ELISA versus Conventional Methods of Diagnosing Endemic Brucellosis

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Abstract. The diagnostic value of enzyme-linked immunosorbent assay (ELISA) was evaluated when blood specimens of 92 patients suspected of brucellosis underwent the ELISA (IgM and IgG), standard tube agglutination (SAT), and 2-mercaptoethanol (2-ME) tests and blood cultures; 38 sera from non-brucellosis patients and 34 sera from blood donors were also subjected to ELISA, SAT, and 2-ME tests. SAT was able to pinpoint only 23 (25%), whereas ELISA confirmed the etiology in 56 (60.9%; $P < 0.001$) patients with brucellosis, including 31 culture-confirmed cases. The sensitivity and specificity of ELISA were 100% and 71.31%, respectively. Because they were confirmed by ELISA, the diagnosis could never be excluded with SAT in 33 cases. ELISA has been found to be more sensitive in acute (28% higher sensitivity; $P < 0.02$) and chronic (55% higher sensitivity; $P < 0.01$) cases. For accurate diagnosis in suspected brucellosis cases detection, we recommend both ELISA IgM and IgG tests. ELISA IgG and 2-ME tests seem to be promising tools in judging prognosis.

INTRODUCTION

Brucellosis is a widespread zoonosis that infects several domestic and wild animals. *Brucella melitensis*, *B. abortus*, *B. suis*, and *B. canis* are considered to be potentially pathogenic to humans. Newly recognized species such as *B. pinnipediae* and *B. cetaceae* have also been reported to cause disease in humans,^{1,2} although little is known of their epidemiology.

Transmission to humans occurs by ingestion of infected raw milk and milk products or by exposure to infected animals.^{3,4} Although brucellosis in animals has been the target of aggressive eradication programs in several countries, the morbidity associated with brucellosis in humans still remains considerably significant with severe economic consequences. Human brucellosis is a protean disease, but most bacteremic patients have the typical presentations of recurrent fever, lassitude, joint pain, and sweating at night. There may be few, if any, constitutional manifestations in the localized form of the disease that have been described in almost every organ and system. The diagnosis is consequently more difficult on clinical grounds alone. Thus, the diagnosis of human brucellosis is invariably based on microbiological and serological laboratory tests.

Blood culture, the recognized gold standard, is an integral test for laboratory confirmation of brucellosis. In most developing countries where brucellosis is endemic, culture facilities are not available, and culture is a time-consuming procedure requiring prolonged periods of incubation. In addition, failure to detect the pathogen is a frequent occurrence, and *Brucella* spp. are class III pathogens posing considerable risk to laboratory personnel. Extensive efforts have been made on the development of molecular diagnostic assays based on amplification of different genomic targets by the polymerase chain reaction (PCR) for the diagnosis of human brucellosis. A recent publication⁵ has indicated the persistence of DNA in the serum, even after completion of standard treatment regimen, ranging

from weeks to months, thus failing to establish these molecular assays as routine diagnostic methods as yet. Hence, the laboratory diagnosis is often serologically directed to detection of *Brucella*-specific antibodies. IgM predominates for the first week of the illness, after which the IgG levels begin to rise, reach a peak after a few weeks, and predominate over the IgM level until adequate therapy eliminates the infection.^{6,7} From among a variety of serological techniques, the Rose Bengal plate agglutination test (RBPT), standard tube agglutination test (SAT), and Coombs tests have been used worldwide for more than a century as aids in the diagnosis of human brucellosis. SAT is the recognized test for obtaining quantitative information about the immune response to brucellar antigens. Despite being the standard method, it is time-intensive and hence, unsuitable as a primary test for laboratories with large specimen workloads. Another drawback is that diagnosis of brucellosis cannot be established on the antibody titer by these classic tests alone, because healthy people engaged with animal husbandry practices in endemic areas may show significant titers of *Brucella* antibodies.^{8,9} Another matter of concern is also the cross-reactions that occur between *Brucellae* and other bacteria. Additionally, SAT is unable to distinguish active cases from chronic ones.¹⁰ Nowadays, the Coombs test is rarely performed in routine clinical laboratories, because the procedure is too complex, time consuming, and labor intensive, necessitating skilled personnel. Enzyme-linked immunosorbent assay (ELISA) is capable of readily identifying individual IgM and IgG antibody to the surface antigen of *B. abortus*, allowing for a better correlation with the clinical situation. Previously, for the diagnosis of human brucellosis, measuring the humoral immune response by ELISA technique was shown to be important. However, very few comparative publications are available on the evaluation of ELISA in bacteriologically proven cases and the standard serological tests for brucellosis.^{11,12} Therefore, the focal points of this work were to evaluate the diagnostic accuracy of ELISA with culture and classical serological methods [SAT and 2-mercaptoethanol (ME) test], which are currently accepted tests for microbiological diagnosis of brucellosis, as well as to determine the utility of ELISA in an area where brucellosis is endemic.

