A High Percentage of Serum Samples That Test Reactive by Enzyme Immunoassay for Anti-*Brucella* Antibodies Are Not Confirmed by the Standard Tube Agglutination Test

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We describe a retrospective analysis of *Brucella* enzyme immunoassay (EIA) IgM and IgG results compared to those of the standard tube agglutination test (SAT). Among 1,091 samples tested, 104 (9.5%) and 24 (2.2%) sera were positive by IgM and IgG EIA, respectively. Supplemental testing by SAT showed that 82.7% (86/104) of IgM EIA-reactive samples and 54.2% (13/24) of IgG EIA-reactive samples were negative by SAT. Testing all EIA screen-reactive samples by SAT is required when evaluating patients for potential brucellosis. Due to the limitations of serology, culture remains the gold standard for detecting *Brucella* infection.

Brucellosis is an uncommon disease in the United States, with an estimated incidence of 100 to 200 cases each year (2). The disease is generally characterized by acute or insidious onset of fever, night sweats, and fatigue but may also result with organ or tissue involvement. Although *Brucella* spp. are most commonly transmitted by ingestion of contaminated food/water (e.g., unpasteurized milk or cheese) or contact with infected animals, *Brucella* spp. are also an important cause of laboratory-acquired infection (7).

Laboratory diagnosis of brucellosis is made by isolating *Brucella* spp. from clinical samples (e.g., blood) or by serology. Due to the safety risk associated with cultivating *Brucella* spp., serology is often used as an initial screening method to assess for potential exposure to this organism. Historically, detection of antibodies to *Brucella* spp. has been performed using conventional methods, such as the standard tube agglutination test (SAT) or immunofluorescence (IFA). The sensitivity of SAT varies between 82.6% (8) and 95.6% (4) depending on the titer used to define a positive result. At a cutoff titer of ≥1:80, as used in this retrospective analysis, SAT has a sensitivity of 95.1% among patients with *Brucella* sp. bacteremia (8). SAT specificity is likewise dependent on the cutoff titer used and the prevalence of the disease in the population tested (1, 5, 8). In recent years, many clinical laboratories have implemented serologic tests based on enzyme immunoassay (EIA) technology for the detection of IgM- and IgG-class antibodies to *Brucella* spp. (6, 9). The use of EIAs has allowed laboratories to automate testing, increase sample throughput, and provide an objective interpretation of results. However, recent data suggest that commercial EIAs for anti-*Brucella* antibodies may demonstrate poor specificity when testing is performed in areas of low disease prevalence (3, 9). Welch et al. compared a *Brucella* IgM and IgG EIA (Calbiotech, Spring Valley, CA) with the SAT and found the specificities of these tests to be 73.7% and 65.0%, respectively (9). The Centers for Disease Control and Prevention (CDC) also described the potential for false-positive results by a *Brucella* EIA (Panbio, Inc., Columbia, Maryland), especially when testing is performed in areas with a low prevalence of this disease (3). Due to the significant clinical and public health ramifications associated with a positive laboratory test for *Brucella*, the CDC recommends that all samples testing positive by EIA for IgM-and/or IgG-class antibodies be confirmed by an agglutination method (3).

In order to evaluate the performance of the anti-*Brucella* IgM and IgG EIAs used in our laboratory, we performed a retrospective review of *Brucella* antibody results over a 5-month period (January 2011 to February 2011 and December 2011 to February 2012). During this time frame, a total of 1,091 sera were submitted to our reference laboratory for *Brucella* serologic testing. Samples were screened for IgM- and IgG-class antibodies using an EIA (Euroimmun U.S., Morris Plains, NJ), with testing being performed according to the manufacturer's package insert. Testing was performed using 3 different EIA kit lots for IgG and 2 different kits lots for IgM. Samples testing positive or equivocal by EIA were reflexed to confirmatory testing by SAT (reagents acquired from the Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA), with a titer of ≥1:80 being considered positive (9). All samples tested by SAT were serially diluted (1:80 to 1:5,120) to control for prozone inhibition occasionally seen during acute brucellosis. In addition to the retrospective analysis, we tested sera collected from healthy blood donors (*n* = 50) and a cross-reactivity panel of sera known to be positive for antibodies to other infectious organisms (e.g., *Borrelia burgdorferi*, *Treponema pallidum*) (Table 1).

Among the 1,091 retrospective samples, 104 (9.5%) were positive (index value, ≥1.1) and 50 (4.6%) were equivocal (index value, 0.8 to 1.0) by EIA for anti-*Brucella* IgM (Table 2). Following testing by SAT, 82.7% (86/104) of the IgM EIA-positive samples were negative by tube agglutination, while 92.0% (46/50) of the IgM EIA-equivocal samples were negative by SAT (Table 2). Of the 18 IgM-positive samples that were confirmed by the agglutination method, 11 (61.1%) had a SAT reciprocal titer of ≤1:160,

TABLE 1 Cross-reactivity panel tested by EIA and the standard tube agglutination test

Potential cross-reacting antibody or control group (no. of sera tested)	No. (%) of sera with a positive or equivocal result by[a]:	
	EIA	SAT
Healthy control (50)	3 (6.0)[b]	0 (0.0)
Lyme (10)	0 (0.0)	0 (0.0)
Syphilis (10)	1 (10.0)[c]	0 (0.0)
Epstein-Barr virus (10)	0 (0.0)	0 (0.0)
Q fever (10)	1 (10.0)[d]	0 (0.0)
Rheumatoid factor (10)	1 (10.0)[c]	0 (0.0)

[a] SAT, standard tube agglutination test; EIA, enzyme immunoassay.
[b] These 3 samples were equivocal by the *Brucella* IgG EIA.
[c] These samples were positive by the *Brucella* IgG EIA.
[d] This sample was equivocal by the *Brucella* IgM EIA.

