

Comparative study of the performance of different commercially available and in-house serological assays for diagnosis of human brucellosis using samples from endemic and non-endemic areas

Moustafa Mohamed Awaden^{1,2}, Robyn A. Stoddard¹, Guillermo Pimentel² and Alex R. Hoffmaster¹

¹Bacterial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA,

²US Naval Medical Research Unit 3 (NAMRU 3), Cairo, Egypt



Abstract

Infection with *Brucella* spp. continues to pose a human health risk globally. Clinical diagnosis of human brucellosis is problematic since the disease has a broad spectrum of manifestations, can be confused with many other acute febrile infections, and must be confirmed by laboratory testing. The gold standard for laboratory diagnosis of brucellosis is the isolation of *Brucella* spp. from blood or other biological fluids by microbial culture. However, microbial culture suffers from low sensitivity, time requirements, risks associated with laboratory handling of highly infectious *Brucella* spp., and lack of availability in areas where brucellosis is endemic. Due to these difficulties, serological methods (agglutination reactions in particular) are primarily used for patient diagnosis. They are less expensive, simple and readily available compared to the other methods. Agglutination methods, however, can have low sensitivity and specificity, and interpretation of the test results may be difficult due to cross reactions with other bacterial species. They also can be labor intensive and thus unsuitable for high volume surveillance and epidemiological studies. ELISA assays, on the other hand, are fast, sensitive, specific, high throughput, and can be cost effective. The aim of this study was to compare the performance of commercially available ELISA kits and an in-house ELISA to each other and to *Brucella* microagglutination test (BMAT) using samples from regions endemic (Egypt, n=214) and non-endemic (United States, n= 268) for brucellosis. Performance evaluation included sensitivity, specificity, cost, time and complexity, throughput and ability to differentiate between acute and chronic infections. A total of 487 serum samples collected from case-patients and controls were included in this study. Case-patients were defined as persons with laboratory-confirmed *Brucella* infection, and included those confirmed by both culture and serology (n=56) and those confirmed by serology alone (n=132). Controls were defined as persons diagnosed with other infections that serologically cross react with human brucellosis (n=46) or with fever of unknown etiology (n=60) who provided samples on admission only. The original serology was performed by tube agglutination on serum provided from all patients at admission and, for case-patients, also on serum provided 2-7 months after treatment for brucellosis. For this evaluation, serum samples were tested by BMAT and BioQuant, IBL and Vircell ELISA kits, while testing using in-house and EUROIMMUN ELISA is currently underway. The complete evaluation and comparison of these assays will be presented.

Methods

Sample Collection

- Case-patients for this study included patients positive by culture and serology (n=56) or serology only (n=132) from locations where brucellosis is endemic (Egypt, n=67) or non-endemic (United States, n=121).
- Serum samples were provided from all patients at admission (acute sample) and 2-7 months after diagnosis and starting therapy (convalescent sample).
- Specificity was tested using sera from persons diagnosed with infections that may serologically cross react with or clinically present similar to brucellosis (n=46) and from persons with fever of unknown etiology (n=60) who provided samples on admission only.

Laboratory methods

- Serum samples were tested by *Brucella* microagglutination test (BMAT), the gold standard for serological diagnosis at the Centers for Disease Control and Prevention, as previously described (8) with minor modifications including use of U-bottom plates, incubation at 28°C, and discontinued use of safranin.
- ELISA assays were performed based on the manufacturers' procedures and recommendations: BioQuant (BioQuant Inc., California, USA), IBL America (Immuno-Biological Laboratories, Inc., Minnesota, USA), Vircell (Vircell, Granada, Spain) and EUROIMMUN (EUROIMMUN US, New Jersey, USA).
- An in-house ELISA originally developed at the Naval Medical Research Unit (NAMRU) in Cairo, Egypt (5) was included with minor modifications including: serum was diluted at 1/160 and using the signal/cut-off ratio (S/CO) for interpretation.