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MATERIALS AND METHODS

Ninety-two consecutive patients reporting to Belgaum Institute of Medical Sciences (BIMS) Hospital, Belgaum, Karnataka, India (between October 2007 and December 2008) with a presumptive diagnosis of brucellosis based on compatible clinical and epidemiological features were selected. Clinical presentations like prolonged fever, joint pain, sweats, anorexia, fatigue, splenomegaly, hepatomegaly, and enlargement of lymph nodes in various combinations were taken into account. Epidemiological indications such as belonging to high-risk groups (farm laborers, farmers, shepherds, butchers, abattoirs, and veterinarians), exposure to animals, and consumptions of high-risk foods in various combinations were also considered. Information obtained from each patient was inclusive of demographic data, symptoms, duration of illness from the onset of symptoms, treatment history, and epidemiological profile like occupational history, history of ingestion of raw milk, and contact with domestic animals. The outcomes of clinical examination were also duly noted and analyzed. According to the duration of symptoms, the patients were classified into three groups: (1) acute group who had the presentation for less than 8 weeks ($N = 57$), (2) subacute group who had the symptoms for more than 8 weeks but less than 52 weeks ($N = 6$), and (3) chronic group who had an evolution period previous to the onset of the illness for more than 52 weeks ($N = 29$). The control group consisted of a total of 72 blood specimens from BIMS, Belgaum, Karnataka, India where brucellosis is prevalent. Of the 72 specimens, sera were obtained from patients diagnosed with illnesses other than brucellosis ($N = 38$), including enteric fever/typhoid fever ($N = 14$), malaria ($N = 9$), pyrexia of unknown origin ($N = 7$), rheumatoid arthritis ($N = 4$), pulmonary tuberculosis ($N = 2$), viral hepatitis ($N = 1$), toxoplasmosis ($N = 1$), and from healthy blood donors ($N = 34$).

For serology, clotted blood samples were centrifuged at $3000 \times g$ for 10 minutes, and then, the serum was divided into aliquots and stored at -20°C until required for testing. SAT, 2-ME test, and IgM and IgG ELISA were performed on serum specimens of all 92 brucellosis-suspected patients and all 72 blood specimens belonging to control groups. SAT and 2-ME tests are tube-agglutination tests. The SAT measures the total amount of agglutinating IgM and IgG antibodies. The 2-ME destroys IgM antibodies. Any agglutination that occurs in 2-ME test, therefore, is caused by IgG antibodies that are resistant to the action of 2-ME. *B. abortus* plain antigen for SAT and 2-ME test was supplied by the Indian Veterinary Research Institute (IVRI), Izatnagar, India. In the SAT, the patient serum was diluted in 0.5% phenol saline. Equal volumes of serial dilutions of the serum (from 1:10 to 1:1,280) and *B. abortus* plain antigen were mixed in test tubes and incubated in an incubator at 37°C for 24 hours. Known negative and positive control sera were used. A set of antigen-control tubes simulating no agglutination, 25%, 50%, 75%, and 100% agglutination using different quantities of antigen and 0.5% phenol saline according to the manufacturer's instructions to compare the results of the test samples were used. A positive end point was defined as the highest dilution of serum yielding $\geq 50\%$ agglutination. The 2-ME test was done with the same antigen, except that 2-ME phosphate-buffered saline solution was added instead of phenol saline to each tube to a final concentration of 0.05 M.¹³ A titer $\geq 1:160$ and $\geq 1:80$ was considered diagnostic for SAT and 2-ME tests, respectively, together

with compatible clinical and epidemiological characteristics mentioned above.

