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Comparison of standard agglutination tests, enzyme immunoassay, and Coombs gel test used in laboratory diagnosis of human brucellosis

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Background/aim: It was aimed to evaluate the results of Rose Bengal (RB), ELISA total tests (IgM and IgG), and the *Brucella* Coombs gel test (BCGT), which are used as screening tests, with the combined results of a tube agglutination test (standard Wright test: SWT) and a tube agglutination test with antihuman globulin (AHG TAT).

Materials and methods: Samples from 97 patients prediagnosed with brucellosis (age ≥18 years) were screened with RB, ELISA, and BCGT. SWT < 160 samples and RB-negative but ELISA- or BCGT-positive samples were tested by AHG TAT. SWT ≥ 160 or AHG TAT ≥ 160 was taken as reference.

Results: Thirty-two of 56 RB-positive samples and one RB-negative but ELISA- and BCGT-positive sample were found to be ≥160 with SWT or AHG TAT. Sensitivity, specificity, accuracy, and agreement (kappa) values according to SWT ≥ 160 or AHG TAT ≥ 160 positivity were as follows, respectively: RB 96.9%, 62.5%, 74.2%, and 0.509; ELISA total 100%, 60.9%, 74.2%, and 0.515; BCGT test 97%, 70.3%, 79.4%, and 0.594.

Conclusion: Sensitivities of the screening tests are similar, but positivities should be confirmed by more specific tests. Positive samples from screening tests should be tested with AHG if the SWT value is <160.

Key words: Serological tests, brucellosis, ELISA, *Brucella* Coombs gel test

1. Introduction

Brucellosis is a systemic zoonotic infection disease in humans affecting various organs and systems, which causes a wide variety of clinical presentations. The acute phase may progress to a chronic disease with relapse or development of persistent localized infection. Subacute brucellosis is a typical form with undulant fever (1–3). Mortality is rare and usually results from infection of the brain or heart, as endocarditis with severe destruction of valve structures is the most frequent cause of death in brucellosis (4,5). Brucellosis incidence reported from endemic regions worldwide is <0.01 and may be as high as >200 per 100,000 people. It is estimated that the real incidence is more than 25 times the reported values (2).

While the gold standard for the diagnosis of brucellosis is culture, it requires a long incubation period, there is a risk of laboratory infection, and isolation of the etiologic agent varies according to disease phase, antibiotic use, *Brucella* species, and culture medium (3,2,6,7). Antibody tests are widely used because of these reasons. Positive results with the Rose Bengal (RB) slide agglutination test, which is used as a screening test, should be supported by other methods such as titrimetric tests (2,6,7–10). There is a problem in defining a diagnostic titer in a single sample and it may change according to the prevalence of the population, but a titer of ≥160 is mostly accepted (6,7–11).

Blocking antibodies found in chronic cases are IgG (IgG_1 and IgG_2) and IgA antibodies, which can specifically bind to the antigen without visible agglutination. Presence of blocking antibodies in the serum can be shown by Coombs test (antihuman globulin: AHG) or BrucellaCapt test (6,7,9,10,12).

Alternative methods, such as the enzyme-linked immunosorbent assay (ELISA) and AHG gel test, with which more samples in shorter time periods can be tested, are presented against standardization problems of agglutination tests with the possibility of different

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evaluations by different people in different laboratories, but evaluation of these tests is necessary before substitution (6–8,13–16).

In this study, it was aimed to evaluate the results of RB, ELISA total tests (IgM and IgG), and the *Brucella* Coombs (AHG) gel test (BCGT), which were used as screening tests, with the combined results of a tube agglutination titration test (standard Wright test: SWT) and the AHG tube agglutination test (AHG TAT).

2. Material and methods

2.1. Samples

Samples from 97 patients prediagnosed with brucellosis and aged 18 years or older, which were sent to the Medical Microbiology Laboratory of the Ege University Medical Faculty between 07.01.2014 and 29.01.2015, were included in the study. The ethical committee approved the study on 07.01.2014 (number 13-12.1/8).

