

## The comparison of Brucella gel agglutination test with other Brucella tests

### *Brusella jel aglütinasyon testinin diğer Brusella testleri ile karşılaştırılması*

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#### ABSTRACT

**Objective:** In this study, it was aimed to compare the sensitivity of diagnostic tests in patients with a preliminary diagnosis of brucellosis.

**Methods:** We have compared the serological methods, standard tube agglutination test (STA), Coombs Test (CT), Rose Bengal (RBT), and the gel centrifugation test. In patients with a preliminary diagnosis of brucellosis, subjects with a positive test result of RBT has been included in the research and other diagnostic tests STA, CT and Coombs Gel centrifugation tests were performed within the range of same titration.

**Results:** Total 132 patient's serums were studied. In RBT positive 92 patients' serums, negative test results were found in 11 with STA, in 9 with CT and in 6 with gel test. While 35 patients were identified to be positive by using Brucella gel test at 1/5120 titer, no positive test results were seen with STA and CT at the same titer. Generally, CT results were one titration below the gel centrifugation test results.

**Conclusion:** In conclusion, RBT and STA were not always adequate to determine the diagnosis of brucellosis. Low titer STA results should be supported by tests such as CT or gel centrifugation and the seroconversion must be monitored. Due to giving fast results, gel centrifugation test can be preferred in diagnosis of Brucellosis.

**Key words:** Brucellosis, gel centrifugation, Coombs test

#### ÖZET

**Amaç:** Bu çalışmada, bruselloz ön tanısı konmuş hastalarda tanı testlerinin duyarlılığını karşılaştırılması ve hassasiyetlerinin ölçülmesi amaçlanmıştır.

**Yöntem:** Serolojik yöntemlerden, Standart Tüp Aglütinasyon Testi, (STA), Coombs testi (CT), Rose Bengal testi (RBT) ve Jel sentrifügasyon testleri karşılaştırılmıştır. Bruselloz ön tanılı hastalarda RBT pozitif çıkan serumlar araştırma kapsamına alınmış ve diğer tanı testlerinden STA, Brucella CT ve Coombs Jel sentrifügasyon testleri aynı titrasyon aralıklarında çalışılmıştır.

**Bulgular:** Rose Bengal testi pozitif çıkan 132 hasta serumu çalışmaya dahil edildi. RBT pozitif 92 hastanın 11'i STA ile, 9'u CT ile, ve 6'sı Jel sentrifügasyon testi ile negatif bulundu. 35 hasta serumu Jel testi ile 1/5120 titrede pozitif bulunurken, CT ve STA ile bu titrede pozitiflik saptanmadı. Genel olarak CT sonuçlarının Jel testi' ne göre bir titrasyon düşük olduğu görüldü.

**Sonuç:** Brusellozis tanısında RBT ve STA her zaman yeterli olmamaktadır. STA'nın düşük titreleri için serokonversiyon takibi veya CT veya Jel sentrifügasyon gibi testlerle laboratuvar tanı desteklenmelidir. Jel sentrifügasyon testinin daha kısa sürede sonuç vermesi nedeniyle Brucella tanısında tercih edilebilir.

**Anahtar kelimeler:** Bruselloz, jel sentrifügasyon, Coombs testi

#### INTRODUCTION

Brucellosis is a zoonotic disease that can occur at any age which takes place in Turkey in endemic areas. It is known to be a public health problem in our country and around the World causing human deaths and significant economic losses [1-4]. It is quite common in animals in the plains of Ankara,

Konya region, the Southeast Anatolia region, especially in Diyarbakir and Urfa. Despite all precautions, such as pasteurization and vaccination, the disease continues to be a problem and to be transmitted by milk and milk products in many parts of the World mainly in Asia and the Mediterranean region [2,5].