ELISA testing was carried out employing reagents supplied by NovaTec Immundiagnostica GmbH, Dietzenbach, Germany. Separate microtiter plates for IgM and IgG were used. The principle of IgM and IgG ELISA was that microtiter strip wells were precoated with *Brucella* antigens to bind corresponding antibodies of the patient specimen. After washing the wells to remove all unbound sample material, horseradish peroxidase-labeled anti-human specific (IgM or IgG) immunoglobulin conjugate was added. This conjugate binds to the captured *Brucella*-specific antibodies. The immune complex formed by the bound conjugate was visualized by adding tetramethylbenzidine substrate, which gives a blue reaction product. The 0.2 M sulphuric acid solution was added to stop the reaction. This resulted in a yellow end-point color. Absorbance at 450/620 nm was measured in an ELISA microwell plate reader. Controls and patient samples were determined in duplicate. The absorbance values found were converted into NovaTec Units (NTU) using the following formula as specified by the manufacturer: patient sample (mean) absorbance value $\times 10$ /Mean absorbance value of cut off controls = NTU (> 11 NTU was considered positive as specified by the manufacturer). Automatic washing and absorption readings were done with Aspen diagnostics LISA washer and Aspen diagnostics LISA plus, respectively.

Paired sera available from 23 suspected brucellosis patients were subjected to SAT, 2-ME, and ELISA tests. Multiple sera were reevaluated during follow-up at different time intervals after diagnosis and start for treatment of SAT, 2-ME, and ELISA tests in the 11 suspected brucellosis group. The follow-up period ranged from 14 to 180 days with a median follow-up of 88 days. These patients were also followed-up clinically for resolution of symptoms and signs.

For blood cultures, 20 mL of venous blood from each patient of the suspected brucellosis group was collected (at one time), and four cultures were done by the classic method of adding 5 mL aseptically into the broth phase of each Castaneda's biphasic medium consisting of brain heart infusion agar and broth (Hi Media, Mumbai, India); 2–10 mL venous blood was collected from pediatric patients and inoculated as mentioned above. The media were incubated at 37°C in an incubator for 30 days and examined for growth one time per day, allowing the broth-blood mixtures to flow over the agar phase every day. The blood cultures were performed in 11 non-brucellosis cases that were positive for *Brucella* IgM ELISA ($>$ diagnostic levels).

The serologic and culture specimens of each patient were processed simultaneously. Identification of *Brucella* strains was carried out using standard classification tests, including growth characteristics, Gram staining, a modified Ziehl-Neelsen stain, oxidase activity, urease activity, H_2S production (4 days), dye tolerance such as basic fuchsin (1:50,000 and 1:100,000) and thionin (1:25,000, 1:50,000, and 1:100,000), and seroagglutination.⁷ Seroagglutination was performed using *B. abortus* and *B. melitensis* monospecific antisera (Murex Biotech Ltd., Dartford, England). Clinical isolates were sent to IVRI, Izatnagar, India for confirmatory identification.

A case of brucellosis was defined as having either blood culture or SAT positive individually or in combination. This was used for calculating sensitivity and specificity of the ELISA test.

Statistical significance was determined by χ^2 tests with Yates correction. The P values less than 0.05 were considered to indicate

statistical significance. Statistical tests were done using Epi-Info version 6.02 software (Centers for Disease Control and Prevention and World Health Organization, Geneva, Switzerland).

RESULTS

We studied a total of 92 patients having presumptive diagnosis of brucellosis. The patients were between 1 and 84 years of age, with the mean age of the patients being 27.46 years with a standard deviation of ± 18.74 years. Seventy-one (77.2%) patients were of the adult age group (> 14 years) and 21 (22.8%) were children, giving an adult to child ratio of 3:1. Sixty-nine (75%) were males (mean age = 26.78 ± 17.86 years), and 23 (25%) were females (mean age = 29.48 ± 17.42 years); the male to female ratio was 3:1. Analysis of cases by age and sex showed that brucellosis principally affected working-age adolescent and adult males between the ages of 15 and 84 years and accounted for 54 (58.6%) of the 92 cases. There was no seasonal variation in the cases studied.

The noticeable symptoms were with patients having fever, joint pain, low backache, headache, and vomiting; fever was the principal presentation. A combination of fever reaching 40°C and arthritis affecting the larger joints was very common. The most consistent findings on physical examination were hepatosplenomegaly (23 patients), followed by splenomegaly alone (8 patients), only hepatomegaly (5 patients), and lymphadenopathy (3 patients). Farmers/farm laborers (45 patients) and shepherds (44 patients) were the major occupational groups seen affected in the present series. Consumption of unprocessed milk and direct contact with domestic animals were recognized as major risk factors for transmission of brucellosis in our study.