2.2. Serological tests

The RB test (Türk Halk Sağlığı Kurumu, Turkey), ELISA IgM and IgG tests (Vircell, Spain), and BCGT (1/40 dilution) (Odak *Brucella* Coombs gel test, Toprak Medikal, Turkey) were used as screening tests. The SWT (Türk Halk Sağlığı Kurumu) and AHG test (Millipore, UK) were used as titrimetric tests.

2.3. Study algorithm

All the samples were screened with RB, ELISA IgM, ELISA IgG, and BCGT. RB-positive samples were tested by SWT (TAT). Samples positive with RB but SWT-negative or with titers lower than 160 and samples negative with RB but positive with ELISA IgM and/or IgG or BCGT were tested by AHG TAT to investigate presence of blocking antibodies. SWT or AHG TAT positivity with titers of ≥160 were accepted for the confirmation of laboratory diagnosis. The study algorithm of the samples is summarized in Figure 1.

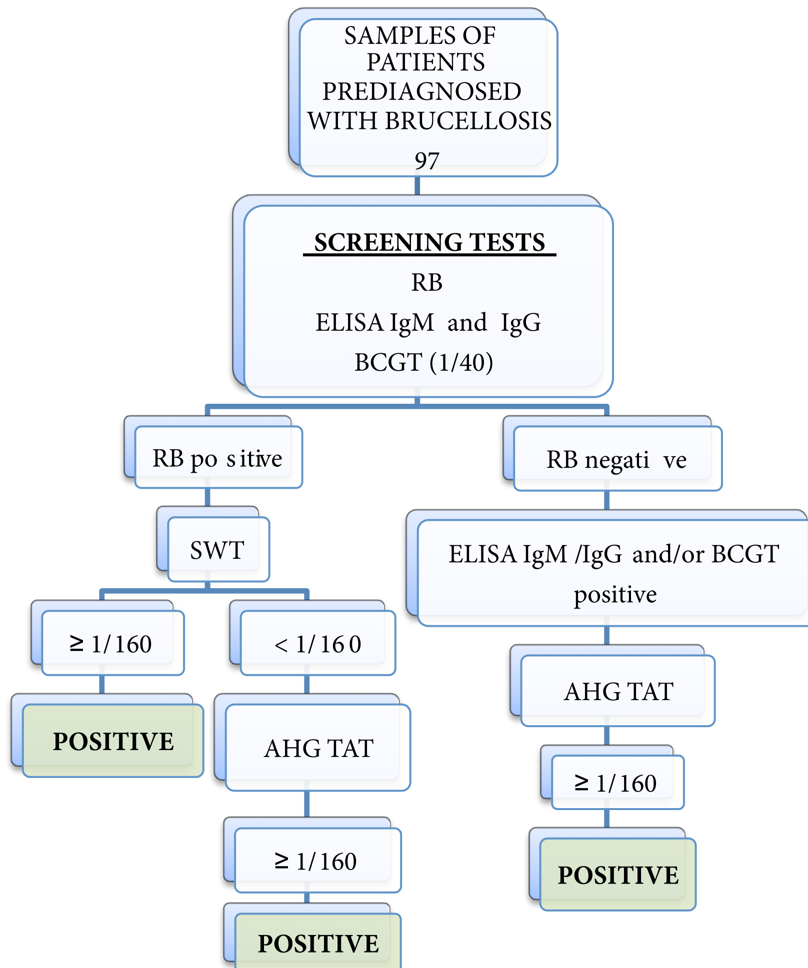

Figure 1. Study algorithm of the samples from 97 patients prediagnosed with brucellosis. RB: Rose Bengal test, SWT: standard Wright test, BCGT: *Brucella* Coombs gel test, AHG TAT: tube agglutination test with antihuman globulin.