Interpretation of serology results:

- BMAT:
 - Samples from Egypt were considered positive if the titer was ≥ 320 , inconclusive for titers between 40 and 160 and negative at < 20 .
 - Samples from the United States were positive if the titer was ≥ 160 , inconclusive for titers between 40 and 80 and negative at < 20 .
- Commercial ELISA kits: Interpreted based on the manufacturer's instructions
- NAMRU ELISA: An S/CO ratio of ≥ 1.0 was positive, $\geq 0.7 < 1.0$ was borderline, and < 0.7 was considered negative

Data analysis

- A sample was considered to be positive on the ELISA assays if either or both IgM and IgG were positive.
- Statistical analyses were performed using 2x2 contingency tables.

Introduction

- Brucellosis is a worldwide zoonosis and a major health problem in the Mediterranean basin, the Middle East, the Arabian Gulf and Latin American countries (1).
- The definitive diagnosis for brucellosis is blood culture, however, problems with false-negative results, the prolonged periods of incubation, and the danger of handling *Brucella* spp. in the laboratory has limited its usage (2, 3).
- Serology tests, agglutination assays in particular, are the primary diagnostic tools for diagnosis of brucellosis because of their low cost, simplicity, and they are readily available compared to the other methods (2).
- Agglutination methods can have low sensitivity and specificity in some cases, and they are time consuming and unsuitable for high volume testing (4, 5).
- ELISA assays have been found to be fast, sensitive, specific, high throughput, and cost effective (6, 7).
- In this study we compare the performance of four commercially available ELISA kits and an in-house ELISA to each other and to *Brucella* microagglutination test (BMAT) using samples from regions endemic (Egypt) and non-endemic (United States) for brucellosis.

Results and Conclusions

Table 1. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of five ELISAs and BMAT tests for detection of anti-*Brucella* antibodies in brucellosis (188) and non-brucellosis (106) patients

Assay	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
BioQuant	100	40	75	100
IBL	99	90	95	99
Vircell	96	94	97	93
NAMRU	95	100	100	91
EUROIMMUNE	100	88	94	100
BMAT	100	97	98	100

Table 2. Cross-reactivity of each assay with serum specimens from patients diagnosed with other diseases

Diagnosed Pathogen	Number of samples tested	Number (%) of samples positive by:					
		BioQuant	IBL	Vircell	NAMRU	EUROIMMUNE	BMAT
<i>Escherichia coli</i> O:157	6	6 (100)	1 (17)	0 (0)	0 (0)	1 (17)	1 (17)
<i>Vibrio cholera</i>	5	3 (60)	0 (0)	0 (0)	0 (0)	1 (20)	1 (20)
<i>Francisella tularensis</i>	5	4 (80)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Salmonella typhi</i>	10	6 (60)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Rickettsia typhi</i>	10	5 (50)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)
<i>Leptospira interrogans</i>	10	2 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Culture and serology negative ^a	60	38 (63)	10 (17)	6 (10)	0 (0)	11 (18)	0 (0)

^aIncluded 50 sera from patients in Egypt with acute febrile illness of unknown etiology

Table 3. Detection of anti-*Brucella* antibodies in acute serum samples collected from endemic and non-endemic areas using five different ELISA assays

Assay	Number and percent (%) of acute specimens testing positive			
	Brucellosis diagnosed by culture and serology		Brucellosis diagnosed by serology	
	Endemic area (n=30)	Non-endemic area (n=26)	Endemic area (n=37)	Non-endemic area (n=95)
BioQuant	30 (100)	26 (100)	37 (100)	95 (100)
IBL	30 (100)	26 (100)	37 (100)	94 (99)
Vircell	30 (100)	25 (96)	35 (95)	90 (95)
NAMRU	30 (100)	25 (96)	36 (97)	86 (91)
EUROIMMUNE	30 (100)	26 (100)	37 (100)	95 (100)

Table 4. Practical and economic evaluations of the different laboratory tests used in the study