TABLE 2 Results of the standard tube agglutination test among samples testing positive or equivocal by a *Brucella* IgM and/or IgG enzyme immunoassay[a]

Brucella EIA test and result (no. of samples)	No. (%) of sera with each SAT result	
	Positive[b]	Negative[c]
IgM		
Positive (104)	18[d] (17.3)	86 (82.7)
Equivocal (50)	4[e] (8.0)	46 (92.0)
IgG		
Positive (24)	11[f] (45.8)	13 (54.2)
Equivocal (5)	0 (0.0)	5 (100.0)

[a] SAT, standard tube agglutination test; EIA, enzyme immunoassay.
[b] ≥1:80 titer.
[c] <1:80 titer.
[d] Four of these 18 samples were also positive by the *Brucella* IgG EIA.
[e] All four of these samples had a SAT titer of 1:80.
[f] Four of these 11 samples were also positive by the *Brucella* IgM EIA.

while all four IgM equivocal samples that were positive by agglutination had a SAT reciprocal titer of 1:80.

In comparison, 24 of 1,091 (2.2%) samples were positive (ratio units [RU]/ml, ≥22) and 5 (0.5%) were equivocal (RU/ml, 16 to 21) by EIA for anti-*Brucella* IgG (Table 2). Among these samples, 13/24 (54.2%) of the IgG-positive and 5/5 (100%) of the IgG-equivocal specimens were negative by SAT. Interestingly, 4 of the 1,091 samples (0.37%) were positive by both IgM and IgG EIA and all 4 were confirmed positive by SAT (Table 2). Two of these 4 samples had SAT reciprocal titers of ≥1:1,280.

These data demonstrate that a significant percentage of samples testing positive by EIA for IgM- or IgG-class antibodies to *Brucella* are subsequently negative by SAT (82.7% and 54.2%, respectively). These rates are higher than those previously reported by Welch et al. (9), who demonstrated 10/37 (27.0%) EIA IgG-reactive samples and 9/37 (24.3%) EIA IgM-reactive samples to be negative by SAT. These differences are likely to be due to (i) different EIA and SAT reagents used between the studies, (ii) different numbers of samples tested (37 versus 1,091), and (iii) unique study populations. Interestingly, all four samples testing positive by both the IgM and IgG EIAs in our study were confirmed to be positive by SAT, indicating that detection of IgM and IgG by EIA may carry a higher positive predictive value than detection of IgM or IgG alone.

Despite the advantages of EIA (e.g., automated testing, higher throughput), the elevated percentage of positive results by this method warrants particular attention to the reporting and interpretation of results. Laboratories using EIA to screen for anti-*Brucella* antibodies should reflex all EIA-positive samples to an agglutination method for confirmation. We analyzed the quantitative data (e.g., index values) for all EIA-positive results and did not identify a statistically significant difference between samples that were confirmed by SAT and those that were not (data not shown). Therefore, an EIA threshold that might predict SAT positivity could not be defined. In addition to reflexing all screen-positive samples to agglutination, clinical laboratories should consider holding the results of screen-positive EIAs until the results of the confirmatory SAT are available. Reporting positive IgM or IgG EIA results prior to the confirmatory agglutination test may result in unnecessary anxiety by health care providers and patients and, perhaps, overtreatment.

This study has several limitations. First, the conclusions are only directly applicable to the anti-*Brucella* EIA (Euroimmun U.S.) used in our laboratory; however, other groups have reported similar findings using EIA methods from other manufacturers (3, 9). Second, only sera that were positive or equivocal by EIA were tested by SAT, so we are unable to assess the sensitivity and specificity of the EIA. Third, samples are submitted to our reference laboratory without accompanying laboratory (e.g., culture) and clinical (e.g., clinical signs, exposure history) data. Therefore, we cannot definitively rule out the possibility that a portion of EIA-positive, SAT-negative samples were from patients with *Brucella* infection. However, the vast majority of samples tested in our laboratory are from patients residing in the United States, and therefore, it is unlikely that a reactive rate of ~9% for IgM is reflective of true, acute brucellosis. Despite these limitations, this study represents the largest comparison of SAT and anti-*Brucella* EIA using consecutive, nonselected serum samples. Furthermore, the original CDC recommendation (3) is based on an investigation using *Brucella* EIA analyte-specific reagents (PanBio, Inc., Columbia, MD). This study provides important supplemental data to the CDC report by describing the performance of an EIA (Euroimmun U.S.) that is labeled "for *in vitro* diagnostic use." Due to the large number of samples tested (*n* = 1,091) and the consecutive study design, these results may more accurately approximate the comparability of EIA to SAT in U.S. reference laboratories testing a population with an overall low prevalence for brucellosis.

In conclusion, screening for anti-*Brucella* IgM and IgG by EIA may result in a high rate of samples testing as reactive by EIA but negative by SAT. Health care providers and public health departments should await the results of a confirmatory agglutination test before making diagnostic or treatment decisions. In addition, clinical laboratories should review their reporting algorithms and interpretive comments to ensure that the results of *Brucella* serology are providing accurate guidance to health care providers evaluating patients for potential *Brucella* infection.

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