2.4. Statistical methods

Sensitivity, specificity, and accuracy of RB, ELISA total tests (IgM and/or IgG positivity), and the BCGT were determined by taking SWT or AHG TAT positivity with titers of ≥160 as a reference. Agreement of test results with SWT or AHG TAT ≥160 positivity was analyzed by calculating kappa evaluation coefficients and kappa values between 0.21 and 0.40 were interpreted as weak, 0.41–0.60 as intermediate, 0.61–0.80 as good, and >0.80 as excellent correlation (17).

3. Results

3.1. Results of screening tests

Among the 97 samples, at least one screening test was positive in 52 cases and in 46 of them all three screening tests (RB, ELISA total, BCGT) were positive. Distribution of samples according to the results of screening tests is shown in Figure 2.

3.2. SWT results

Of the 56 RB-positive samples, 22 were found to be ≥160 positive and 34 were <160 or negative.

3.3. AHG TAT results

Thirty-four RB-positive samples that were SWT negative or <160, two RB-negative samples that were ELISA total- and BCGT-positive, and four samples that were only ELISA total-positive were tested by AHG TAT to investigate the presence of blocking antibodies. Among these 40 samples, five of the RB-positives increased to 160, four increased to 640, and one increased to 320, and one RB-negative sample that was ELISA total- and BCGT-positive increased to a titer of 160.

Results of the 97 samples with the study algorithm are summarized in Figure 3.

3.4. Sensitivity, specificity, accuracy, and agreement (kappa value)

These values for RB, ELISA total (IgM + IgG), BCGT, and BCGT of ≥160 according to SWT ≥160 or AHG TAT ≥160 positivity are shown in the Table.

4. Discussion

Diagnosis of probable brucellosis is made by confirmation of clinical and laboratory findings with specific microbiological tests. Isolation of *Brucella* species form blood, bone marrow, and tissue samples is accepted as the gold standard. However, the isolation rate of the etiological agent varies according to stage of the disease, antibiotic use, *Brucella* species, culture medium, and technique used and it may be low in relapses (2,3,8). Identification of the agent by polymerase chain reaction has not been accepted as a standard diagnostic tool (2,3,7). For these reasons, antibody tests are widely used in brucellosis diagnosis. The specificity of the RB test, which has been used as a screening test for many years, varies according to the prevalences of the populations and confirmation of positive results with titrimetric tests is required (1,2,6,7,11,18–21). Even though a SWT titer of ≥160 in the presence of associated epidemiological (exposure history) and clinical findings is generally accepted for the diagnosis of brucellosis, the diagnostic titer for the confirmation of the disease has not been clearly identified (2,8,19,22). The diagnostic titer needs to be evaluated according to the prevalence of the disease in the area where the patient lives (rural or urban) and the features of the studied population (for example, occupational risk factors) (2,6,7,9,10,12).

In this study, a single serum sample was evaluated and a titer of ≥160 by SWT or AHG TAT was added to the algorithm for samples negative or with low titers to investigate the blocking antibodies. Thirty-two of 56 RB-positive samples were confirmed with ≥160 SWT or ≥160 AHG positivity. One sample that was RB-negative but positive by BCGT (1/40) and ELISA total tests was found to be 1/160 positive by AHG TAT. RB sensitivity and specificity were determined as 96.9% and 62.5%, respectively by taking SWT ≥160 or AHG TAT ≥160 positive samples as a reference. RB sensitivity and specificity rates were reported between 75% and 100% in studies done with active brucellosis cases (11,18–21). It is observed that RB sensitivity and specificity rates