Diagnostic yields of culture, SAT, and ELISA are depicted in Table 1. The diagnosis of brucellosis was confirmed in 56 (60.9%) of 92 provisionally diagnosed brucellosis patients. Blood culture identified only 31 (33.6%) cases with the isolation of *B. melitensis* biotype 1, thereby leading to 31 bacteriologically proven and 61 bacteriologically non-proven cases. SAT was positive in 23 (25%) cases, and 2-ME was positive in 21 (22.8%) cases only, whereas ELISA IgM and IgG together were found positive in 56 (60.9%) cases. The sensitivity and specificity for ELISA were found to be 100% and 71.31%, respectively. A statistically significant difference was noticed in the performance of ELISA over traditional agglutination tests in the diagnosis of brucellosis ($P < 0.001$, χ^2 Yates corrected = 13.78) (Table 1). Acute patients showed IgM ELISA NTUs in a range of 24.83–139.01 (mean = 63.66 NTUs), whereas IgG ELISA NTUs were found in a range between 11.44 and 35.04 (mean = 19.8 NTUs). Subacute cases recorded IgM ELISA NTUs in a range of 21.94–123.36 (mean = 52.61 NTUs), whereas IgG ELISA NTUs were between 11.73 and 21.86 (mean = 16.2

NTUs). Chronic illness patients documented ELISA IgM NTUs in a range of 13.18–48.2 (mean = 24.81 NTUs), whereas IgG ELISA NTUs were seen in a range of 27.26–124.7 (mean = 61.52 NTUs). A noteworthy point was the detection of all 56 (100%) cases by a single method, namely a combination of IgM and IgG ELISA. Only IgG was not detected by ELISA in any of the blood specimens tested. Only IgM (NTU range = 11.39–16.15; mean = 13.79) detected by ELISA was seen in 11 suspected cases. However, SAT recorded completely negative in 18 cases and insignificant titers (range = 1:20–1:40) in 19 blood specimens, and the 2-ME test was found completely negative in 37 cases. Table 2 summarizes the results of the performance of SAT and ELISA at various phases of illness. ELISA was found to be more sensitive than SAT in detecting brucellosis in acute ($P < 0.02$, χ^2 Yates corrected = 6.4) (Table 2) as well as chronic forms ($P < 0.01$, χ^2 Yates corrected = 8) (Table 2). Of 57 acute cases, ELISA detected 28 (49.1%), whereas SAT identified only 12 (21%) cases. Among 29 chronic brucellosis patients, only 8 (27.5%) were detected by SAT, whereas ELISA could identify 24 (82.7%).

Analysis of 23 cases for which paired sera were available showed six cases where both samples were negative and four cases where both samples were positive by all three serological tests. Blood cultures were positive in two of four cases positive for serology. In the other 13 cases, the initial samples showed insignificant titers in SAT alone but were negative by 2-ME and ELISA tests. In 9 of 13 cases, second samples showed appearance of IgM and IgG at diagnostic levels as determined by ELISA, whereas findings of SAT and 2-ME tests remain unchanged. Blood culture yielded *B. melitensis* in all of these nine cases. The remaining four cases showed rising SAT titers (to $\geq 1:160$) and the appearance of 2-ME antibodies and diagnostic levels of IgM and IgG ELISA in the second samples along with blood culture being positive in two cases.

Zero of eleven patients reevaluated during follow-up experienced relapse after the completion of treatment. On clinical examination, none had a palpable spleen or liver. In all these, SAT titers and IgM ELISA NTUs remained measurable at diagnostic levels, despite elimination of illness. However, steady fall was noticed in 2-ME antibodies and ELISA IgG NTUs in all, and all had negative 2-ME and IgG ELISA tests in the last follow-up.

