

^aBrucella serum agglutination assay.

^bThe Calbiotech IgG ELISA had an overall agreement, sensitivity, and specificity of 56.3%, 41.7% (95% CI, 22.0–62.1%), and 65.0% (95% CI, 53.2–77.3%), respectively.

^cThe Calbiotech IgM ELISA had an overall agreement, sensitivity, and specificity of 77.4%, 83.3% (95% CI, 61.7–94.8%), and 73.7% (95% CI, 60.0–80.9%), respectively.

^dThe Calbiotech IgG and IgM ELISA assays had a combined agreement, sensitivity, and specificity of 69.7%, 92.3% (95% CI, 72.7–98.6%), and 55.0% (95% CI, 42.3–59.1%), respectively.

being positive. The combined ELISA agreement, sensitivity, and specificity were 69.7%, 92.3% (95% CI, 72.7–98.6%), and 55.0% (95% CI, 42.3–59.1%), respectively (Table 1).

DISCUSSION

Neither the IgG nor IgM assay compared well with the agglutination method, which has been shown to be sensitive and specific (6,8–10). Although sensitivity increased when the combined IgG and IgM results were compared with agglutination, the accuracy and specificity were still poor. Despite the small sample size, the 95% CI's upper limit for the combined specificity was still well below acceptable limits at 59.1%. With poor specificity, the probability of reporting out a false positive result is high, potentially leading to unnecessary treatment. The current treatment for brucellosis is multiple antimicrobials administered over a 6-week period (11,12) and the burden associated with treatment can include side effects related to medication use, the emergence of antibacterial resistant microbes, and the cost of medication (13,14).

It should be noted that our study was conducted on serum samples obtained in the US, an area with a low rate of brucellosis. It has previously been reported that the Panbio ELISA assays for the detection of anti-*Brucella* antibodies have high sensitivities in endemic regions (15). However, these assays are no longer available in the US and may not perform the same in regions where disease is not endemic (4).

As a result of the complications associated with a false positive diagnosis, the Centers for Disease Control (CDC) has published laboratory criteria for diagnosis, which states that “nonagglutination-based tests [do] not meet the current CDC/CSTE (Council of State and Territorial Epidemiologists) case definition for brucellosis” (4,16). Our study supports the CDC's conclusion by showing ELISA assays suffer from poor sensitivity and specificity when compared with agglutination.

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