Validation of Two Rapid Serological Tests for Human Brucellosis Detection

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Abstract

As brucellosis have several non-specific clinical phases, the search for reliable diagnosis of the disease is necessary. Therefore, for serological determination of brucellosis, rapid immunofiltration tests designated as ERIFA and NERIFA and ELISAs were developed and evaluated for diagnostic purposes. The sera were collected from Clinical Microbiology Department of Kars State Hospital between 2007 and 2009. These sera were used for determination of sensitivity of ELISAs, ERIFA and NERIFA in comparison to rose bengal test, Brucellacapt and serum agglutination tests. In the study, highest sensitivity was detected by ELISA, ERIFA and NERIFA, respectively. Specificity was highest with ELISA as 95.2% and with both ERIFA and NERIFA as 92.9% and the lowest was with RBT as 90.5%. When two ELISAs were compared based on the IgG sensitivity, there was no difference (p<0.05), but the difference was important (p>0.05) when IgM was accepted as a criteria for comparison. The current study reports the first evaluation of ERIFA and NERIFA in human beings. It is concluded that the RIFA models can be accepted as an ancillary test for diagnosis of human brucellosis in endemic countries.

Keywords: Human Brucellosis, Rapid Tests, Serology

1. Introduction

Brucellosis is a zoonotic disease caused by Brucella genus and the disease is widespread, and highly endemic in the Mediterranean, Middle East, Latin American, and Asian countries[1,2]. The incidence of the disease differs between countries with up to 2-3 cases per 1000 population/year[3]. As human brucellosis has different clinical symptoms,[4] laboratory diagnosis based on isolation, mostly from blood culture or the antibody detection is essential. However, isolation of the bacteria has several drawbacks such as delay in reproduction in primary cultures that may detain diagnosis longer than 7 days[43]. Moreover, sensitivity of blood culture is often low, changing from 50 to 90% based on the period, Brucella species, culture medium, amount of disseminating bacteria, and the blood culture technique used[44]. Therefore, sero-diagnostic tests are mostly used especially in people with persistent infection and recurrences, and in the regions where antibodies against brucellosis are prevalent[45-9]. Most frequently applied diagnostic tests for human brucellosis are the Serum Agglutination Test (SAT), the Coombs anti-Brucella test, the Rose Bengal test (RBT), and Complement Fixation Test (CFT)[7,8]. For a long time, radioimmunoassay[9,10] and enzyme immunoassay[7,11-15] have also been used. The diagnostic performance of SAT
and Brucellacapt has been studied thoroughly\cite{16-19} and defined as useful tests. However, there are problems in interpretation of the results of these tests, especially for low titers obtained from healthy people who had the infection earlier in endemic areas\cite{11,18}, also for early diagnosis or in patients with chronic brucellosis or recurrence\cite{19}. Immunochromatographic lateral flow assays\cite{20} and immunofiltration based test models known as enzymatic (ERIFA) and non-enzymatic (NERIFA) rapid immunofiltration assays\cite{21,22} were suggested for rapid detection of Brucella-specific antibodies in different species in place of highly complicated and more expensive tests.

As brucellosis has several non-specific clinical phases, the search for reliable diagnosis of the disease is necessary. Although much improvement has been made for diagnostic purposes, there is still need for the accurate detection of brucellosis. In the study, the objective was to evaluate rapid immunofiltration based tests designated as ERIFA and NERIFA as alternatives to other common serological tests and develop and evaluate in-house ELISA tests for IgG, IgM and IgA detection in comparison with commercially available ELISAs and Brucellacapt.

2. Materials and Methods

2.1 Human Sera

In the study, the groups of sera below were used: (i) a total of 136 sera included from brucellosis suspicious people. The samples were from a retrospective study performed in Clinical Microbiology Department of Kars State Hospital between 2007 and 2009. These sera were used for detection of sensitivity of ELISA, ERIFA, NERIFA and RBT. Positive sera used in the study were selected from the patients with the clinical symptoms (prolonged fever, joint pain, anorexia, fatigue, lymph adenopathy).

ii) 30 negative control sera confirmed by blood culture and serology were used for the detection of test specificity.

iii) In addition to the samples stated above, 73 positive and 37 negative with a total of 110 RBT, Brucellacapt and SAT tested sera were used for comparison of ELISAs and ERIFA.

The Rose Bengal Test (RBT)

RBT was conducted as defined by Diaz et al.\cite{8}. For the test, serum was handed out on a card and mixed with the same quantity of the antigen (Veterinary Control Laboratory; Pendik, Turkey). The card was later moved gently at 22 °C for 8 minutes and any visible agglutination was evaluated as positive.

