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Research Article
Evaluation of a New and Rapid Serologic Test for Detecting Brucellosis: *Brucella* Coombs Gel Test

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Abstract

Background: Many serological tests have been used for the diagnosis of human brucellosis. A new serological method is identified as *Brucella* Coombs gel test based on the principle of centrifugation gel system similar to the gel system used in blood group determination. In this system, if *Brucella* antibodies were present in the serum, antigen and antibody would remain as a pink complex on the gel. Otherwise, the pink *Brucella* antigens would precipitate at the bottom of the gel card system. **Objective:** In this study, we aimed to compare the *Brucella* Coombs gel test, a new, rapid screen and titration method for detection of non-agglutinating IgG with the *Brucella* Coombs test. **Materials and Methods:** For this study, a total of 88 serum samples were obtained from 45 healthy persons and 43 individuals who had clinical signs and symptoms of brucellosis. For each specimen, Rose Bengal test, standard agglutination test, Coombs test and *Brucella* Coombs gel test were carried out. **Results:** Sensitivity and specificity of *Brucella* Coombs gel test were found as 100.0 and 82.2%, respectively. **Conclusion:** *Brucella* Coombs gel test can be used as a screening test with high sensitivity. By the help of pink *Brucella* antigen precipitation, the tests' evaluation is simple and objective. In addition, determination of *Brucella* antibody by rapid titration offers another important advantage.

Key words: *Brucella*, gel test, serology, SAT, ELISA

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Brucella is an important zoonotic pathogen in the world and is associated with a high degree of morbidity but minimal mortality in the endemic areas[1,2]. The transmission of brucellosis to humans occurs by contacting the infected animals or ingesting their products[2,3]. Brucellosis, an important public health problem in many developing countries is a systemic disease affecting various organs or body systems and can cause periods of chronicity, re-infection and relapse[4-6].

Brucellosis can mimic several infections[7]. The absence of specific symptoms makes it difficult to distinguish brucellosis from typhoid, mononucleosis, leishmaniasis and tuberculosis[8]. Because of these reasons, specific laboratory tests are needed to confirm the diagnosis[7].

The diagnosis of this disease usually relies on isolation of the bacteria or detection of the anti-*Brucella* antibodies in blood. For diagnosis of brucellosis, blood culture is the gold standard method but it is hazardous, expensive, dangerous, insensitive and requires long incubation period[4,7,8]. Serological methods are easier to implement and provide a great aid in diagnosis, therefore, they are also used for determination of potential exposure to this microorganism. Most commonly, these tests are used in the laboratory for diagnosis of brucellosis[8-10].

Many serological tests have been used for the diagnosis of human brucellosis, including Rose Bengal (RB) test, complement fixation test, the Standard Agglutination Tube (SAT) test, anti-human globulin (indirect Coombs) test, Indirect Fluorescence Antibody (IFA) test, enzyme-linked immunosorbent assay (ELISA) and more recently, an immunocapture-agglutination test (*Brucella* capt test). However, most of these tests require incubation time at least 18-24 h[11-16].

Brucella Coombs gel test is a new and very rapid serological method for detecting non-agglutinating *Brucella* IgG antibodies. In this study, we aimed to compare the novel *Brucella* Coombs gel test with the conventional *Brucella* Coombs test.

MATERIALS AND METHODS

In this study, a total of 88 serum samples were obtained from 43 individuals who had clinical symptoms and signs of brucellosis and also from 45 healthy people at Atatürk University Medical Faculty Research Hospital and Palandoken Public Hospital in Erzurum. Serological tests (Rose Bengal test, standard agglutination test and Coombs test) were carried out in microbiology laboratory. Then, the serums were stored at -20°C until required.

