

Evaluation of Brucella Coombs Gel Test with Blood Culture

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ABSTRACT

Objective: To evaluate the correlation between Brucella spp. blood culture, which is accepted as the gold standard in the diagnosis of brucellosis, and the Brucella coombs gel test (BCGT), a new and rapid test developed in our country.

Material and method: Brucellosis is suspected in 100 patients from various clinics of our hospital microbiology laboratories, simultaneous blood culture results (Bact/allert 3D bioMerieux, France) and serum standard tube agglutination test (STA Seromed, Turkey), Brucella Coombs gel test (BCGT, Islab, Turkey) It was evaluated retrospectively.

Results: Serology and blood culture growth were negative in 82/100 of the patients. STA/BCGT results were consistent in 18/100, and $\geq 1/320$ was positive. Brucella melitensis was isolated from blood cultures of seven patients and concurrently taken BCGT was positive (100%). There are 11 patients with positive serology with culture negative.

Conclusion: Although blood culture is accepted as gold standard, it is not always possible to catch blood culture positivity. Serology tests, especially BCGT, should be preferred routinely because it gives early results, is cheap and safe.

Keywords: Brucellosis, Blood cultur, Serology

INTRODUCTION

Brucellosis is the most common zoonotic disease in the world. Brucella species (B.abortus, B.melitensis and B.suis) that cause infection in humans and animals are intracellular, aerobic, gram-negative, small bacilli (1, 2). Brucella involves many tissues and organs and progresses with nonspecific symptoms. In cases with fever of unknown cause and nonspecific symptoms, clinicians are recommended to investigate brucellosis in diagnosis. The incubation period of the disease is approximately 2-3 weeks, and nonspecific findings such as fever (often corrugating), night sweats, muscle pain, back pain and loss of appetite occur. It is also known that various complications such as osteomyelitis, hepatomegaly, splenomegaly, meningitis, endocarditis and epididymo-orchitis can develop during Brucellosis (3, 4). One of the two important criteria in the diagnosis of brucellosis is Brucella spp. isolation; the other is the detection of the presence of high titer specific antibodies in the serum accompanied by clinical findings and the demonstration of seroconversion. The specificity and sensitivity of serological tests differ, so it is recommended that the tests be combined. For example, since the standard tube agglutination (STA) test may produce false negative results due to the presence of blocking antibodies, this test should be validated with Coombs anti-Brucella (CAB) or capture agglutination (ICA) tests.

BCGT (ODAK Brucella Coombs Gel Test, Toprak Medikal, Istanbul), which has been developed in our country in recent years, has been introduced as a new and rapid method based on agglutination. This test is performed in wells with a gel matrix containing Coombs antibodies (anti-human IgG). During the test, the blocking antibodies are bound by centrifugation at high speed and Coombs serum, and the results are evaluated visually within 2 hours. In the present study, it is aimed to determine the efficiency of BCGT in serological diagnosis of brucellosis by comparing it with blood culture results.

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MATERIAL AND METHODS

A total of 100 patients, from whom STA and BCGT were simultaneously requested from the patient serum with blood culture, were selected and the growth and results were evaluated retrospectively. Blood culture bottles (Bact/allert 3D BioMerieux, France) samples that signaled in the blood culture device were inoculated in blood, chocolate and EMB medium.

Growths were evaluated after 24.48 hours 37 °C incubation. Colonies with gram negative cocci on gram stain were identified by VITEK 2 (BioMeriux, France) when catalase and oxidase were positive. STA (Seromed, Turkey) method and BCGT (Islab Turkey) were studied in serum samples.

BCGT: In this method, first serum dilutions were made in the wells reserved for each patient in the dilution plate. After adding 100 µl dilution fluid to the first well and 50 µl dilution fluid to the other wells, 5 µl of each patient's serum was added to the first wells and mixed; Serial dilutions (1 / 40-1 / 5120) were prepared by passing 50 µl to the other wells; 50 µl was taken from the last well and discarded. Then, 50 µl of Brucella antigen suspension was added to all wells, mixed and incubated at 37 °C by covering the plate. The gel matrix microtubes to be used at this time were marked with their respective serum numbers.

After the incubation, after shaking the plate well, 50 µl of the mixture in the relevant well was taken and pipetted into the relevant microtube in the gel matrix. Microtubes were incubated for 20 minutes at 37 °C, and then centrifuged for 20 minutes at the appropriate speed recommended by the manufacturer.

Results were evaluated visually. In the evaluation; In the absence of antibody, the precipitation of pink brucella antigens at the bottom of the tube was considered negative, and the presence of the pink antigen and antibody complex on the gel in the presence of antibodies was accepted as positive (**Figure 1**).