	BioQuant	IBL	Vircell	NAMRU	EURO	BMAT
Sera volume needed (μ l)	10.0	2.0	10.0	1.5	2.0	40.0
Assay time (hours) ^a	2.5	3.5	3.0	3.5	2.75	21 ^b
Cost/sera sample (US\$)	4.40	7.20	3.40	0.30	4.40	1.40
% samples tested two/three times ^c	46/27	44/32	18/0	9/0	13/0	0/0

^aTime needed to test one ELISA plate (96 tests) for both IgM and IgG antibody by one person which includes setting up and incubation times for each assay

^bTime needed for setting up 61 samples by one person to test for the total and IgG antibody titers

^cSamples required repeat testing due to a result that was over the optical density limit or because of a borderline result in which a repeat test is recommended by the manufacturer's recommendations.

Conclusions

- All assays showed a high sensitivity level ($\geq 95\%$), with no statistically significant differences noted between the assays ($P > 0.05$).
- A wide variation was seen in the specificity of the ELISA assays with the BioQuant ELISA having a much lower specificity when compared to all other assays ($P < 0.001$).
- No one pathogen seemed to cause more false-positives on the assays, but there were more false-positives with sera from persons whose health status or etiology of illness was unknown.
- When comparing the performance of the ELISA assays on acute sera from case-patients where *Brucella* is endemic and non-endemic, there was no statistically significant difference ($P > 0.05$) (Table 3). There was also no significance between case-patients diagnosed based on culture and serology or serology alone ($P > 0.05$).
- The ELISA assays showed little variation in percentage (95% to 100%) of specimens found to be positive in acute samples, however, there was greater variation in the percentage of samples found to be positive with the assays for convalescent samples (80% and 100%) (Figure 1).
- The ELISA assays required a similar volume of serum and time for completion of the assay, but they varied greatly on the cost per sample.
- All of the kits required some retesting of samples, however BioQuant and IBL assays required a significant amount of retesting which increases both the time and cost per sample tested.
- In-house assays have the advantage that they can be less expensive and still perform well, however, they require additional time for development, validation, and quality control and assurance assessment.

