

ELISA Cut-off Point for the Diagnosis of Human Brucellosis; a Comparison with Serum Agglutination Test

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Abstract

Background: Brucellosis is a world-wide disease, which has a diverse clinical manifestation, and its diagnosis has to be proven by laboratory data. Serum agglutination test (SAT) is the most-widely used test for diagnosing brucellosis. The enzyme linked immunosorbent assay (ELISA) can also determine specific antibody classes against brucella. It is a sensitive, simple and rapid test, which could be an acceptable alternative to SAT with fewer limitations, however, like any other new test it should be further evaluated and standardized for various populations. This study was planned to determine an optimal cut-off point, for ELISA which would offer maximum sensitivity and specificity for the test when compared to SAT.

Methods: Four hundred and seven patients with fever and other compatible symptoms of brucellosis were enrolled in the study. Serum agglutination test, 2-Mercaptoethanol test, and ELISA were performed on their sera.

Results: The cut-off point of 53 IU/ml of ELISA-IgG yielded the maximal sensitivity and specificity comparing to the other levels of ELISA-IgG, and was considered the best cut off-point of ELISA-IgG to diagnose acute brucellosis. At this cut-off, the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio were 84.09%, 85.38%, 62.20, 94.90, 5.75, 0.18, respectively.

Conclusion: The best cut-off point of ELISA-IgG is 53 IU/ml, which yields the maximal sensitivity and specificity to diagnose acute brucellosis.

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Keywords • ELISA • agglutination test • brucellosis

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Introduction

Brucellosis affects about 500000 individuals annually world-wide.¹⁻³ Although the epidemiological data on the disease is frequently incomplete, it has been recognized as one of the most common zoonoses in the Eastern Mediterranean Region, with more than 45000 cases reported annually.^{4,5} Brucellosis is an important health problem in Iran, and according to the data derived from active surveillance during 2001-2005, the incidence

of the disease is between 120-400 per 100,000 people.⁶ According to the surveillance program, most of the cases are among farmers, slaughterers and butchers, or those who have an occupational risk factor.⁶ Furthermore, a large study in 1986 revealed that approximately 7.4% of cows in Iran were infected with Brucellosis.⁷ Since 83% of cases with brucellosis in this country are less than 40 years old,⁷ the importance of occupational exposure, especially during adolescence and young adulthood, cannot be overemphasized.^{8,9}

Because of its nonspecific and diverse clinical manifestations, the clinical diagnosis of brucellosis must be certainly ascertained with laboratory confirmation. Although, culture of the bacteria is the gold standard for a definite diagnosis, attempts at isolation of the bacteria are frequently unsuccessful, and brucellosis is usually diagnosed serologically.^{10,11} Furthermore, despite the fact that some laboratories use rapid isolation techniques such as BACTEC, DuPont isolator, polymerase chain reaction methods or immunoblotting techniques, these techniques are not available in most developing countries, and conventional methods of isolation are too slow to be routinely used for diagnosis.^{8,12-15} Consequently, in the absence of bacteriologic confirmation, a presumptive diagnosis can be made on the basis of a single high or rising titer of specific antibodies.^{6,8,12}

Among serological methods, serum agglutination test (SAT) is the most widely-used one. It is the standard and highly sensitive method for the diagnosis of diseases.^{11,16} In a study in which the sensitivity of enzyme linked immunosorbent assay (ELISA) IgG vs positive culture was 81.3%, the sensitivity of SAT was 93.7%.¹⁷ The higher sensitivity of SAT was also demonstrated in other studies, especially in studies from Saudi Arabia, which demonstrated that the SAT sensitivity was 100%.¹⁸⁻¹⁹ Despite the high yields of SAT, it has some limitations like false positive and negative results.¹⁹⁻²² When SAT is used to diagnose brucellosis, false-positive reactions occasionally result from cross-reactions with antibodies to *Salmonella spp.*, *Yersinia spp.*, *Vibrio cholera*, *Francisella tularencis* or *Escherichia coli* O:157. False-positive and false-negative reactions can be avoided by routinely diluting the serum above 1/320.^{12,23-25} Another problem with using SAT is difficult interpretation of the test results. In various regions, different threshold titers, varying from 1:40 to 1:320, have been taken as an indicator of active Brucellosis. In Saudi Arabia, where brucellosis

is endemic, a titer of 1:320 or higher has been found to be indicative of active Brucellosis.^{19,26} Based on a study by Karimi et al. in Iran, a positive SAT titer of 1:80 was present in 2.4% of the general population, and a 2-mercaptoethanol (2ME) test titer of 1:20 was present in less than 1% of the general population. Accordingly, in Iran a single titer of SAT 1:80 or more in the presence of a 2ME titer of 1:20 or more can be taken as a positive test result for brucellosis in the general population.²⁷ This would increase the overall diagnostic specificity at the cost of sensitivity.