Of 38 non-brucellosis patients, 21 patients were found positive for *Brucella* antibodies. Of the 21, SAT (1:20–1:80) alone was positive in 10 cases, whereas 5 cases recorded IgM-ELISA alone (> 11 NTU). The remaining six cases were positive for SAT titers (1:20–1:80) and IgM-ELISA (> 11 NTU) along with two cases being positive for 2-ME antibodies (1:20–1:40). Two specimens yielded *Escherichia coli* on 11 blood cultures. In a total of six blood specimens positive for antibodies from blood donors, five samples showed SAT titers (1:20–1:80) along with

TABLE 1

Results of culture, SAT, and ELISA performed on 92 patients with clinical suspicion of brucellosis

Group	SAT N (%)		ELISA (both IgM and IgG) N (%)	
	+ve	–ve	+ve	–ve
Culture positive (N = 31)	12 (38.7)	19 (61.3)	31 (100)	0 (0)
Culture negative (N = 61)	11 (18)	50 (82)	25 (41)	36 (59)
Total (N = 92)	23 (25)	69 (75)	56 (60.9)	36 (39.1)

χ^2 value = 13.78; $P < 0.001$.

TABLE 2

Diagnostic yield by two different serological assays according to evolution of illness

Group	No. of patients	Positive by SAT N (%)	Positive by ELISA (both IgM and IgG) N (%)
Acute (< 8 weeks)	57	12 (21)	28 (49.1)
Subacute (8–52 weeks)	6	3 (50)	4 (66.6)
Chronic (> 52 weeks)	29	8 (27.5)	24 (82.7)

χ^2 values: (a) acute = 6.4, $P < 0.02$; (b) subacute = 0.14, $P > 0.5$ (not significant); (c) chronic = 8, $P < 0.01$.

one specimen being positive for ELISA IgM (> 11 NTU). The remaining one blood sample was positive for ELISA IgM only (> 11 NTU). All six blood samples were negative for 2-ME antibodies.

DISCUSSION

The noticeable finding of the study was SAT negative or insignificant titers in a considerable number of patients ($N = 33$) on whom the diagnosis was confirmed with a positive blood culture and/or positive ELISA IgM and IgG. This implies a serious limitation of conventional agglutination tests for disease diagnosis, because prompt therapy is very essential for successful treatment. We would have missed these cases if conventional tests alone had been performed. This would have led to underdiagnosis and underreporting. This fact underscores the importance of ELISA in the diagnosis of brucellosis in endemic countries. Our data support the results of several previous studies,^{11,12,14,15} because those reports also showed the occurrence of a falsely low or equivocal SAT titer in patients with brucellosis and consequently, the diagnosis was made with the detection of antibodies by ELISA. Gad and Kambal¹¹ reported false-negative agglutination results in 10% of culture-positive cases that were all found interestingly positive by ELISA. Ariza and others¹⁴ did comparative evaluation of SAT and ELISA on 761 sera collected from a total of 75 brucellosis cases and concluded that ELISA was a more sensitive and specific technique as opposed to standard serological tests for diagnosing brucellosis.

We pinpointed a significant number of cases by ELISA—a finding that has a relevance to the laboratory diagnostic tool. In all 56 brucellosis-confirmed cases, both raised IgM and IgG detected by ELISA were noted. This serological picture is very similar to that reported by researchers elsewhere in the world.^{11,15–17} Gad and Kambal¹¹ also reported ELISA IgM and IgG positive in 17 brucellosis patients, in addition to recording only IgM antibodies in 10 (7.4%) patients. Gazapo and others¹⁵ noted the presence of both IgM and IgG antibody levels in a greater than normal range in 6 of 10 occupationally exposed patients and 21 of 36 patients belonging to the non-high-risk group, although they had also reported only IgM and IgG antibodies in a few patients of both groups. Reddin and others¹⁶ found that patients with acute disease had elevated IgM and IgG levels. Sippel and others¹⁷ recorded elevated IgM and IgG levels in their large group of acute patients studied. IgM levels were recorded three times more than the IgG in all of our acutely infected patients. Subacute patients also showed similar antibody patterns to that of acute cases; however, because the number is small, further study would be required for valid conclusions. All these cases had already lapsed in the duration of illness ranging between 2 weeks and 4 months when they presented to the hospital. Of 28 acute patients picked up by ELISA, 6 had duration of illness between 2 weeks and 1 month, whereas the remaining 22 had more than 1 month but less than 2 months. Conversely, IgG predominated over IgM in all chronically infected patients. It is known that both IgM and IgG antibodies appear promptly after brucellar infection, and their concentration rises during the following days. This could be the reason for our cases showing both antibodies, irrespective of stage of illness. Our data also clearly indicate that it is the combination of IgG and IgM levels that allows a proper serological diagnosis at any stage of the illness. However, our