Serum Agglutination Test (SAT)

In the test, whole B. abortus antigen from Pendik Veterinary Control Institute was used and the result was evaluated after 24 hours of incubation at 37 °C. The titre was accepted as serum dilution disclosing more than 50% agglutination\cite{5,17,22,23}.

Immunocapture-Agglutination (Brucellacapt) Test:

Brucellacapt test (Vircell SL, Spain) was carried out to the producer’s directions. Serum dilution was made in a U-shaped microplate coated with anti-human immunoglobulins. After adding the antigen suspension the plates were set at 37 °C for 24 h in a damp place\cite{4,13,23,24}.

2.2 ELISAs

Commercial Novatech (NT) ELISAs were applied to the producer's directions. The set of negative sera was used for precision of cut-off points in both home-made and NT-ELISAs, besides internal control sera were used for NT-ELISA. As an additional test, IgA antibody response was determined by ELISA performed according to Genç et al.\cite{15}.

Commercial ELISA test

Novatech ELISA kits were used for antibody detection of IgG and IgM in sera and the results were compared with home-made IgG and IgM ELISA results. The cut-off values were determined with 20 Brucella negative control sera.

Home made ELISAs

ELISAs were implemented to detect IgG, IgM, and IgA classes antibodies against LPS of the B. abortus S19 strain\cite{15,25}.

Antigen preparation

S-LPS antigen was prepared from B. abortus S19 broth culture by hot phenol method described by OIE\cite{26}. OPS antigen for ERIFA test was obtained from Dr. Ignacio Moriyon at University of Navarra, Pamplona, Spain.
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**Rapid Immunofiltration Assays (RIFAs)**

Two models of RIFA designated as enzymatic (ERIFA) and non-enzymatic (NERIFA) as described earlier for *Mycoplasma gallisepticum* infection detection in poultry and for brucellosis diagnosis in domesticated animals were used\[21,22,27\]. Both models were implemented on nitrocellulose (NC) membrane (Schleicher & Schuell, Germany) in the cassette. In this test system, NC membrane was also used for capturing all reagents. In both formats, all steps are identical except for the detection step.

### 2.3 ERIFA

This model was performed by Genç et al.,\[28\]. In this model, Brucella antigens were spotted onto the membrane. Test cassette was also included human IgG serum (1 mg/ml) for internal control. Blocking and washing steps were applied with 1% PBST/FG. In all test steps, solutions flowed through the membrane. This test was maintained with anti-human Ig (A+G+M) mix (Sigma-Aldrich) conjugate and the color development was run with BCIP/NBT (Sigma-Aldrich) substrate. Color development was stopped after 1 min of absorption and the results were categorized according to intensity and visualization time.

### 2.4 NERIFA

NERIFA was performed as described by Genç et al.,\[28\]. This model is based on the conjugate, “protein G/colloidal gold” (CG/G conjugate) (Arista Biologicals, USA) binding to the anti-Brucella antibodies against LPS antigens that were separately dotted onto NC filter membrane. After addition of serum and washing process, the conjugate is added. Following washing, the result is evaluated as that of ERIFA in comparison with an IgG as internal control. Optimization of the NERIFA was realized with monoclonal anti-Brucella LPS antibody (IgG2a, clone 4B5A), reference strong and weak Brucella positive sera and sera obtained from suspicious patients as well as negative control.

**Evaluation of Test Results**

In ELISAs, threshold value in each plate was established by calculating average means of 20 negative control sera plus 2 Standard Deviation (SD). Results above the threshold absorbance level were assessed as positive and those below it as negative. The SAT and Brucellacapt test were performed by 2 fold dilution starting at 1:20, using a commercial *B. abortus* antigen (Pendik and Vircell). The agglutinating titer was detected as the serum dilution showing 50% agglutination. In the Brucellacapt test, a titer of twice the SAT titer was considered as positive, that is ≥1:80 for SAT titer and ≥1:160 for Brucellacapt were equally evaluated as positive. In RIFA tests, color reaction with the internal control and antigen dots indicated that the serum was positive, no color reaction with internal control showed that the test was invalid.

### 3. Results

In the study, highest sensitivity was detected by homemade ELISA, ERIFA and NERIFA respectively. Lower sensitivity was detected as 83% with RBT as shown in Table 1. Positivity was confirmed by diagnosis of clinically suspicious individuals and serologically by Brucellacapt and SAT results. Negative sera were obtained from healthy individuals and confirmation was done by SAT and Brucellacapt. Specificity was highest with ELISA as 95.2% and the same as 92.9% was with both ERIFA and NERIFA and the lowest was with RBT as 90.5%.