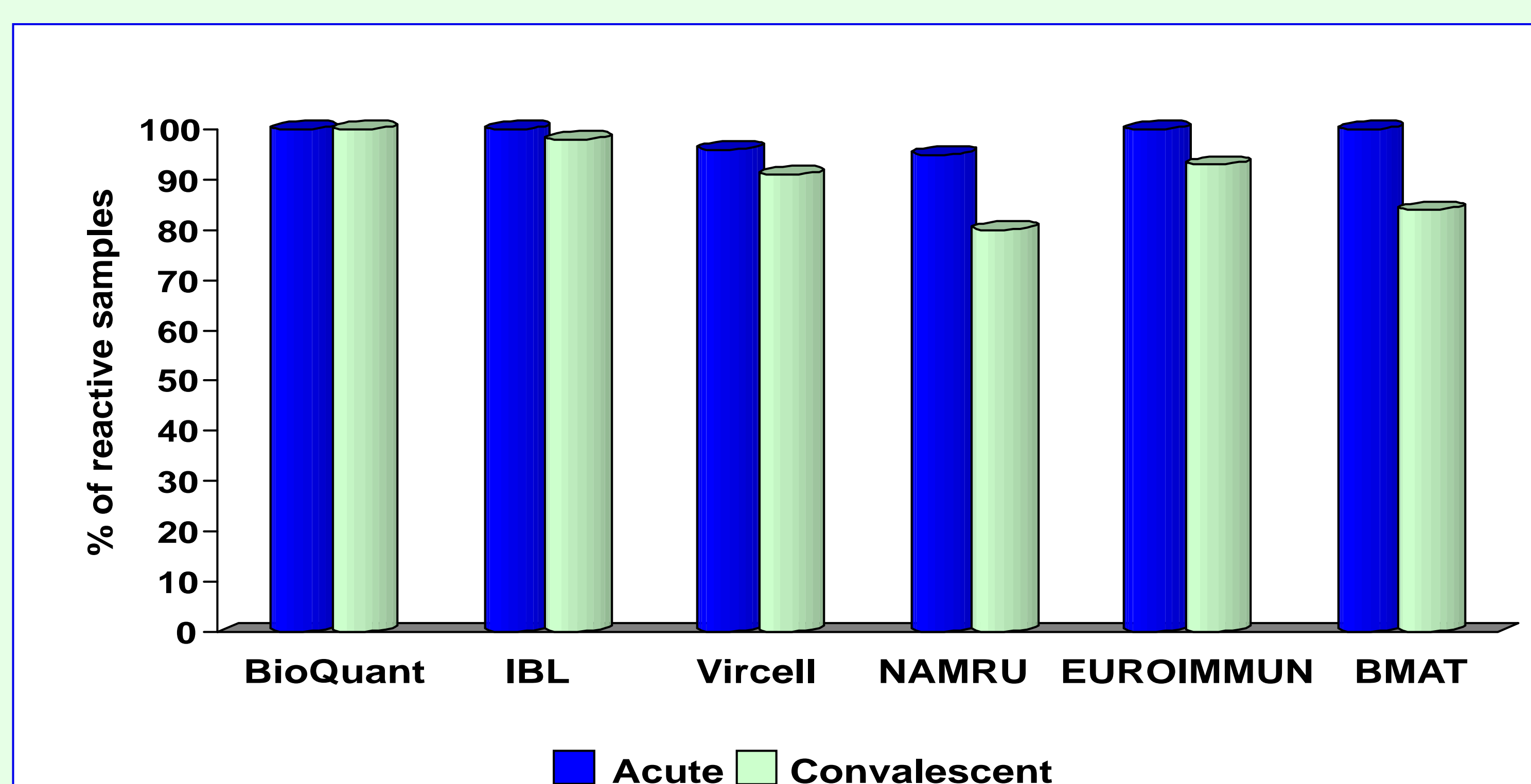


Figure 1. Percentage of reactive samples detected in acute and convalescent serum samples from 188 case-patients with anti-*Brucella* antibodies using six different laboratory methods

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References

- Mantur BG, Amarnath SK, Shinde RS. 2007. Review of clinical and laboratory features of human brucellosis. *Indian J Med Microbiol.* 25(3):188-202.
- Al Dahouk S, Tomaso H, Nöckler K, Neubauer H, Frangoulidis D. 2003. Laboratory-based diagnosis of brucellosis—a review of the literature. Part II: serological tests for brucellosis. *Clin Lab.* 49(11-12):577-89.
- Yagupsky P. 1999. Detection of Brucellae in blood cultures. *J Clin Microbiol.* 37(11):3437-42.
- Gad El-Rab MO, Kambal AM. 1998. Evaluation of a *Brucella* enzyme immunoassay test (ELISA) in comparison with bacteriological culture and agglutination. *J Infect.* 36(2):197-201.
- Fadeel MA, Wasfy MO, Pimentel G, Klena JD, Mahoney FJ, Hajjeh RA. 2006. Rapid enzyme-linked immunosorbent assay for the diagnosis of human brucellosis in surveillance and clinical settings in Egypt. *Saudi Med J.* 27(7):975-81.
- Araj GF. 1997. Enzyme-linked immunosorbent assay, not agglutination, is the test of choice for the diagnosis of neurobrucellosis. *Clin Infect Dis.* 25(4):942.
- Araj GF, Kaufmann AF. 1989. Determination by enzyme-linked immunosorbent assay of immunoglobulin G (IgG), IgM, and IgA to *Brucella melitensis* major outer membrane proteins and whole-cell heat-killed antigens in sera of patients with brucellosis. *J Clin Microbiol.* 27(8):1909-12.
- Brown SL, Klein GC, McKinney FT, Jones WL. 1981. Safranin O-stained antigen microagglutination test for detection of *Brucella* antibodies. *J Clin Microbiol.* 13(2):398-400.