The recently-introduced test, ELISA, can determine specific class of IgG, IgM and IgA antibodies against brucella. The assay is a sensitive, simple and rapid test with less limitation, and might be an acceptable alternative to SAT.^{11,25,28} Nevertheless, there are some contradictory reports regarding the diagnostic ability of ELISA in acute brucellosis. Therefore, it is reasonable to further evaluate and standardize the test according to the various geographical regions and populations.

The objective of the present study was to determine an optimal cut-off point for ELISA and compare the test outcome with that of SAT. The optimal cut-off was defined as a point at which, the sum of the sensitivity and specificity are the uppermost.

Materials and Methods

The study was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences. Four hundred and seven patients with fever and other symptoms of brucellosis from an area endemic for brucellosis in Northwest of Iran were enrolled in the study. After obtaining written informed consent, 5 ml of blood sample was taken from each patient, and a short questionnaire comprising questions regarding the epidemiological information was filled out for everyone. Sera of the samples were separated, and were analyzed for brucellosis using SAT, 2ME and ELISA-IgG tests.

Serum Agglutination Test

The procedure was done using serial dilutions from 1/20 to 1/5120 in tubes to overcome possible prozone phenomenon. Abortus Antigen produced by Pasteur Institute of Iran was used.

2-Mercaptoethanol Test

The test was performed like serum agglutination test except for the addition of the 2-mercaptoethanol.

ELISA-IgG

The test was performed according to the manufacturer's (IMMUNOLAB GmbH, Germany) instructions. The absorbance was measured at 450 and 620 nm with Anthos 2020 ELISA reader.

Patients with a SAT titer of 1/80 or greater plus a 2ME titer of 1/20 or greater were considered to have brucellosis, and the remaining patients were considered to have other febrile illnesses mimicking brucellosis.

Data including age, sex and antibody titers were analyzed using Statistical Package for Social Sciences (SPSS version 16, Chicago, Illinois, USA,). Data were analyzed using Student t, Chi-squared, or Mann-Whitney U-test. Receiver Operating Characteristic (ROC) curve was utilized to establish the best cut-off point, which yielded the best sensitivity and specificity for ELISA. A P value of <0.05 was considered statistically significant.

Results

From the 407 sera, 11 had missing data and were not included in data analysis. 41% of the patients were male and 59% were females.

The mean age of the patients was 38.14 years.

Based on clinical symptoms as well as a SAT titer of 1/80 or greater and a 2ME titer of 1/20 or greater, 88 patients (21.9%) had brucellosis and 308 patients (77.7%) had other febrile illnesses. In subjects who deemed positive for the disease, the SAT titers were between 1/80 to 1/5120 and 2ME titers between 1/20 to 1/640 (table 1).

The mean serum level of ELISA-IgG in the brucellosis-positive group was 103.96 ± 11.08 IU/ml, which was significantly ($P < 0.001$) higher than that of the brucellosis-negative group (69.10 ± 3.93 IU/ml).

The area under the ROC curve to differentiate the brucellosis-positive and brucellosis-negative groups was 0.858, which was significantly ($P < 0.001$) different from 0.5 (figure 1).