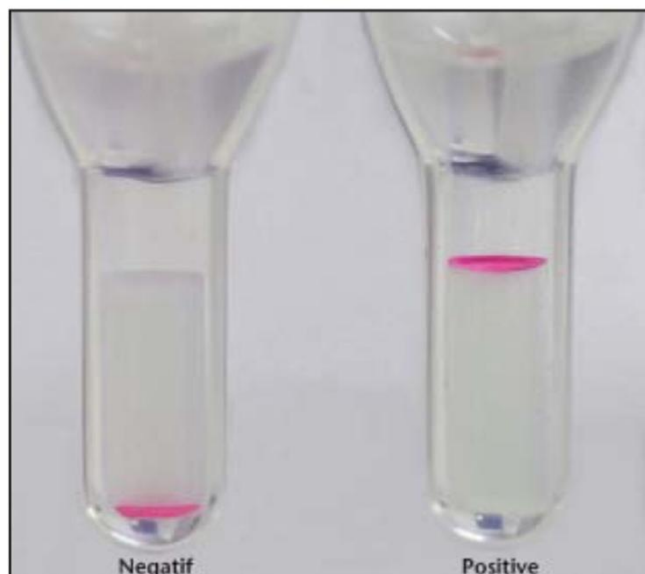


Figure 1:

RESULTS

The average age of the patients is 30.9 and it consists of 54 male and 46 female. The results of Brucella STA and BCGT were found to be 100% compatible in 100 patients. Brucella melitensis grew in simultaneous blood culture of 7 out of 18 patient sera with Brucella STA / BCGT; 1/320 and above. When the titers are evaluated; Culture was negative in 1 patient with 1/320, and culture was negative in 3 patients with 1/640; Culture growth was positive in 4 patients with 1/1280, negative in 4 patients, positive in 1 patient with 1/2560; 1/5120 was positive in 2 patients and negative in 3 patients. Blood culture positivity 1-7. It has been detected between days. (**Table 1**)

Table 1. Correlation of BCGT / STA test with blood culture growth

Sample no	STA	BCGT	Blood culture signal	Positive signal day of blood culture
1	1/320	1/320	Negative	
3	1/640	1/640	Negatif	
4	1/1280	1/1280	Negatif	
4	1/1280	1/1280	Pozitive (<i>Brucella melitensis</i>)	5.day 6.day 6.day 7.day
1	1/2560	1/2560	Pozitive (<i>Brucella melitensis</i>)	5.day
2	1/5120	1/5120	Pozitive (<i>Brucella melitensis</i>)	1.day 7.day
3	1/5120	1/5120	Negative	
82	Negative	Negative	Negative	

DISCUSSION

Culture is accepted as the gold standard in the laboratory diagnosis of brucellosis. However, the sensitivity of blood culture varies according to the clinical condition of the patient, the amount of bacteria circulating in the blood, the method used, the laboratory's practice, the amount of bacteria circulating in the blood and the type of bacteria, and the positivity rate is between 15-70% (5, 6). The higher concentration of bacteria in the reticuloendothelial system increases the chance of isolation in bone marrow cultures. However, since relapse develops in 5-40% of acute brucellosis cases, these cases may not always have culture positivity (6).

In our study, according to the high titer positivity, the growth in culture was detected as 7/18 and 30.8%. However, it was determined that bacterial incubation was not kept for a long time in patients who were positive for STA / BCGT, but there was no growth in blood culture, and it was reported as no growth after 7 days. Although it is estimated that the chance of production may increase after a longer incubation, the company recommendations are that there will be growth in the blood culture bottle in the first 5 days and that long incubation is unnecessary. It is thought that the bacteria passes from the blood to the reticuloendothelial system, so it is not detected in the blood. Although nucleic acid amplification tests or detection of antigens in the diagnosis of brucellosis are diagnostic for new brucellosis cases, the presence of DNA in the blood of the cases even after successful treatment is not useful in determining the recurrence of brucellosis (7, 8). In the first week of the infection, IgM antibodies against lipopolysaccharide (LPS) antigens appear in the serum, and IgG antibodies appear within two weeks. Both types of antibodies reach their highest level in the fourth week. However, the time of serological change during relapse varies from patient to patient. In this case, clinical findings and serological tests are important.

Brucella agglutination tests play an important role in the serological diagnosis of brucellosis. One of the two important criteria in the diagnosis of brucellosis is *Brucella* spp. isolation; the other is the detection of the presence of high titer specific antibodies in the serum accompanied by clinical findings and the demonstration of seroconversion (9). In routine practice, samples found positive by the Rose Bengal test are evaluated by tube agglutination and dilution tests. STA test is a common method used in the diagnosis of brucellosis all over the world and still in our country.

In this test, total antibodies, especially against S-LPS, are detected on the surface of the bacteria. The disadvantage of the STA test is that false positive and false negative results create difficulties in interpretation. False positivity may occur due to cross reactions with other gram-negative bacteria, while false negativity may be due to the very early stage of the infection, the presence of blocking antibodies or the prezone event.

This can be overcome by the complement binding test, the 2-mercaptoethanol test, the CAB test and the ICA test. It is stated that BCGT reacts with *B. abortus*, *B. melitensis* and *B. suis*, is 99% compatible with the Coombs test and is a fast

and economical test that can be used for both screening and titration (10). Studies have reported that the specificity and sensitivity of CAB and ICA methods are close to each other. Irvem et al. In his study, CAB or ICA was found to be perfectly compatible with BCGT, but since it was the first study, more different studies were required (11). In different studies from our country; In the comparison of BCGT with the CAB test and ICA test, it was concluded that BCGT was perfectly compatible with these tests (12-16).

CONCLUSION

BCGT method shows excellent compliance with both CAB and ICA tests; the application of the test is practical; It was determined that it gave results in as little as two hours and it was visually easier to evaluate than other methods. In this study, when the serum samples of patients with growth in blood cultures were evaluated, it was found that they were 100% compatible in 7/7. Since BCGT is a new method, there is no other study evaluated together with blood culture. The result of the test in 2 hours shows that it is routinely preferable due to its ease of diagnosis, cheap and safe.

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