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Research Paper

# A Comparison of Immuncapture Agglutination and ELISA Methods in Serological Diagnosis of Brucellosis

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## Abstract

**Background:** Different serological tests are used in serologic diagnosis of brucellosis. The most widely used of these are Standard Tube Agglutination and Coombs anti-brucella tests. Whereas ELISA Ig M and Ig G tests have been in use for a long time, immuncapture agglutination test has been recently introduced and used in serological diagnosis. The aim of this study was to compare diagnostic values of ELISA Ig M and Ig G and immuncapture agglutination tests with Coombs anti-brucella test.

**Methods:** Sera from 200 patients with presumptive diagnosis of brucellosis were included into the study. Coombs anti-brucella test, ELISA Ig M and Ig G tests and Immuncapture test were investigated in these sera. Then, sensitivity, specificity, negative predictive and positive predictive values were calculated.

**Results:** Sensitivity, specificity, negative predictive and positive predictive values were found to be 90,6 %, 76,3 %, 94,2 %, and 65,9 % respectively for the Immuncapture test, whereas they were found to be 73,7 %, 58,9 %, 84,2 %, and 42,8 % for Ig G and 72,2 %, 67,8 %, 85,2 %, and 48,7 % for Ig M. The Immuncapture test was found to be compatible with ELISA Ig M and Ig G tests but it was statistically incompatible with Coombs anti-brucella test.

**Conclusions:** Immuncapture agglutination test yields similar results to those of Coombs anti-brucella test. This test is a useful test by virtue of the fact that it determines blocking antibodies in the diagnosis and follow-up of brucellosis.

Key words: Brucellosis, immuncapture, ELISA, IgG, IgM, serologic diagnosis

## INTRODUCTION

Brucellosis is a zoonotic infection that is transmissible from animals to humans and it affects various organs and leads to different clinical symptoms. It progresses with symptoms and signs such as high temperature, sweating and pain in the joints but it is also a disease that leads to clinical pictures imitating rheumatic and psychiatric diseases. *Brucella* is a gram negative staining, immotile, non spore forming, aerobic, microaerophile and coccobacillus bacteria that has microcapsules when it is newly separated from the

organism. Isolation of the microorganism from the culture ensures diagnosis of the disease but sensitivity of this method is correlated 30-90 % with the stage of the disease (1). When the culture is found negative, investigation of classic serologic tests and antibodies occupy an important place in diagnosis of brucellosis. Antibodies begin to form 2 weeks after the beginning of disease. Those who engage in animal husbandry may have normal antibodies at 1/80 titer. Immunglobulin (Ig) M type antibodies appear in one week and reach a peak in three months. Ig G antibodies, on the other hand, appear in three weeks and reach a peak in six to eight weeks. Coombs test is needed to investigate blocking antibodies. Dilutions need to be performed in very high ratios in order to remove occurrence of prezone (2). In recent years, the immuncapture agglutination test, which is based on sandwich ELISA system, has been introduced. In this method, microwell is covered with Coombs antibodies against human origin Ig G, Ig M and Ig A antibodies. This method is brucella agglutination test that occurs in microwell and performed with Coombs antiserum and determines the three antibodies that form against brucella.

The purpose of this study is to compare the diagnostic values of Immuncapture agglutination and ELISA methods, which are used for the diagnosis of brucellosis with reference to Coombs test.

## MATERIAL AND METHOD

Sera samples from 200 patients with presumptive diagnosis of brucellosis which were sent to Central Microbiology Laboratory of Selcuk University Meram Faculty of Medicine from various clinics were included in the study and kept at -70ºC until performing laboratory study. Coombs anti-brucella test (Vircell, S.L., Spain), ELISA Ig G and Ig M (Vircell, S.L., Spain) and Brucellacapt (Vircell, S.L., Spain) tests were studied simultaneously in these sera.

Brucellacapt agglutination test was conducted in the following manner: All reactives were brought to room temperature (18-25°C). 95 µl serum diluents was put in the first microwell in the microplate whereas 50 µl serum diluents was put in others. 5 µl serum was pipetted into the first microwell and mixed. 50 µl was taken from this microwell and diluted in order and finally 50 µl was removed. 50 µl brucella antigen was added to all microwell. The plate was covered with the protective cover in the box so that the liquid in the microwell would not dry up and the required reaction would take place and incubated at 37°C for 18-24 hours. The results were assessed visually as the first microwell being at 1/160 titration. Since the antigens fall to the bottom without attaching to the wall if brucella antibodies do not exist, they were seen in the form of blue dots in the serum being studied. The blue dot was assessed to be negative whereas homogenous blue appearance was considered to be positive.

1/320 and higher values were taken to be positive for Brucellacapt whereas values above the cut-off value were considered to be positive for ELISA. The results were read on spectrophotometer at 450 nm absorbance. The results obtained via the three methods were recorded.