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The definitive diagnosis of brucellosis is done possible by the isolation of the agents from blood and bone marrow samples. However isolation of bacteria may vary duration of disease, the circulating amount of bacteria, the length of incubation time and antibiotic usage by the patient. In brucellosis, serological diagnosis has come to the fore for reasons such as to wait a long time for bacteria to produce, culture studies are at risk of infection, blood culture not done in all health institutions. Although, high sensitivity and specificity in a short time leads to results, which is easy and inexpensive several serological methods can be used in the diagnosis of brucellosis, all over the world as the most common Rose Bengal test (RBT) and standard tube agglutination test (STA) methods are preferred (The sentence should be written most clearly) [3,6].

Standard tube agglutination test and Brucella agglutination test with Coombs are laboratory methods commonly used in diagnosis. One of the tests that is used currently, at the laboratory diagnosis; Brucella Coombs gel test has a similar principle with other diagnosis tests. This test is a Brucella agglutination test that happens in small wells filled with gel matrix and Coombs antibody, but after the first manipulation without 18-24 hours incubation, it results after 20 minute of centrifuging.

Our aim was to compare these tests used in the diagnosis of brucellosis and showing variability sensitivity and specificity. Promote achieve reliable results in a short time will allow early initiation of treatment and it will increase patient and physician satisfaction.

## METHODS

The serum samples of 132 patients diagnosed as having brucellosis sent from various clinics to Diyarbakir Education and Research Hospital Microbiology Laboratory, Turkey were included in the study.

When examined epidemiologically, It has been determined that 58.2% of the patients reside in Diyarbakir, 41.8% in the rural region of the Diyarbakir.

It has been determined that 58.2% of the patients reside in Diyarbakir, 41.8% in the rural region of the Diyarbakir, when examining the distributions according to the age groups, 43.1% (57) of them was found respectively as 40 years of age and

above, 38.6 (51) as 21-40 years of age, 18.1 (24) as 0-20 years of age. 34% (45) of the patients were female and 66% of them (87) were male.

With patient sera, Rose Bengal test (RBT- Refik Saydam Hygiene Center Presidency Antigen Turkey), standard tube agglutination test (STA- Refik Saydam Hygiene Center Presidency Antigen Turkey), Brucella Coombs test (MCBT-Microagglutination test) and Brucella Coombs gel (ODAK Diagnostics, Turkey tests were performed. Tests have been studied in accordance with manufacturer's working principle.

The procedure of studying of Brucella coombs gel test: Brucella antibody added to the serum samples which were diluted on dilution plates. The samples were pipetted to the 12x8 gel microtubes where are included antihuman IgG gel matrix. The results are evaluated on agglutination after centrifuging for 20 minutes at 3000 rpm. The samples were resulted as negative if pink colored brucella antibody subsides at the bottom of microtubes or resulted as positive if pink colored antibody floats over the gel.

132 serum samples, which was sent to the laboratory and in which Rose Bengal test was positive, were studied respectively in the same titer with the other tests, STA,CT , Brucella CT and Brucella Gel test. Sera were stored at -20 ° C until studying. Before starting the study, all the reagents used and the sera was brought to room temperature (18-25°C). Dilutions were made up 1/40 from 1/5120 to titer.

## RESULTS

In the serum of 132 patients with negative results were as follows: STA 57, CT 49, and Gel test 51. Only 3 patients were found 1/40 titer with Gel test .7 patients with STA, 2 patients 1/80 titer with gel test, 25 with STA, 2 with CT, 1 patient 1/160 titer with gel, 22 with STA, 24 with CT, 14 patients 1/320 titer with gel were determined.

**Table 1.** Sensitivity and specificity of tests

	Positive	Negative	Total serum samples
CT	83	49	132
STA	75	57	132
Brucella Coombs Gel Test	81	51	132
Rose Bengal Test	92	40	132

1/640 titer on 20 patients with STA, 14 with gel test, 11 patients with CT, at 1/1280 titer, 10 with STA, 22 with CT, 8 patients with gel, at 1/2560 titer,

5 with STA, 32 with CT, 17 patients with gel were determined to be positive. At 1/5120 titer only 35 patients had positive results with gel tests (Table 2).