In Table 2, home made ELISAs were compared with commercial ones based on the IgG and IgM detections and home-made ELISA and ERIFA were also evaluated based on the Ig A, M, G mix with RBT, Brucellacapt and SAT confirmed sera. When two ELISAs were compared based on the IgG sensitivity, there was no difference.

| Brucellacapt-SAT | RBT | ELISA<sub>ns</sub> | ERIFA<sub>ns</sub> | NERIFA |
|------------------|-----|-------------------|-------------------|--------|
| Positive (n=94)  | +   | +                 | +                 | +      |
| Negative (n=42)  | -   | -                 | -                 | -      |
| +ve              | 78  | 91                | 90                | 89     |
| -ve              | 16  | 3                 | 4                 | 5      |
| Sensitivity (%)  | 83  | 96.8              | 95.7              | 94.7   |
| Specificity (%)  | 90.5| 95.2              | 92.9              | 92.9   |
(p<0.05), but the difference was important (p>0.05) when IgM was accepted criteria for comparison. ERIFA and home-made ELISA were found concordant with each other according to sensitivity and specificity values for mix antibody detection.

4. Discussion

The diagnosis of brucellosis requires several approaches which are not sufficient for accurate diagnosis\cite{12,14,29}. Culture is a gold standard method, which is necessary for typing of isolates and it is not preferred in routine applications due to the long incubation period, infectiousness and low sensitivity\cite{1,6,30}. Numerous commercial and home-made tests have been developed to identify brucellosis patients. Although, agglutination, complement fixation, globulin test, Brucellacapt, ELISA have been common\cite{4,14,23,24,31} recently, immunochromatographic\cite{20} and immunofiltration tests\cite{21,22} are used as ancillary rapid and accurate tests.

Serological detection of Brucellosis in humans involves serious problems. The duration of the antibodies in patients treated after illness is long and it is difficult to distinguish the previously suffered infection. Although consensus is available with most of the tests, some concerns are also available with dilutions of agglutination tests for determination of cut-off value\cite{1,17}. Manteco et al.\cite{17} reported that the most sensitive and specific results were obtained with SAT, Coombs and Brucellacapt in serum

| RBT/ B. capt /SAT | home-made | ELISA | Comm. ELISA | ERIFA |
|-------------------|-----------|-------|-------------|-------|
| Positive (n=73)   | IgA 36    | IgM 23 | IgM 40      | Mix 68 |
| + ve              | IgG 58    | mix 70 | IgG 60      |       |
| Negative(n=37)    | 34        | 31    | 29          | 32    |
| - ve              | 34        |       | 28          |       |

Representative presentations of ERIFA (Fig 1) and NERIFA (Fig 2) are shown below in respect to this study.

![ERIFA results](image1)

**Figure 1.** Representative presentation of ERIFA results.

![NERIFA results](image2)

**Figure 2.** Representative presentation of NERIFA results.
dilution of 1:40, but also stated that accepting this value as threshold value could be a problem in interpreting Brucella serology in endemic regions. This dilution provides more than 95% specificity with these 3 tests, but it may lead to misdiagnosis of low-titred healthy individuals against Brucella. Nevertheless, some authors suggest titrations on SAT and Coombs in endemic areas above 1:320 (1), but titrations are often applied in 1:160 for SAT and 1:320 for Coombs. In these studies, all positive sera in the SAT were found positive in Coombs and Brucellacapt test, but 4% in the negative samples were found positive at 1:80 and lower dilutions with the Brucellacapt test. In addition, at the beginning of the acute phase of the disease, Brucellacapt titers may be high in chronic or long-term disease states, while SAT titres may be low\[4\].

Because of some drawbacks of agglutination tests, co-administration of 2 assays such as brucellacapt and ELISA for IgG and IgM is recommended\[32,33\]. Some studies detects IgM specificity as 100% by ELISA for the detection of acute brucellosis. However, it was shown that IgM positivity was detected in non-infected individual by different serological methods. To increase the specificity of the test, sera should be preabsorbed with cross-reacting agent prevailing in the region\[34\]. It allows detection of antibodies at various periods of disease. Tests such as Coombs, Brucellacapt and ELISA IgG may be positive during the acute course of the infection, however, in chronic, mixed and focal conditions, SAT can be negative\[29\]. IgGs that are present in the body for longer periods after treatment are difficult to distinguish from reinfection, recurrence or pre-exposed infection. A standard threshold can not be reported since there is no standard to determine it precisely. Therefore, it was aimed to determine immunoglobulin isotypes with higher sensitivity. On the other hand, the use of specific tests that detect IgM alone for the diagnosis of brucellosis in humans may lead to misdiagnosis\[34\]. This is particularly important in the endemic areas where a significant number of people have had Brucella infection. So, tests that will detect all immunoglobulin isotypes and concentrations correctly are required.