The results were analyzed by using the paired t test method on SPSS for Windows 13.0 software. This study was approved by the local institutional ethics committee of the Selcuk University Meram Faculty of Medicine.

## RESULTS

The immuncapture results in the 200 sera samples were classified as negative, 1/320 positive, 1/640 positive, 1/1280 positive, 1/2560 positive, 1/5120 positive and 1/10240 positive. ELISA results, on the other hand, were divided into positive and negative and a distribution table was structured according to the results of immuncapture (Table 1). A total of 144 samples were determined to be positive for immuncapture and 122 for Ig M, and 123 for Ig G. Sensitivity, specificity, negative predictive and positive predictive values for ELISA and immuncapture test are given in Table 2. The compatibility of the results of the three tests was analyzed on the basis of evaluation and statistical evaluation with reference to Coombs test. The groups emerged as Group I (ELISA), Group II (Coombs) and Group III (Brucellacapt). According to the results of the paired t-test conducted at 95 % confidence interval between Group I and Group II, t value was found to be -0,84, and correlation 0,439. Accordingly, Groups I and II were not statistically compatible. According to the results of the paired t-test conducted at 95 % confidence interval between Group II and Group III, t value was found to be -1,26, and correlation 0,551. Accordingly, the values between Group II and Group III were found to be statistically compatible. According to the results of the paired t-test conducted at 95 % confidence interval between Group I and Group III, t value was found to be 0,32, and correlation 0,397. Accordingly, the values between Group I and Group III were found to be statistically compatible.

Sensitivity, specificity, negative predictive and positive predictive values were found to be 90,6 %, 76,3 %, 94,2 %, and 65,9 % respectively for the Immuncapture test, whereas they were found to be 73,7 %, 58,9 %, 84,2 %, and 42,8 % for Ig G and 72,2 %, 67,8 %, 85,2 %, and 48,7 % for Ig M respectively.

**Table 1.** Distribution of the results of Immuncapture and ELISA tests

| Immuncapture | | ELISA Ig G | | ELISA Ig M | | ELISA Ig M and G | |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Negative | Positive | Negative |
| Negative | 56 | 23 | 33 | 18 | 38 | 34 | 22 |
| 1 / 320 | 44 | 35 | 9 | 23 | 21 | 37 | 7 |
| 1 / 640 | 18 | 13 | 5 | 16 | 2 | 17 | 1 |
| 1 / 1280 | 37 | 18 | 19 | 27 | 10 | 30 | 7 |
| 1 / 2560 | 14 | 11 | 3 | 12 | 2 | 13 | 1 |
| 1 / 5120 | 21 | 19 | 2 | 18 | 3 | 20 | 1 |
| 1 / 10240 | 10 | 4 | 6 | 8 | 2 | 8 | 2 |
| Number of Positive sample | 144 | 123 | 77 | 122 | 78 | 159 | 41 |

**Table 2.** Sensitivity, specificity, negative predictive and positive predictive values of tests used in comparison

| Test | Sensitivity | Spesifity | PPD | NPD |
|---|---|---|---|---|
| ELISA | 90,0 | 66,7 | 91,1 | 63,6 |
| Immuncapture | 90,6 | 76,3 | 94,2 | 65,9 |
| IgG | 73,7 | 58,9 | 84,2 | 42,8 |
| IgM | 72,2 | 67,8 | 85,2 | 48,7 |

PPD: positive predictive value

NPD: negative predictive value

## DISCUSSION

Brucella agglutination tests have an important role in the diagnosis of brucellosis. Main antigenic structure which is imported in the diagnosis of brucellosis is the smooth lipopolysaccharide structure of the antigen cell surface. *Brucella*, which is a gram negative bacterium, has a lipopolysaccharide structure in the outer membrane in S colony phase and has a surface that is in contact with the outer surface. This smooth lipopolysaccharide structure plays a very important role in agglutination tests. Ig M and G type antibodies that form against this structure are identified through agglutination tests. ELISA test which is among these tests and makes it possible to determine the type of antibody (3).

Obtaining negative results in agglutination tests is a common phenomenon. One of the reasons for this is blocking antibodies. One of the methods used to show existence of blocking antibodies is the Coombs test. Brucellacapt test, on the other hand, is an immuncapture agglutination test which is based on sandwich ELISA method.

In a study conducted by Orduna et al. (4) on the serum samples from 82 patients diagnosed with brucellosis, 157 patients presumed to have brucellosis and 412 control patients, 82 patients were found to be positive with brucellacapt test and Coombs test in initial sera whereas 75 patients were found to be negative with standard tube agglutination (SAT). When 1/160 and higher titers were taken to be positive, sensitivity of brucellacapt test, Coombs anti brucella test and SAT are respectively 95.1 %, 91.5 % and 65.8 %. The correlation of brucellacapt test and Coombs anti brucella test was found to be r =0,866 in their study. This correlation was found to be 0,551 in our study and lower in comparison. Orduna et al. found that since the brucellacapt could determine all three of the antibodies and blocking antibodies that form against brucella, the titers that it has determined were higher in number than STA and Coombs methods has higher sensitivity and specificity (4).