**Table 2.** According to the tests studied the distribution of antibody titers Brucella

	Negative	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
STA	11		7	25	22	20	10	5	-
Coombs Brucella	9			2	24	11	22	32	
Brucella Coombs Gel	6	3	2	1	14	14	8	17	35

STA: Standard tube agglutination test

A patient's serum found negative with STA, which was found positive with gel centrifugation test at 1/1280 titer before, three patient's serums found at 1/80 titer with STA which was found at

1/320 titer before, 15 patient's serums found at 1/160 titer with STA which was found positive at 1/160 titer before (Table 3).

**Table 3.** The distribution of antibody titers of Brucella according to standard tube agglutination test and Brucella gel test

	Brucella gel								
	(-)	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
STA (-)	5	2	2				2		
STA 1/40									
STA 1/80					3		1	1	1
STA 1/160	1			1	4	1			15
STA 1/320		1			5	6	1	1	10
STA 1/640					2	5		8	5
STA 1/1280						1	2	4	3
STA 1/2560					1			1	1
STA 1/5120									

STA: Standard tube agglutination test

By using gel centrifugation test, 4 patient's serums found negative with CT which was found at 1/80 titer and 1/40 titer before, 12 patient's serum

was found same with CT which was found at 1/320 titer. Generally, it is seen that Gel Centrifugation test results are one titer below the CT results (Table 4).

**Table 4.** The distribution of antibody titers Brucella according to Coombs and Brucella gel test

	Brucella Gel Testi								
	(-)	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
Coombs (-)	5	2	2						
Coombs 1/40									
Coombs 1/80									
Coombs 1/160				1		1			
Coombs 1/320	1	1			12	3	2	3	2
Coombs 1/640					1	7	1	2	
Coombs 1/1280					1	3	5	12	1
Coombs 1/2560									32
Coombs 1/5120									

The cut-off value for each of the three tests was considered to be 1/160 titer and Brucella Coombs test (CT) was considered as a reference test.

The sensitivity and specificity of standard tube agglutination test (STA, Wright) and Brucella Gel Coombs Test (CT) were determined as follows: STA: Sensitivity: 90.3% Specificity: 100%; Brucella Coombs Gel Test: Sensitivity: 97.5% Specificity: 100%

## DISCUSSION

Brucellosis is endemic in Turkey. Patients, especially are concentrated in Central Anatolia, East and Southeast Anatolia. Although the number of patients steadily has decreased over the years, human and animal brucellosis has not yet taken under control in our country [7].

There are some serious problems about developing the process of treatments, responses of treatment, duration of illness, the signs that show the level of illness and understanding of pathogenic mechanisms of kinds of Brucella. Because of the clinical pictures can be asymptomatic and especially there are so many clinic symptoms that shows rheumatic diseases, it is being resulted with the using of false treatments. This failures result in losing of work force and loose of money [8,9].

Various serological processes are used to diagnose brucellosis and the comparisons of these processes inform different amount of sensitivity and specificity. The first test to do on the clients that are thought to have brucellosis is RBT. Even though RBT is a sensitive method, easy to apply, short time to get results and economic, RBT itself cannot detect the clinic form (acute, subacute, chronic, recurrent, and local infection), also the test stays positive after it is completed for a long time which cause inadequate to conclude [3]. RBT is a quick, cheap and easy test on brucellosis pre-diagnosed clients of serologic diagnosis. But sometimes wrong results may occur (tularemia, humoral immunodeficiency, Rheumatoid Factor positive, etc.). Because of these reasons RBT must be supported with a second test which is capable of capturing and detecting blocker antibodies on high titer is required [10].