With this study, it was planned to study diagnostic validity of rapid immunofiltration-based tests in human brucellosis detection based on different immunoglobulin subtypes either with conjugate combination or protein G preference. Different tests and methods were evaluated and compared for these rapid tests. As a guidance of SAT, a titer of \(\geq 1:80\) is accepted as positive, in the study this titer of SAT was compared with the titer of 1:160 of Brucellacapt to increase the sensitivity of the test. Other tests used in the study were evaluated based on optimal titers. It was shown in this study that sensitivity was not affected with the dilution. Besides, higher sensitivity was obtained with ELISA and ERIFA/NERIFA compared to others, respectively.

Regarding IgG, antibodies can not be considered as good serological markers for determining active infection as they may remain at high titers for many years\[11\]. As ELISA has capability of determining IgM and IgG antibody to the surface antigen of B. abortus, it allows better association with the clinical presentation\[13\].

To understand the course of infection based on immunoglobulin isotype, specific tests replaced with IgM ELISA. In the study, anti-Brucella IgG and IgM antibodies to LPS were found concordant with IgG (p<0.05), but the difference was important (p>0.05) when IgM parameter was concerned. Actually, there are numerous tests available, but a few publication on the assesment of ELISA in bacteriologically proven state for human brucellosis diagnosis\[13,15,17,19\]. Therefore, to detect the specificity of the rapid tests more accurately, brucellacapt and SA tests were selected and used together with ELISA.

Renovations in rapid test technology for brucellosis detection is on the development of membrane based field. In this respect, Lateral Flow (LFA)\[36\] and Rapid Immunofiltration tests (RIFAs) have been recently developed for brucellosis diagnosing in different species\[21,22\]. As the diagnostic performance of the RIFAs and ELISA were found to be statistically similar, these tests can be applied as “an individual quick test”. Besides, the ERIFA/RIFA’s advantages over ELISA are high performance, simplicity and quickness and applicability in 2 minutes, no need to particular material and specialization and offering possible naked-eye evaluation (seen in Figure 1 and 2).

In this study, we compared these models with commonly used tests in clinical trials. In the study, it is concluded that SAT or Brucellacapt should be combined with either ELISA\(_{\text{mix}}\) or ERIFA\(_{\text{mix}}\)/NERIFA in place of two agglutination tests. Additionally, there was no difference between ERIFA and NERIFA with regard to sensitivity and specificity (p<0.05).
5. Conclusion

It is concluded that it would be appropriate to select NERIFA based on the colloidal gold conjugate in lieu of enzymatic conjugate in ERIFA. The current study reports the first estimation of these test models in human. Although limited number of serum samples was used, consequently the RIFA models can be accepted as an ancillary test for reliable detection of human brucellosis in developing countries where the disease is prevalent.