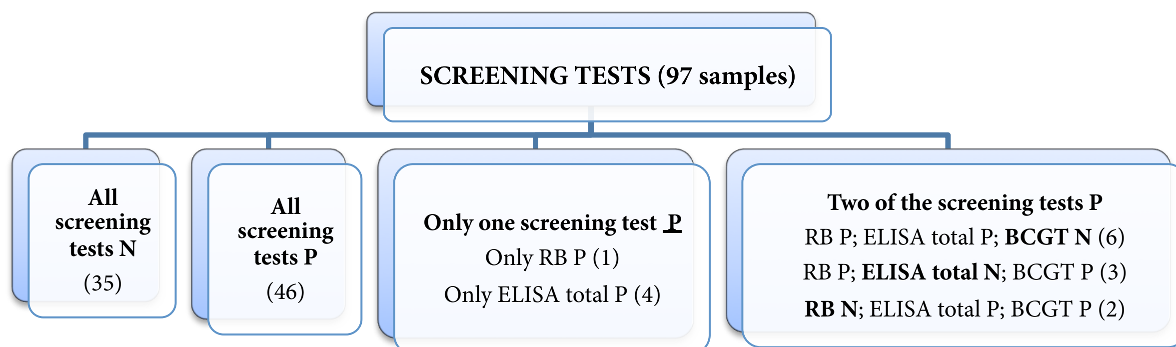

Figure 2. Distribution of samples according to the results of screening tests. P: Positive, N: negative.

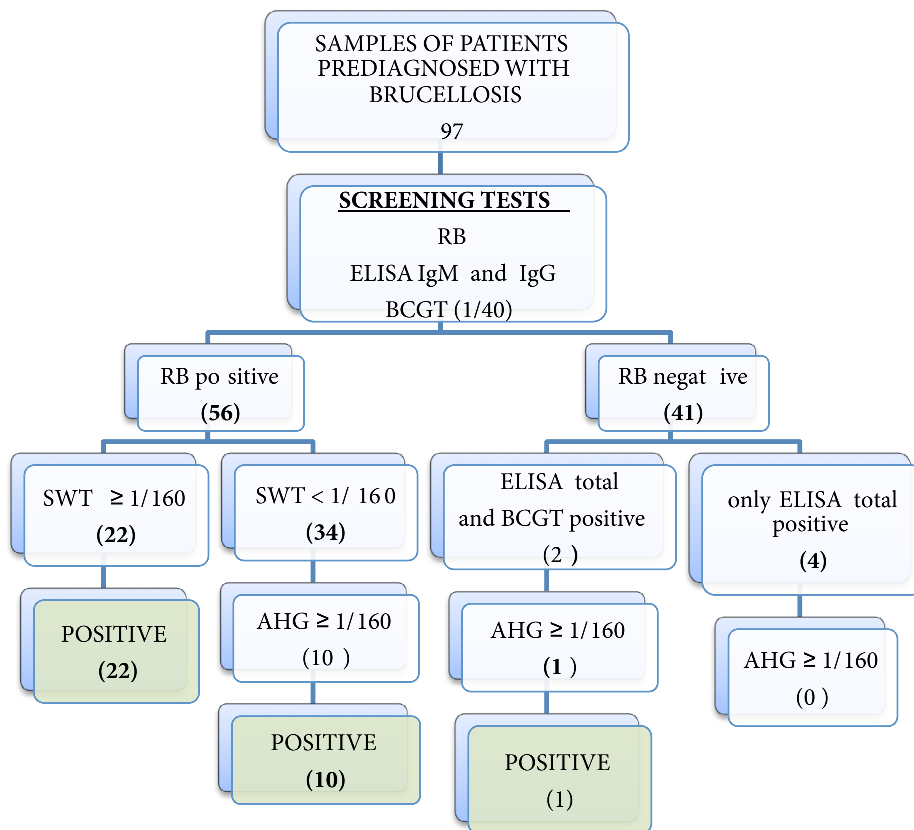

Figure 3. Algorithm of serological tests used for brucellosis serodiagnosis and the number of samples found to be positive (number). RB: Rose Bengal test, SWT: standard Wright test, AHG: tube agglutination test with antihuman globulin, BCGT: *Brucella* Coombs gel test.

Table. Sensitivity, specificity, accuracy, and kappa values according to SWT ≥160 or AHG TAT ≥160 positivity.