In a study conducted by Casao et al. on 123 sera samples, the compatibility ratio between the brucellacapt test and the Coombs test was found to be correlated (r=0.14), (2). The correlation coefficient was found to be higher in our study (r=0,551).

Ardic et al. (5) compared immuncapture and STA with reference to Coombs test. When 1/160 and higher titres were considered positive, they found sensitivity of the brucellacapt test was 97.3 %, its specificity was 55.6 %, its positive predictive value was 90 % and its negative predictive value was 83.3 %. When they took the threshold value to be 1/320, they calculated these values to be 100 %, 59.1 %, 88.6 % and 100 % respectively. The Coombs test was taken as the reference test in our study, and the sensitivity, specificity and positive and negative predictive values

of the other two methods were calculated and their compatibility with one another was investigated statistically.

In a comparative study conducted by Prado et al (6), immuncapture agglutination test (Brucellacapt), SAT and Coombs anti-Brucella test were compared with Ig G, Ig A and Ig M ELISA tests. It was determined that as diagnostic tests, the sensitivity and specificity of immuncapture-agglutination test (Brucellacapt) and Coombs anti-brucella were similar to one another; in the follow-up of the treatment, the antibody titers determined via these tests were close to one another and it was concluded that they were well correlated. Though we tested similar methods in our study, the exclusion of ELISA Ig A test from our study was a shortcoming.

In a study conducted by Gomez et al., on the other hand, a direct correlation was observed between the Brucellacapt test and Coombs test in negative and positive sera samples. Similar results were obtained in positive sera between the Brucellacapt test and the Coombs test titers within the range of 1 or 2 dilutions (7).

In another study conducted by Serra et al., statistical difference was not observed between the Brucellacapt and Coombs tests in terms of sensitivity and specificity in the diagnosis and follow-up of brucellosis and it was concluded that the results were similar in the follow-up of patients with brucellosis (8).

Araj noted that it was not uncommon for agglutination tests to yield false negative results in patients with neurobrucellosis and claimed that the ELISA method was the most reliable method in these patients (9). However, agglutination and Coombs tests have been used as standard tests in the diagnosis of brucellosis and their correlation with clinics were quite good. Whether the ELISA test is the best method in the treatment of patients with neurobrucellosis or not needs to be investigated with similar studies.

In a similar study conducted by Memish et al., which included 68 patients with brucellosis and 70 control group, sensitivity and specificity were found to be 45.5 % and 97.1 % for Ig M and 79 % and 100 % for Ig G respectively (10). When the two ELISA Ig positivity were evaluated together, sensitivity and specificity were found to be 94.1 % and 97.1 % respectively. Evaluation of two Ig's together rather than one by one increases their sensitivity and specificity values. The Ig G and Ig M sensitivities found in our study were higher in comparison to the study in question but specificity is lower. This situation may be related with the phase of the brucella infection.

In a study conducted by Ciftci et al. (11) on the basis of blood culture results, sensitivity was calculated to be 97.1 % for ELISA Ig G and 71.4 % for ELISA Ig M. They found the compatibility of ELISA Ig M and Ig G test results with STA at the level of 75.3 % for Ig M and 84.4 % for Ig G. These results were high in comparison to our results but the number of samples is lower. The fact that blood culture and Ig A were also investigated using the ELISA method in that study is its advantage.

While specific Ig M rises alone or with Ig A in acute brucellosis, Ig G rises alone or with Ig A in chronic brucellosis (12). The sensitivity of Ig M ELISA test was 80 % in acute cases whereas the sensitivity of Ig G and Ig M together was determined to be between 90 and 100 % (13). Therefore, these two antibodies should be evaluated together in patients presumed to have brucellosis.

The ELISA method has higher positivity, higher titers and the advantage of identifying different classes of antibodies in comparison to other agglutination methods. Different results may be obtained depending on the nature of anti-globulin. This situation has an effect on the sensitivity, specificity and ultimately applicability of the method (12,14). ELISA tests are relatively costlier tests in comparison to agglutination tests that require equipment and experience. In a comparative study conducted by Araj et al, it was argued that the ELISA method should be preferred because in chronic and complicated cases, STA and Rose Bengal tests might miss a serious portion of positive cases (15).

Coombs test is necessary for an investigation of blocking antibodies in the serologic diagnosis of brucella infection. Among the tests that can be used alone or together with other tests, immuncapture agglutination and ELISA Ig M and Ig G tests, which are based on sandwich ELISA system, are standardized tests that have high diagnostic value and can be used interchangeably. Titer is found higher in the immuncapture method because Ig G, Ig M, Ig A antibodies and blocking antibodies are identified.

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## Conflict of Interest

The authors have declared that no conflict of interest exists.

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