***Brucella* coombs gel test methods:** The ODAK *Brucella* Coombs gel test (ISLAB, Turkey) was performed according to the manufacturer's manual. For screening of *Brucella*, 5 µL of serum were mixed with 50 µL of *Brucella* diluent in the well of dilution plate. Fifty microliters of *Brucella* antigen were then added to the suspension in the well and stirred. The plate was shaken and 50 µL of mixture were transported by pipette to the related well in *Brucella* gel matrix. The gel matrix was placed in the centrifuge and spun for 60 min in the proper adjustment. The results were evaluated by two different researchers. In this application, the final titration of the serum is 1/20. The samples that were found to be positive by the screen method were taken to titration process. For the titration of *Brucella* antibodies, 8 wells for each patient's serum in the dilution plate were saved. *Brucella* diluent was added the wells (100 µL for the first well and 50 µL for the other wells). Five microliters of serum was added to the first well and stirred. Then 50 µL were taken from the first well and added to the second well. After this consecutive dilution, 50 µL were taken from the well and discarded. Fifty microliters of *Brucella* antigen suspension were added to all wells and stirred. The plate was shaken and 50 µL from the related well were transferred by pipette to the related well in *Brucella* gel matrix. The gel matrix was placed in the centrifuge and was spun for 20 min in the proper adjustment. The result was visually evaluated. In this application, the first dilution was 1/40 and the second was 1/80 and therefore, the other wells could follow the same pattern in terms of two fold dilution. If *Brucella* antibodies were not present in the serum, the pink *Brucella* antigens would precipitate. If *Brucella* antibodies were present in the serum, antigen and antibody would remain as a pink complex on the gel (Fig. 1). In titration method, dilution values of 1/160 and above were evaluated as positive results (Fig. 2).

Statistical analysis: The results of the *Brucella* Coombs gel test were compared with the classic Coombs test. The sensitivity and specificity of the *Brucella* Coombs gel test were calculated using Receiver Operating Characteristic (ROC) curve according to clinically suspected and positively accurate Coombs test.

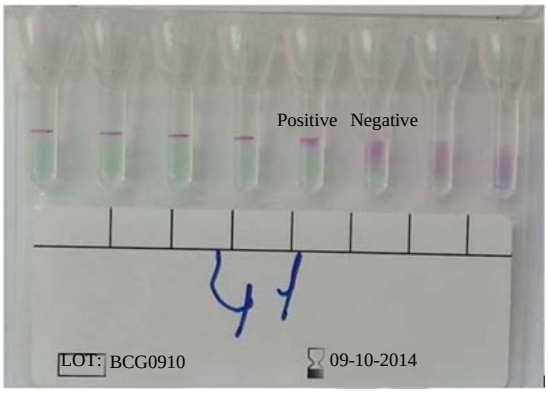

Fig. 1: Evaluation of positive and negative test results

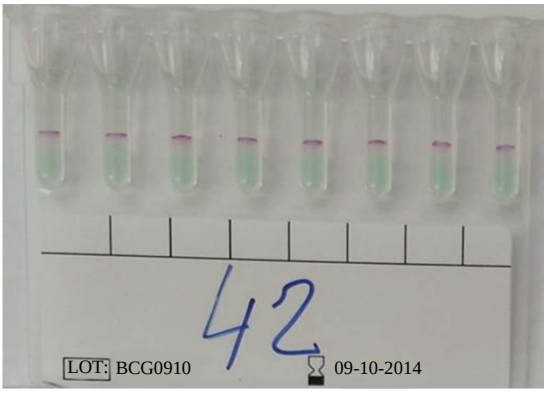

Fig. 2: 1/5120 positive titration

RESULTS AND DISCUSSION

Sensitivity and specificity of the *Brucella* Coombs gel test and standard agglutination test for determination of the sera from patients clinically suspected of brucellosis were confirmed by Coombs test.

The sera of 22 patients whose Coombs tests were negative were also found to be negative by Coombs gel tests. Also, the 7 samples that gave agglutination in Coombs test titrations of 1/80 and under were also shown to be positive at different titrations by Coombs gel test (Table 1).

Sensitivity, specificity, positive predictive value and negative predictive value of *Brucella* Coombs gel test were 100.0, 82.2, 84.3 and 86.0% and of standard agglutination test were 53.4, 100.0, 100.0 and 69.2%, respectively (Table 2).

Several serological tests that determine the presence of antibodies against *Brucella* have an important role in the diagnosis of brucellosis. The most commonly used tests are the Rose Bengal test, the Serum Agglutination Tube (SAT) test and the Coombs anti-*Brucella* test[17].