Sensitivity and specificity were calculated for different levels of ELISA-IgG. Compared to other cut-off points ELISA-IgG, the cut-off point of 53 IU/ml for ELISA-IgG yielded the highest sensitivity and specificity. Therefore, it was considered the best cut-off point of ELISA-IgG to diagnose acute brucellosis. At this cut-off, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio, and negative likelihood ratio were 84.09%, 85.38%, 62.20, 94.90, 5.75, and 0.18, respectively (table 2)

Table 1: Distribution (in percentage) of titers of serum agglutinin test (SAT) and 2-mercaptoethanol (2ME) in brucellosis patients

	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
SAT	0	0	35.6%	21.2%	22.2%	13.3%	5.6%	0	2.2%
2ME	17.8%	30.0%	30.0%	10.0%	8.9%	3.3%	0	0	0

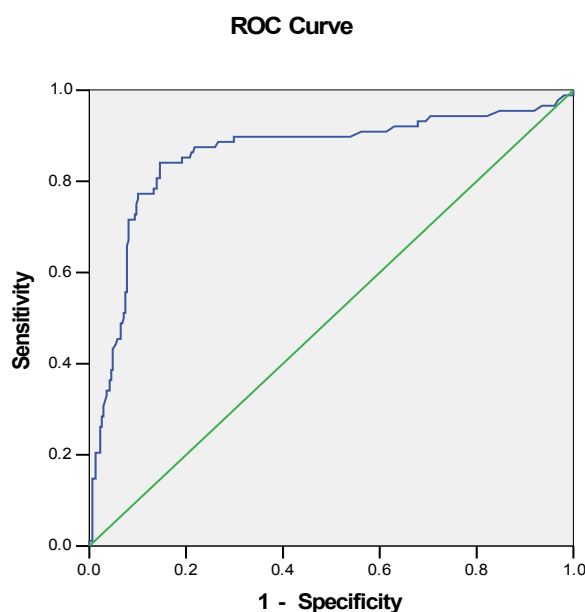


Figure 1: Receiver Operating Characteristic (ROC) curve distinguishing between patients with brucellosis and those without the disease (control patients) diagnosed using ELISA IgG assay.

Table 2: The power of different ELISA titers in diagnosing acute brucellosis

ELISA titers (53 IU/ml)	Sensitivity	Specificity.	LR+	LR-
10.25	87.50	76.94	3.79	0.16
53.00	84.09	85.38	5.75	0.18
110.5	77.27	87.66	6.26	0.25
202.5	34.09	96.42	9.54	0.68
301.00	10.22	99.35	15.75	0.90

LR+: Positive likelihood ratio; LR-: Negative likelihood ratio

There was a significant correlation between SAT and ELISA-IgG titers ($r=541$, $P<0.001$) or 2ME and ELISA-IgG titers ($r=0.534$, $P<0.001$).

In another analysis, patients with a SAT titer of 1/160 or greater and a 2ME titer of 1/40 or greater were considered to have brucellosis, and the remaining patients were considered to have other febrile illnesses mimicking brucellosis, and the maximum sensitivity and specificity was attained at ELISA-IgG level of 53 IU/ml (table 3).

Discussion

Serum agglutination test is the most widely-used serological test for the diagnosis of brucellosis. It is very sensitive, and considered the most reliable method for the diagnosis of brucellosis.^{10,18,19,27}

According to Ciftci et al.²⁹ if culture-positivity is accepted as the gold standard in the diagnosis of brucellosis, the sensitivity of the serum agglutination will be 94.3%.

Enzyme linked immunosorbent assay test has been recently introduced for the diagnosis of brucellosis. Although, conflicting studies have been published regarding the diagnostic accuracy of ELISA, it has been reported to be rapid, highly sensitive, and specific in determining the brucella IgG, IgM, and IgA antibodies in blood and CSF.^{19,30}

Guneri,¹⁷ compared SAT and ELISA, and reported that their sensitivities was 80% and 72%, respectively. Esalatmanesh,³¹ reported that the sensitivity, specificity, PPV, and NPV of ELISA-IgG were 100%, 72.7%, 86.9% and 100%, respectively.

Etrek and colleagues,²⁵ reported that the sensitivity and the specificity of ELISA were, 81.3% and 95%, respectively. However, the sensitivity and specificity of SAT was 93.7% and 100%, respectively.

