Comparison of a New and Rapid Method: Brucella Coombs Gel Test With Other Diagnostic Tests

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Background: The aim of this study was to detect reliability of Brucella Coombs gel test (BCGT) by comparing with with ELISA (IgG + IgM), Standard agglutination test, and Brucella immunocapture agglutination methods in serological diagnosis of brucellosis. **Methods:** Brucella Coombs gel test (BCGT), Brucella ELISA (IgG + IgM), Standard agglutination test, and Brucella immunocapture agglutination tests of 78 patients with presumptive diagnosis of brucellosis which were sent to Microbiology Laboratory of Konya Numune Hospital from

various regions of Konya were studied. **Results:** Results: Of 78 patients with ELISA IgG and IgM, STA, BICA and BCGT; 26, 21, 10, 12 and 12 were positive. When compared with BICA, the sensitivity and specificity of BCGT were 100% and 100%, respectively. **Conclusion:** According to results BCGT can be used as a diagnostic test in routine laboratories after more comprehensive studies in control groups and patients. J. Clin. Lab. Anal. 30:756–759, 2016. © 2016 Wiley Periodicals, Inc.

Key words: Brucellosis; Brucella immunocapture agglutination; ELISA; Brucella Coombs gel test; Standard agglutination test

INTRODUCTION

Brucellosis is primarily a disease of herbivores (1). However, it is accidentally transmitted to humans from animals and causes various clinical findings. Worldwide, Brucellosis is still a significant health problem and endemic in the Mediterranean region, Asia, and developing countries (1–3). Turkey is one of the areas where Brucella infection is commonly seen, though a significant reduction of the infection has been obtained by the vaccination of animals, isolation of infected animals, and other control strategies in the recent years (1, 4–6). The agent of the disease is small, aerobic, Gram-negative rod known as Brucella that causes undulant fever, arthralgia, and even in 5–10% cases, the neurological system may be affected (1, 2, 7, 8). The source of infection in humans is commonly ill animals infected by Brucella; *Brucella melitensis* is the commonly observed pathogen (1, 9).

Diagnosis of brucellosis is very important, as it requires a long-term therapy (10). For a definite diagnosis of brucellosis, though blood culture is the gold standard, it takes a long time, for example, 7 or more days. This method

can have low sensitivity (10–12). Serological tests such as Rose Bengal latex agglutination test, Enzyme-Linked ImmunoSorbent Assay (ELISA) IgG and IgM, standard tube agglutination (STA) test, Coombs gel test, and Brucella immunocapture agglutination (BICA) methods are mostly used for the diagnosis of brucellosis. STA is an easy and cheap test, but blocking antibodies may lead to false-negative results (10, 11). Coombs gel test is useful for detecting blocking antibodies, but it is a time-consuming test and is not routinely used in many laboratories (3). BICA is easy to perform and interpret, and also, detects acute and chronic infection (11).

The combination of the tests is highly recommended for true diagnosis, as sensitivity and specificity of tests vary.

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Also, STA and BICA require at least 18–24 h of incubation time. Recently a new method, Brucella Coombs gel test (BCGT) with incubation time of 2 h has been introduced. This rapid method is based on the agglutination method. The microtubes contain matrix and Coombs antibodies (anti-human IgG). If there is antibody in the serum of patient, the pink antigen–antibody complex appears after centrifugation, which cannot be converted into gel and remains at the top of microtubes. If there is no antibody, pink antigen accumulates at the bottom of the microtube after centrifugation. The aim of this study is to detect reliability of BCGT by comparing ELISA (IgG + IgM) with STA and BICA methods in serological diagnosis of brucellosis.

MATERIALS AND METHODS

Sera samples from 78 patients with a presumptive diagnosis of brucellosis sent to Microbiology Laboratory of Konya Numune Hospital from various regions of Konya during June 1–30, 2015 were included in the study, prospectively. This study was approved by the local institutional ethics committee of Konya Numune Hospital. For BICA test (Metser-MetserLAB, Istanbul, Turkey), 50 µl serum diluent, 5 µl serum sample, and 50 µl colored antigen suspension were added to each U-shaped well precoated with anti-human immunoglobulin on a microplate, which was later covered to prevent evaporation. The entire solution was incubated for 18–24 h at 37°C, according to the manufacturer's instructions. For Brucella agglutination test (Brucella Coombs Gel Test, ISLAB, Turkey), a value of 1/320 and more was considered positive. STA was performed by dilution in a tube, incubated for 18–24 h at 37°C, and titer was evaluated according to agglutination. For STA (Cromatest-LINEARChemicals, Barcelona, Spain), a value of 1/160 and more was considered positive.

ELISA (IgG and IgM; NovaLisa-NovaTec, Germany) tests were performed according to instructions of the commercial kit manufacturer. Values above cutoff were taken as positive.

For BCGT (ODAK Brucella Coombs Gel Test, ISLAB, Turkey) method; first serum dilutions from each patient were prepared and incubated. After incubation, mixture in the plate was added to microtubes containing gel matrix. These microtubes were incubated at 37°C for 20 min, and were then centrifuged at appropriate revolutions per minute for 20 min according to the manufacturer's instructions. Results were visually assessed. For positive results, pink antigen–antibody complex was at the top of the gel. For negative results, pink antigen–antibody complex was at the bottom of the gel because of the absence of antibody. For BCGT, a value of 1/40 and more was considered positive. Serial dilutions were studied for BCGT-positive patients. In our study, BICA was considered as the refer-

TABLE 1. Distribution of ELISA IgG, ELISA IgM, STA, BICA, and BCGT Test Results

	ELISA IgG (n)	ELISA IgM (n)	STA (n)	BICA (n)	BCGT (n)
Positive	26	21	10	12	12
Negative	52	57	68	66	66

STA, standard tube agglutination; BICA, Brucella immunocapture agglutination; BCGT, Brucella Coombs gel test.

ence test; and sensitivity, specificity, positive and negative predictive values of the tests were calculated (3).