6. References

1. Corbel MJ. Brucellosis: an overview. Emerg Infect Dis. 1997; 3:213–21. https://doi.org/10.3201/eid0302.970219 PMid:9204307 PMCid:PMC2627605
2. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsisanos EV. The new global map of human brucellosis. Lancet Infect Dis. 2006; 6:91–9. Available from: http://infection.thelancet.com https://doi.org/10.1016/S1473-3099(06)70382-6
3. Dean AS, Crump L, Greter H, Schelling E, Zingstagg J. Global burden of human brucellosis: A systematic review of disease frequency. PLOS Neglected Tropical Diseases. 2012; 6(10):1865. DOI: 10.1371/journal.pntd.0001865. https://doi.org/10.1371/journal.pntd.0001865
4. Orduna A, Almaraz A, Prado A, Gutierrez MP, Garcia-Pascual A, Duenas A, Cuervo M, Abad R, Hernandez B, Lorenzo B, Bratos MA, Torres AR. Evaluation of an immunocapture-agglutination test (brucellacapt) for serodiagnosis of human brucellosis. J Clin Microbiol. 2000; 11:4000–5.
5. Alton GG, Jones LM, Angus RD, Verger JM. Techniques for the brucellosis laboratory. Paris: Institut National de la Recherche Agronomique; 1998.
6. Yagupsy P. Detection of Brucella in blood cultures. J Clin Microbiol. 1999; 37:3437–42. PMid:10523530 PMCid:PMC85661
7. Maichomo MW, McDermott JJ, Arimi SM, Gathura PB. Assessment of the Rose-Bengal plate test for the diagnosis of human brucellosis in health facilities in Narok district, Kenya. East Afr Med J. 1998; 75:219–22. PMid:9745838
8. Diaz R, Casanova A, Ariza J, Moriyon I. The rose bengal test in human brucellosis: A neglected test for the diagnosis of a neglected disease. Plos Neglected Tropical Diseases. 2011; 5(4):1–7. https://doi.org/10.1371/journal.pntd.0000950 PMid:21526218 PMCid:PMC3079581
9. Hewitt WG, Payne DJ. Estimation of IgG and IgM Brucella antibodies in infected and non-infected persons by a radio-immune technique. J Clin Pathol. 1984; 37:692–6. https://doi.org/10.1136/jcp.37.6.692
10. Parrat D, Nielsen KH, White RG, Payne DJH. Radioimmunoassay of IgM, IgG and IgA Brucella antibodies. Lancet. 1977; 309(8021):1075–8. https://doi.org/10.1016/S0140-6736(77)92334-0
11. Ariza J, Pellicer T, Pallares R, Gudiol F. Specific antibody profile in human Brucellosis. Clin Infect Dis. 1992; 14:131–40. https://doi.org/10.1093/clinids/14.1.131 PMid:1571417
12. Gomez MC, Nieto JA, Rosa C, Geijo P, Escribano MA, Munoz A, Lopez C. Evaluation of seven tests for the diagnosis of human Brucellosis in an endemic area. Clin Vaccine Immunol. 2008; 15:1031–3. https://doi.org/10.1128/CVI.00424-07 PMid:18448622 PMCid:PMC2446629
13. Mantur B, Parande A, Amarnath S, Patil G, Walkevar R, Desai A, Parande M, Shinde R, Chandrashekar M, Patil S. ELISA versus conventional methods of diagnosing endemic Brucellosis. Am J Trop Med Hyg. 2010; 83(2):314–8. https://doi.org/10.4269/ajtmh.2010.09-0790 PMid:20682874 PMCid:PMC2911177
14. Fadeel MA, Hoffmaster AR, Shi J, Pimentel G, Stoddard RA. Comparison of four commercial IgM and IgG ELISA kits for diagnosing Brucellosis. J Med Microbiol. 2011; 60:1767–73. https://doi.org/10.1093/jmm/jmm.0.033381-0 PMid:21835974
15. Genç O, Büyüktenant Ö, Yurdusev N. Development of qualitative and quantitative ELISA models for bovine Brucellosis diagnosis. KAU Vet Fak Derg. 2010; 16(2):287–91.
16. Young EJ. Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination test and review of the literature. Rev Infect Dis. 1991; 13:359–72. https://doi.org/10.1093/clinids/13.3.359 PMid:1866536
17. Mantecon MA, Gutierrez P, Zarzosa MP, Duenas AI, Solera J, Fernandez-Lago L, Vizcaino N, Almaraz A, Bratos MA, Rodriguez Torres A, Orduna-Domingo A. Utility of an immunocapture-agglutination test and an enzyme-linked immunosorbent assay test against cytosolic proteins from Brucella melitensis B115 in the diagnosis and follow-up of human acute brucellosis. Diagn Microbiol Infect Dis. 2006; 55:27–35. https://doi.org/10.1016/j.diagmicrobio.2005.11.003 PMid:16500069
18. Moreno MS, Guinea Esquero L, Carrero Gonzalez P, Visedo Orden R, Garcia Carbajosa S, Calvo T, Reverte Cejudo D. Diagnosis of brucellosis in an endemic area. Evaluation of routine diagnostic tests. Medicina Clinica (Barcelona). 1992; 98(13):481–5.
19. Pellicer T, Ariza J, Foz A, Pallares R, Gudiol F. Specific antibodies detected during relapse of human brucellosis. J Infect Dis. 1988; 157(5):918–24. https://doi.org/10.1093/infdis/157.5.918 PMid:3361155
20. Smits HL, Abdoel TH, Solera J, Clavijo E, Diaz R. Immunochromatographic Brucella-specific immunoglobulin M and G lateral flow assays for rapid serodiagnosis of
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human brucellosis. Clin Diagn Lab Immunol. 2003; 10: 1141–6. https://doi.org/10.1128/CDLI.10.6.1141-1146.2003