	Sensitivity	Specificity	Accuracy	Kappa*	P**
RB	96.9%	62.5%	74.2%	0.509	0.000
ELISA IgM	75.5%	87.5%	83.5%	0.633	0.000
ELISA IgG	87.8%	68.8%	75.3%	0.507	0.000
ELISA total	100%	60.9%	74.2%	0.515	0.000
BCGT (1/40)	97%	70.3%	79.4%	0.594	0.000
BCGT ≥160	78.8%	93.8%	88.7%	0.742	0.000

*Kappa value of 0.21–0.40 is defined as weak, 0.41–0.60 as medium, 0.61–0.80 as good, and >0.80 as near to excellent agreement.
**P-value for kappa analysis.

increase to 100% in studies that take culture positivity as a reference, while they decrease even to 33%–50% in special patient groups such as chronic, complicated, and focal infections (7,20,21). It is also suggested to increase the test time to 8 min for blocking antibodies in chronic patients (8,20). However, we performed the test in 4 min and have not tried 8 min.

The presence of blocking antibodies in the serum can be demonstrated by Coombs agglutination test (AHG TAT) or BrucellaCapt test. Binding of blocking antibodies

can be induced by adding AHG IgG (Coombs reagent) mechanically (centrifugation at high speed) (2,7,8–10,12). AHG is included in the BCGT that we used in our study and binding of blocking antibodies with AHG is induced with high speed centrifugation to make it possible to get higher titers than in the SWT. In our study, sensitivity of BCGT screening with 1/40 dilution of sera as suggested by the producer was found as 97%, while the specificity was 70.3%. Sensitivity rates of BCGT reported in other studies from Turkey (94%–100%) are similar to ours, while the specificity rate we found is lower than the reported 82%–100% rates (13-16). İrvem et al. (13) reported excellent agreement with AHG TAT and an immunocapture agglutination test. In our study, medium agreement (kappa = 0.594) with SWT/AHG TAT ≥160 positivity and 79.4% accuracy were found. When we evaluated the BCGT with a titer of ≥160, sensitivity decreased to 78.8% while the specificity increased to 93.8%, accuracy increased to 88.7%, and agreement became good (kappa = 0.742). It is necessary to evaluate the titrimetric results of the BCGT, which was developed in Turkey and has started to be used recently, with more studies and longer follow-up periods to demonstrate its clinical value.

Brucella ELISA tests, which were presented as an alternative for laboratory diagnosis to overcome the problems of agglutination tests, have varying sensitivity and specificity rates depending on different kits; in some tests rates as low as 50% were reported (22–25). IgM and IgG sensitivity rates in active brucellosis cases are reported as 80% separately, while when IgM and IgG results are evaluated together, the sensitivity of the test increases to 90%–100% (22–25). It is suggested to perform rheumatoid factor (RF) absorption routinely to prevent false positive results due to RF in the serum (23). RF absorption is used in the test we used in our study. In our study, IgM and IgG sensitivities were found as 75.5% and 87.8%, while specificities were found as 87.5% and 68.8%, respectively. When IgM and IgG were evaluated in total to increase the sensitivity, sensitivity was found as 100% but the specificity decreased to 60.9%. Reported sensitivity rates with the kit we used are similar, but our specificity rate is lower. Binnicker et al. (26) found 82.7% of IgM-positive and only 54.2% of IgG-positive samples as negative with TAT, but they did not use AHG. One of the important problems of cross-reactions due to OPS structure also occur with ELISA tests that use S-LPS antigen. This problem is seen less with ELISA tests that use the whole cell (6,7,25). The *B. abortus* S-99 LPS antigen is used in the kit we used.

In this presented study, the BCGT with ≥160 has the highest accuracy and agreement values, followed by ELISA IgM values. These results seem to be the reflection of their high specificities. However, when their low sensitivities are considered, it is not found to be appropriate to use them as single tests alone. Both of the tests missed seven cases. Limitations of our study can be summarized as the lack of culture results and no follow-up of the patients. Titrimetric values of the BCGT need to be evaluated in broader case-based studies.

In conclusion, a sensitive screening test should be used and then confirmed with a more specific test. Samples positive by screening test but SWT-negative or <160 should be tested with AHG.

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