Rose Bengal (RB) slide agglutination test based on the agglutination reaction of the serum when mixing with the suspension of whole *B. abortus* cells which stained with Rose Bengal dye is a simple, rapid test (within 5-10 min) and gives relatively good results for diagnosing patients with acute brucellosis, however, it gives a high rate of false-negative results, especially in chronic and complicated cases[10,18].

The RB test is widely used to screen *Brucella* infections but WHO guidelines recommend that RB test results must be confirmed by other tests[8,19].

The Standard Agglutination Tube (SAT) test is the most widely used serologic test for confirmation of human brucellosis in many clinical laboratories. The SAT measures total *Brucella* antibody (IgG, IgM and IgA). The highest serum dilution which resulting more than 50% agglutination in test tube is considered as agglutination titer. The detection of seroconversion or high antibody titers ($\geq$1/160) are considered diagnostic results together with an accompanying clinical presentation[20,21].

Particularly, the interpretation of RB and SAT tests are difficult for patients with chronic brucellosis or reinfections and relapses who live in endemic areas where a high portion of the population has antibodies against brucellosis. In these conditions, the immune responses are characterized by the non-agglutinating IgG antibodies predominance. The negative results in serological tests because of the blocking antibodies are common phenomenon that limits the sensitivity of these tests. These kinds of antibodies can be determined only by Coombs test and enzyme immunoassay[22]. On the other hand, ELISA assay has poor sensitivity and specificity when compared with agglutination tests[23].

Due to the presence of blocking antibodies, the Coombs' test, an extending version of SAT may be used instead of SAT[6]. Coombs test is used for the detection of incomplete, blocking or non-agglutinating IgG[21]. This test is also limited because of several disadvantages such as using intensive labor due to centrifugation and washing procedures as well as subjective results[24].

In the recent years, the new immunocapture agglutination test (*Brucella* capt test), a modification of Coombs test has been reported to detect incomplete or blocking IgG and IgA antibodies, with similar sensitivity and specificity for diagnosis of brucellosis. The advantage of this immunocapture agglutination technique (*Brucella* capt test) is its ease to carry out[25,26]. Despite all these fast and simple performance of immunocapture agglutination test, requirement of 24 h for evaluating the results is its significant disadvantage.

Table 1: Distribution of the results of *Brucella* Coombs test and *Brucella* Coombs gel test

	Brucella Coombs gel test								
	Negative	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
***Brucella* Coombs gel test**									
Negative	22		13	1	1				
1/20						1			
1/40				1	1	2			
1/80		1	1	1					
1/160					2	3	8	2	5
1/320					2	1		2	2
1/640							2	2	4
1/1280								1	2
1/2560							1		3
1/5120									1

Table 2: Sensitivity, specificity, PPV and NPV of the *Brucella* Coombs gel test (%)

Test	Sensitivity	Spesifity	PPV	NPV
Brucella Coombs gel test	100.0	82.2	84.3	86.0

PPV: Positive predictive value and NPV: Negative predictive value

In this study, the aim was to determine the sensitivity and specificity of a new rapid serological method that is used for diagnosis of human brucellosis in our country recently. This new serological method is identified as *Brucella* gel test, which is based on the principle of centrifugation gel system similar to the gel system used in blood group determination.

In this system, if *Brucella* antibodies were present in the serum, antigen and antibody would remain as a pink complex on the gel. Otherwise, the pink *Brucella* antigens would precipitate at the bottom of the gel card system. There are only a few studies about this system and the results indicate that the system has a high sensitivity and specifity[27]. In our study, sensitivity and specificity of *Brucella* Coombs gel test were found as 100.0 and 82.2%, respectively.

CONCLUSION

As a result of this study, *Brucella* gel test can be used as a screening test with high sensitivity. By the help of pink *Brucella* antigens precipitation, the tests' evaluation is simple and objective. Additionally, determination of *Brucella* antibody titration in a short time (about half an hour) offers another important advantage. However, the low specificity of this test also needs to be revised in terms of assessment of the positive titration range. Therefore, we believe it would be an appropriate approach to evaluate this test with further studies in larger groups.

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