21. Genç O, Büyüktanır Ö, Yurdusev N. Development of an individual rapid test based on enzymatic immunofiltration assay for detection of anti-Brucella abortus antibody in bovine sera. J Vet Diagn Invest. 2011; 2:49–56. https://doi.org/10.1177/104063871102300107 PMid:21217027

22. Büyüktanır Ö, Genç O, Çelebi Ö, Yurdusev N. Rapid immunofiltration assay as a field diagnostic tool for ovine brucellosis. J Immun and Immunochem. 2012; 33(1):35–47. https://doi.org/10.1080/15321819.2011.591478 PMid:22181819

23. Gupte S, Kaur T. Diagnosis of human brucellosis. J Trop Dis. 2015; 4(1):1–6.

24. Bosilkovski M, Spasovska K, Sopova Z, Vidinic I. The role of Brucellacapt test for follow-up patients with brucellosis. Comp Immun Microbiol Infect Dis. 2010; 3:345–42. https://doi.org/10.1016/j.cimid.2009.06.001 PMid:19625085

25. Şahin M, Genç O, Ünver A, Otlu S. Investigation of bovine brucellosis in the Northeastern Turkey. Trop Anim Health Prod. 2008; 40:281–6. https://doi.org/10.1007/s11250-007-9092-3 PMid:18557191

26. OIE Terrestrial Manual. Bovine Brucellosis; 2012. p. 630–1.

27. Büyüktanır Ö, Yıldırım T, Yakyıcıer C, Genç O, Yurdusev N. Recombinant PvpA protein-based diagnostic prototype for rapid screening of chicken mycoplasma gallisepticum infections. Vet Microbiol. 2008; 129(1-2):139–49. https://doi.org/10.1016/j.vetmic.2007.11.028 PMid:18248920

28. Genç O, Büyüktanır Ö, Yurdusev N. Rapid immunofiltration assay based on colloidal gold-protein G conjugate as an alternative screening test for bovine and ovine brucellosis. Tropical Animal Health and Production. 2012; 44(2):213–215. https://doi.org/10.1007/s11250-011-0019-7 PMid:22102014

29. Araj GF. Update on laboratory diagnosis of human brucellosis. Int J Antimicrob Agents. 2010; 36:s12–7. https://doi.org/10.1016/j.ijantimicag.2010.06.014 PMid:20692128

30. Geresu MA, Mamo G. Review on diagnostic methods of brucellosis. J Veterinary Sci Techno. 2016; 7:3. DOI: 10.4172/2157-7579.1000323. https://doi.org/10.4172/2157-7579.1000323

31. Araj GF, Lulu AR, Mustafa MY, Khatteeb MI. Evaluation of ELISA in the diagnosis of acute and chronic brucellosis in human beings. J Hyg. 1986; 97:457–69. https://doi.org/10.1017/S00222172400063634 PMid:3794323

32. Peeridogahen H, Golmohammadi MG, Pourfarzi F. Evaluation of ELISA and Brucellacapt tests for diagnosis of human Brucellosis. Iran J Microbiol. 2013; 5(1):14–8. PMid:23467496 PMCId:PMC3577553

33. del Pozo JSG, Ortono SL, Navarro E, Solera J. Detection of IgM antibrucella antibody in the absence of IGGS: A challenge for the clinical interpretation of brucella serology. PLoS Negl Trop Dis. 2014; 8(12):e3390. https://doi.org/10.1371/journal.pntd.0003390 PMid:25474572 PMCId:PMC4256177

34. Arabaci F, Oldacay M. Evaluation of serological diagnostic tests for human Brucellosis in an endemic area. J Microbiol Infect Dis. 2012; 2(2):50–6. https://doi.org/10.5799/ahinjs.02.2012.02.0042

35. Memish ZA, Almuneef M, Mah MW, Qassem LA, Osoba AO. Comparison of the Brucella standard agglutination test with the ELISA IgG and IgM in patients with Brucella bacteremia. Diagn Microbiol Infect Dis. 2002; 44:129–32. https://doi.org/10.1016/S0732-8893(02)00426-1

36. Abdoel T, Dias IT, Cardoso R, Smits HL. Simple and rapid field test for brucellosis in livestock. Vet Microbiol. 2008; 130:312–9. https://doi.org/10.1016/j.vetmic.2008.01.009 PMid:18321664