RESULTS

Of 78 patients who attended polyclinics, 36 were men and 42 were women. In our study, 12 patients were BCGT positive, which was a reference test. With ELISA IgG and ELISA IgM, 26 and 21 patients were positive, but most of the patients were less positive—very close to a cutoff value that gave rise to cross reaction. With STA only 10 of 12 patients were positive. Twelve BICA-positive sera were also positive with BCGT. Distribution of ELISA (IgG and IgM), STA, BICA, and BCGT test results are provided in Table 1. The sensitivities of ELISA IgG, ELISA IgM, ELISA total (IgG + IgM), STA, BICA, and BCGT were 92.8%, 100%, 71.4%, 71.4%, 100%, and 100%, respectively. The specificities of ELISA IgG, ELISA IgM, ELISA total, STA, BICA, and BCGT were 79.7%, 89%, 100%, 100%, 100% and 100%, respectively (Table 2).

DISCUSSION

Brucella bacterium is an intracellular bacterial pathogen, which survives in the reticuloendothelial system. Lipopolysaccharide structure of bacterium is important for virulence and antibodies formation against this substance are important in serologic tests used for the diagnosis of Brucella (13–15). Brucella causes undulant fever, and the infection may be acute or chronic. Severe complications may occur in chronic infection (14, 16). Mostly, *B. melitensis* is the infectious agent (14). This disease is more prevalent in endemic areas (16). Turkey lies between Europe and Asia. In Turkey, brucellosis is still an important public health problem, especially in rural areas (4, 14). Brucellosis is also common in neighboring countries of Turkey such as Iran, Iraq, Syria, etc. (14). Our study region is in the middle of Turkey. Animal husbandry is an important source of income in this region. Animal hosts play important role in transmission of this disease, so precautions should be taken to control the disease in animal hosts (13). Protection of animal health is essential for public health (1). Consumption and

TABLE 2. Sensitivity, Specificity, PPV, and NPV of Tests

	ELISA IgG (%)	ELISA IgM (%)	ELISA total (%)	STA (%)	BICA (%)	BCGT (%)
Sensitivity	92.8	100	71.4	71.4	100	100
Specificity	79.7	89	100	100	100	100
NPV	98	100	94.1	94.1	100	100
PPV	50	66.7	100	100	100	100

STA, standard tube agglutination; BICA, Brucella immunocapture agglutination; BCGT, Brucella Coombs gel test; PPV, positive predictive value; NPV, negative predictive value.

traditional preparation of dairy products are other important factors. Yet, there is no vaccine for preventing infection of this disease in humans (17). As brucellosis is endemic in this region, it is very important to educate the population about the ways of transmission and risks of brucellosis. In order to decrease the frequency of the disease and eradicate the disease, public health control strategies should be planned.

Generally, serological tests are used for the diagnosis of brucellosis. Commonly used STA method achieves false-negative results because of blocking antibodies and prozone (10), but STA is not alone sufficient for the diagnosis. True and early diagnosis is very important to decrease morbidity and mortality of brucellosis. So a sensitive, specific, and rapid method is required (10). Peeridogaheh et al. compared BICA test with ELISA tests (IgG + IgM) in blood culture positive patients, reported a high specificity for BICA, and suggested the use of combination of both tests (11). The use of IgG or IgM alone showed low sensitivity for diagnosis (11). In our study, the specificity of ELISA tests was low. Orduna et al. reported that all patients with the diagnosis of brucellosis were positive with BICA test and Coombs gel test with good correlation between these two tests. In the same study, only 75 of 82 patients were positive with STA indicating less correlation between STA and BICA (3). In our study, BICA was considered the reference test because of a good correlation with Coombs gel test (3). Memish et al. evaluated results of STA and ELISA tests in patients with *Brucella* bacteremia. They reported that the sensitivities of ELISA IgM or ELISA IgG alone were lower than STA (19). Casanova et al. also reported BICA as a sensitive and specific test (2). Gómez et al. compared titrated Rose Bengal test, microagglutination test, microtiter-adapted Coombs gel test, BICA, IgG, IgM, and IgA tests and found that sensitivity of ELISA tests was lower than other tests (12). BICA can be used for the diagnosis of all stages of infection, relapses, and reinfections of brucellosis (3). ELISA systems are more costly and need more equipment (13). For true diagnosis, the combination of tests may be useful (18, 20). In this article, we studied a new method, but only one study was conducted for BCGT method in Turkey. İrvem et al. reported that BCGT was an easy and rapid method for *Brucella* diagnosis, and found

that test results of BICA and BCGT in their study were perfectly consistent (18). Sensitivity, specificity, negative predictive, and positive predictive values of BCGT were reported to be relatively high compared to other tests (5). It is less time-consuming and requires less preprocessing. Therefore, results can be obtained within 2 h. It is easy to evaluate results visually because of colored antigens. Our study has some limitations. We could not provide any clinical description of patients, and hemoculture could not be performed as these patients attended polyclinics. New techniques that are easy to use and provide rapid detection are very important for diagnosis. Therefore, BCGT test seems to be a promising technique for the diagnosis of brucellosis. BCGT can be used as a diagnostic test in routine laboratories after more comprehensive studies in control groups and patients.

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