data disagree with those of Magee,¹⁸ who could find elevations of IgM antibody only in the acutely infected patients presented in the study. Our findings are also in disagreement with the results shown in a recent publication by Osoba and others.¹² The authors reported increased IgM titer in 80% of bacteremic patients, whereas many had elevated IgM alone.¹² In a few patients, IgG titer alone was determined positive from among a total of 53 non-bacteremic patients studied.¹² We believe that our 11 cases with ELISA IgM alone were not true cases, because the diagnosis was excluded based on negative blood culture and negative SAT and 2-ME tests. ELISA may give us erroneous interpretation on the specificity, because it is a highly sensitive technique. Probably, this could be caused by cross-reacting antibodies, because similar findings were also noted in 11 patients from diseases other than brucellosis. We would like to postulate that such cases should be disregarded in an endemic country. However, such low positive IgM titers require further elucidation with repeat IgM and IgG tests and need to be intensively investigated for culture; additionally, standard serologic tests need to be repeated after a minimum of 2 weeks to get a meaningful diagnostic recommendation.

The ELISA test was potentially useful in establishing the etiology in 13 initially sero-negative cases when paired serum specimens were reevaluated. It is very interesting to note that conventional tests proved negative, despite the isolation of *B. melitensis* in all nine patients. However, ELISA recorded high NTUs of IgM and IgG antibodies, indicating the value of ELISA in confirmation of the diagnosis. Also, no single titer is diagnostic, and paired sera must be sought to show rise in the antibody levels or appearance of detectable level of antibodies to confirm the etiological agent. In some studies^{7,19} where clinical evidence suggested brucellosis, even values of SAT < 1:160 did not rule out the diagnosis. Four cases with non-diagnostic SAT titers and negative 2-ME and ELISA tests revealed rising SAT titers and positive 2-ME and ELISA tests in paired sera, along with positive blood cultures in two cases. The data clearly indicated that SAT titers of < 1:160 and negative 2-ME and ELISA tests cannot always be disregarded without follow-up, especially if high clinical suspicion is entertained in endemic areas of the world. This also gives us reason to propose use of a combination of IgM and IgG ELISA for the accurate serological confirmation of human brucellosis, an area that needs further clinical research.

The present study has shown persistence of various levels of SAT antibodies and IgM ELISA antibodies in 11 clinically cured patients. This emphasizes the overdiagnosis and diagnostic challenges faced in an area where typhoid, malaria, tuberculosis, and rheumatoid arthritis clinically simulate brucellosis, thereby exposing/denying patients access to specific management. However, our study highlighted the importance of 2-ME and ELISA IgG tests in follow-up of brucellosis cases in *Brucella*-endemic countries. Although the figures herein presented are not large enough to reach statistical significance, adoption of 2-ME and ELISA IgG will prove better in assessing the favorable response to treatment, thereby avoiding unnecessary prolongation of therapy. We have shown similar findings on the performance of 2-ME tests in a previous paper.⁷ Effective clinical cure with anti-brucellar agents could reduce IgG faster than IgM, as shown by ELISA,^{14,15} and Gazapo and others¹⁵ claimed ELISA to provide a very useful tool for follow-up of brucellosis. However, we recommend the use of the 2-ME test rather than IgG ELISA to assess the prognosis, because the former is inexpensive without requiring

specialized equipment and technologically simple to use. This can be readily applied in the developing world, especially to those cases where brucellosis diagnosis has been confirmed by SAT and 2-ME test findings. However, further work should be done to establish the clinical efficacy of these tests. The strong agreement between combination of IgM and IgG ELISA antibody detection and blood culture when applied to *Brucella*-infected blood samples suggested that the former method may be an alternative to cultural isolation for the diagnosis of brucellosis. The ELISA test also has the advantage of being quantitative with assay-to-assay reproducibility and is capable of being automated if samples are large in number.

In conclusion, the ELISA test has offered a significant diagnostic advantage over conventional agglutination methods in the diagnosis of brucellosis in an area where brucellosis is endemic. Applying a combination of IgM and IgG ELISA testing could be of value for the definitive diagnosis of brucellosis in developing countries, where diagnostic capabilities for culture, including automated culture systems and PCR, are poor. The results of our study clearly indicate that both the 2-ME and ELISA IgG tests are valuable tools in the follow-up of cases to monitor and justify the prolonged therapy of patients.

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