Memish et al.¹⁹ compared the diagnostic

ability of SAT with Brucella ELISA-IgG and IgM tests in patients with Brucella bacteremia. Sixty eight patients with clinical features suggestive of brucellosis, who had positive blood cultures for Brucella species, and a control group including 70 healthy military personnel, who were blood donors and had no symptoms of brucellosis, were enrolled in the study. The sensitivity and specificity of the SAT for the bacteremic patients were 95.6% and 100.0%, respectively, while those of the ELISA-IgG were 45.6% and 97.1%, and those of the ELISA-IgM were 79.1% and 100.0%, respectively. The sensitivity and specificity of either IgG or IgM positivity were 94.1% and 97.1%, respectively. Assuming that the prevalence of active brucellosis in Saudi Arabia was 5%, the PPV and NPV of SAT were 100% and 99.7%, those of ELISA-IgG were 45.2% and 97.1%, and those of ELISA-IgM were 100% and 98.9%, respectively. When both the ELISA-IgG and IgM were combined, the PPV and NPV were 63% and 99.6%, respectively. In patients with Brucella bacteremia, the sensitivity of either ELISA-IgM or IgG were lower than those of SAT, however, combining IgM and IgG resulted in a sensitivity and specificity similar to those of SAT.

The higher sensitivity of SAT in comparison with ELISA was also documented in other studies by others.³²⁻³⁵ However, we found only two published studies that had compared quantitatively these two tests.^{36,37} In the present study, patients with a SAT titer of 1/80 or greater and a 2ME titer of 1/20 or greater were considered to have brucellosis, and the remaining patients were considered to have other febrile illnesses mimicking brucellosis. Such criteria would increase the overall diagnostic specificity at the expense of sensitivity. Since we compared patients with brucellosis with patients with other febrile illnesses that should be discriminated from brucellosis, the results of our study

Table 3: The power of ELISA in diagnosing acute brucellosis in different titers of serum agglutinin test

	Sensitivity	Specificity	PPV	NPV	Accuracy	LR+	LR-	True PTs	True healthy	Total
Gold 1	84.09%	85.39%	62.18%	94.95%	85.10%	5.75	0.18	88	308	396
Gold 2	87.27%	79.18%	40.34%	97.47%	80.30%	4.19	0.16	55	341	396

Gold 1: SAT titer of 1/80 or more plus 2ME titer of 1/20 or more; Gold 2: SAT titer of 1/160 or more plus 2ME titer of 1/40 or more; PPV: positive predictive value; NPV: negative predictive value; LR+: Positive likelihood ratio; LR-: Negative likelihood ratio; TP: true positive for the disease

are potentially more useful in practice.

Hasibi et al. studied 37 patients with brucellosis and 78 healthy control individuals, and performed SAT and ELISA on their sera.³⁶ The levels of ELISA-IgG was significantly different in the two groups. Furthermore, the optimal cut-off point for ELISA at 167.35 IU/ml, which is significantly different from our result. Their cut-off point had a sensitivity, specificity, PPV, and NPV of 89.2%, 100%, 100% and 795.1%, respectively.

Soodbakhsh et al.³⁷ compared SAT and ELISA-IgG in 56 brucellosis patients with a control group consisting of healthy individuals and patients with febrile illnesses other than brucellosis, and found that at the IgG level of 50 IU/ml, the sensitivity and specificity were 75 and 100%, respectively. At IgG level of 10 IU/ml the sensitivity and specificity were 92.9% and 92.1%, respectively. Therefore, the first level of ELISA-IgG was better in terms of sensitivity, and the second level was better in terms of specificity. In the present study, we chose a level of ELISA-IgG (53 IU/ml) that provided the highest sum of the sensitivity (84%) and specificity (85%).

In Soodbakhsh and colleagues' study,³⁷ the area under ROC curve of ELISA-IgG for discriminating brucellosis patients from other febrile patients were 0.97. This area in our study was 0.85. One reason for the difference between the results of our study and that of Soodbakhsh et al.³⁷ might be the method of selection of patients with brucellosis. In their study, patients who had a SAT titer of 1/160 or more and a 2ME titer of 1/40 or more in addition to related clinical manifestations were defined to have brucellosis.

In the present study, there was a significant correlation between ELISA-IgG and SAT ($r=0.541$, $P<0.001$), which does not agree with the findings of El-Rab and Kambal.¹¹ They found ELISA-IgM to have a significant positive correlation ($r=0.494$, $P<0.001$) with SAT, but found no correlation between ELISA-IgG and SAT.

Conclusion

The best cut-off point of ELISA-IgG is 53 IU/ml, which yields the maximal sensitivity and specificity to diagnose acute brucellosis. At this cut-off, the sensitivity, specificity, PPV, NPV, positive likelihood ratio, and negative likelihood ratio are 84.09%, 85.38%, 62.20, 94.90, 5.75, and 0.18, respectively.

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Conflict of Interest: None declared

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