Even though STA is the most chosen method to diagnose brucellosis serologically, it is not enough

to detect the blocker antibodies so this process may cause some false negativities [8]. Coomb's test is a diagnosing way to determine the incomplete, blocker or those which are not capable of being agglutinated after the STA test. The anti-human globulin that was injected to the environment makes bonds between antibodies to specify the real seropositivity [11,12]. Nowadays Immuncapture agglutination test, which is more practical and may detect total antibodies without having difficulties with blocker antibodies not like Coomb's test, has been developed.

Even though ELISA (Enzyme-Linked ImmunoSorbent Assay) is fast, sensitive and specific on the diagnosis, it's shown that its performance is not better than other test that are used on routine according to IgM ELISA tests. This situation affects the sensitivity, specificity and applicability of the process. [13].

Brucella test with Coombs and Brucella gel Coombs test includes STA and Coombs tests inside them. Brucella test with Coombs is the test that is used on the wells and Brucella gel test with Coombs is the test to make agglutination by using gel matrix.

It is recommended to apply Coombs Brucella test or ELISA test to confirm such a frequently used test like STA which mostly was used on serological diagnosis of brucellosis [1,2,14]. The tests with Coombs are faster, have 24 hour resulted periods and have high standardizations compared to STA and ELISA tests. It is an advantage that Brucella Coombs Gel test has 30 minute working time. Especially, it is recommended to use it in laboratories on endemic areas. In STA, 1/40 and 1/80 titer have some difficulties to contrast. In the study it is seen that Brucella Gel Coombs test is more sensitive than other tests. If it is compared to the principles of work, all diagnostic tests have similar spent times and similar dilution systems working on them. Even though there is a comparison criterion Brucella Coombs Gel is preferred on diagnosis of Brucella because of its shorter time.

Gultekin and at al. found positive results on 81 (95.3%) patients with RBT, 53 (62.3%) patients with STA and 64 (75.3%) patients with CT of total 117 patients (15). A similar study that was made in Konya resulted positive on 56 (%78.8) patients with Rose Bengal, 30 (42.2%) patients with STA and 52 (73.2%) patients with Brucellacapt of total of 71 pa-

tients. In this study it has been expressed that STA was not enough by itself so in addition to STA, Brucellacapt and/or ELISA tests must be combined used together to diagnose Brucellosis on the patients, who are not be able to have culture tests [8].

A direct correlation observed between the Brucellacapt test and Coombs test on the comparison of these tests [16]. Gomez and at el. found similar amount of titer results in a range of one or two dilution on positive serums with Brucellacapt test and Coombs test [17].

In Kayseri, in a study where Brucella gel test and STA with Coombs were compared; sensitivity and specificity of Brucella gel test was determined to be 100%. It has been specified that performances of both tests were similar [18].

In the study where Irvem et al. compare STA, Brucella gel test, Coombs brucella test and immunocapture agglutination test; Brucella gel test showed excellent correlation with both method hem immunocapture agglutination tests. Our study was found compatible with Brucella gel test and Coombs brucella test [19].

Brucella gel test, which used brucellosis diagnosis, has started recently, therefore studies in the literature on this subject were limited. According to information provided by the manufacturer; Brucella gel test reacts to *B.abortus*, *B.melitensis* ve *B.suis*, coombs test, which were compatible with 99%. It was stated that both rapid and economical test can be used for both titration and screening [20].

In conclusion, in this study it has been seen that when two of serologic tests of diagnoses and chases of brucellosis, which are Coombs Brucella test and Brucella Coombs gel test are compared, they result with similar performances. In addition, 2 hours resulting period of Brucella Coombs gel test makes it more advantaged compared to other tests. At the daily routine of study, it is an important fact to get results on the same day to start to the treatment, which is valuable and important for patients and clinicians.

## REFERENCES

- Colak H, Usluer G, Ozgüneş I, et al. Comparison of method Wright, indirect Coombs and enzyme immunoassay IgG in the diagnosis of chronic brucellosis. Mikrobiol Bul 1992;26:56-60.
- Pappas G, Akritidis N, Bosilkovski M, et al. Brucellosis. N Engl J Med 2005;352:25-36.
- Young EJ. Brucella species. In: Mandell GL, Bennett JE, Dolin R eds. Principles and Practice of Infectious Diseases 5<sup>th</sup> ed. New York: Churchill Livingstone,
- Pappas G, Papadimitriou P, Akritidis N, et al. The new global map of human brucellosis. Lancet Infect Dis 2006;6:91-99.
- Sofian M, Aghakhani A, Velayati AA, et al. Risk factors for human brucellosis in Iran: a case-control study. Int J Infect Dis 2008;12:157-161.
- Cetinkaya Z. Francisella ve Brucella. Basustaoğlu A, Kubar A, Yıldırım ST, Tanyüksel M kitabında (çev ed). Klinik Mikrobiyoloji. Ankara: Atlas Kitapçılık, 2009;815:34-37.
- T.C. Sağlık Bakanlığı Temel Sağlık Hizmetleri Genel Müdürlüğü, Zoonotik Hastalıklar Daire Başkanlığı, Zoonotik Hastalıklar Hizmetiçi Eğitim Modülü Ankara, 2011;2012:29-42.
- Güzelant A, Kurtoglu M.G Kaya M., et al. Comparison of Brucellacapt with Other Serological Tests In the diagnosis of brucellosis. Selçuk Tıp Dergisi 2009;25:125-131.
- Tasova Y, Saltoglu N, Yılmaz G, Inal S, Aksu HSZ. Brucellosis: the evaluation of clinical, laboratory, and treatment characteristics in 238 adult patients. Enfeksiyon Dergisi 1998; 12: 307-312.
- Tünger A, Cavaşoglu C, Kork M. Microbiology (Bacteriology - Virology-Parasitology-Mycology Immunology). ASYA Tıp Kitapevi 2005;183-184.
- Bilgehan H. Small aerobic gram-negative bacilli, Brucella. Bilgehan H, ed. Special Bacteriology and Bacterial Infections Clinical Microbiology. 10. baskı.Izmir: Baris Yayınları Fakülteler Kitabevi, 2005;224-229.
- Meyer ME, Morgan WJ. Metabolic characterization of Brucella strains that show conflicting identity by biochemical and serological methods Bull. World Health Organ 1962;26:823-827.
- Alışkın H. The value of culture and serological diagnosis in human brucellosis. Mikrobiyoloji Bülteni 2008;42:185-195
- Alışkın H, Colakoglu S, Turunc T, et al. Determine the value of Brucellacapt test in the diagnosis of brucellosis. Mikrobiyoloji Bülteni 2007;41:591-595.
- Gültekin E, Uyanık M H, Albayrak A, et al. Comparison of several serological methods for the diagnosis of brucellosis. Türk Mikrobiyoloji Cemiyeti Dergisi 2012;42:142-147.
- Serra J, Velasco J, Godoy P, Mendoza C. Can the Brucellacapt test be substituted for the Coombs test in the diagnosis of human brucellosis? Enferm Infec Microbiol Clin 2001;19:202-205.
- Gomez MC, Rosa C, Geijo P, Escibano MA. Comparative study of the Brucellacapt test 14versus the Coombs test for Brucella. Enferm. Infec. Microbiol Clin 1999;17:283-285.
- Türk Dağı H, Fındık D. A New method for the diagnosis of brucellosis : Brucella Coombs gel test.. 2. Ulusal Mikrobiyoloji Kongre kitabı 2013;PS236; s.303.
- Irvem A, Yücel F, Aksaray S, Bor E. The comparison of New and rapid method for the diagnosis of Brucella Brucella Coombs gel test with of other serological methods. Mikrobiyoloji Bulteni 2015;49:181-187.
- Brucellosis and diagnosis. [http:// www.toprakmedikal/documents/Brucella.ppt](http://www.toprakmedikal/documents/Brucella.ppt).