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The evaluation of a user-friendly lateral flow assay for the serodiagnosis of human brucellosis in Kazakhstan

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Abstract

Serum samples from all patients with culture-confirmed brucellosis including those with chronic disease from Kazakhstan tested positive in the serum agglutination test for titers ≥1:25 and reacted in the *Brucella* immunoglobulin M/immunoglobulin G lateral flow assay (LFA) confirming the high sensitivity of these assays. The strong reactivity in the LFA observed for the majority (92.1%) of the samples from the patients with culture-confirmed brucellosis together with the user-friendliness of the assay procedure makes the LFA ideal for the confirmation of brucellosis in endemic areas in Kazakhstan. The Rose Bengal test lacked sensitivity in particular for patients with chronic brucellosis therefore limiting its value as a quick screening assay. The study emphasizes the importance of the LFA as a useful, rapid, and easy-to-perform tool in the diagnostic testing of brucellosis.
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1. Introduction

Brucellosis is a zoonosis of worldwide importance that is caused by small gram-negative coccobacilli belonging to the genus *Brucella* (Young, 1995a). Human brucellosis is a systemic infection with protean acute, subacute, or persistent clinical manifestations (Young, 1991). Characteristically, patients with brucellosis present with nonspecific signs and symptoms such as fever, sweats, fatigue, and joint pain. Abnormal physical findings are few with the exception of hepatosplenomegaly. The infection may become localized affecting any organ system, and on rare occasions, localized disease becomes chronic with periodic relapses of symptoms. Because no characteristic constellation of symptoms and signs exists, the diagnosis may be readily missed; laboratory testing by culture or serology is essential to confirm the disease (Young, 1995b). Brucellosis is transmitted to man mainly by direct contact with infected livestock and the consumption of nonpasteurized contaminated milk and dairy products. Cattle, goats, sheep, camels, and other livestock may be infected and transmit the disease to the human population. Three species, *Brucella melitensis*, *Brucella abortus*, and *Brucella suis*, cause the majority of the infections in humans. Brucellosis is treated with a combination of doxycycline with either rifampicin or streptomycin for a minimum of 6 weeks (Ariza et al., 2007; Skalsky et al., 2008).

Brucellosis is a major veterinarian, clinical, and public health problem in Kazakhstan. Much of Kazakhstan is semiarid rangeland unsuitable for agriculture and, traditionally, Kazakhs are nomadic livestock producers. During the Soviet time, most of the farming activities were regulated and concentrated within collective and state farms (Robinson and Milner-Gulland, 2003). After independence in 1991, farms became privatized, but because of the economic decline, existing vaccination and disease surveillance programs rapidly disintegrated. Presently, although vaccines are available, the high costs prohibit their use. In a study performed in 1997–1998 among 17 ex-collective farms, all

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but 2 located in Central Kazakhstan revealed serological evidence of brucellosis in cattle, sheep, or goat at 9 farms (Lundervold et al., 2004). The detection of seropositive animals was expected as villagers were aware that brucellosis was endemic and, in the 6 villages where these farms were located, brucellosis had already been diagnosed in humans. In Kazakhstan, most human cases of brucellosis are caused by infection with *B. melitensis*. However, as highest seroprevalence rates were observed in cattle, *B. abortus* seems to be present as well. Brucellosis also is common in neighboring countries such as Tajikistan and Kyrgyzstan where major risk factors include exposure to aborted home-owned animals and consumption of home-made milk products obtained from bazaars and neighbors (Jackson et al., 2007; Kozukeev et al., 2006).

In Kazakhstan, suspected cases of human brucellosis are confirmed by laboratory testing using Rose Bengal (RB) test, the Huddleson test, and the Wright serum agglutination (SAT) test. Testing is done at a regional laboratory, and for confirmation, a sample is sent to the Republican Sanitary and Epidemiological Station (RSES) in Almaty. At the RSES, the same set of tests is performed and recently blood culture became available. In this study, we investigated the potential use of a simple and rapid field test, the *Brucella* immunoglobulin M (IgM) and immunoglobulin G (IgG) lateral flow assays (LFAs), for the diagnosis of human brucellosis in Kazakhstan (Franco et al., 2007). In brucellosis, specific immunoglobulin M (IgM) antibodies develop during the acute phase of the disease, while *Brucella*-specific IgG antibodies predominate in the serum of patients with a more persistent infection and in relapsing patients (Ariza et al., 1992). The *Brucella* IgM/IgG LFA allows the separate detection of specific IgM and IgG antibodies and therefore may aid to distinguish between patient with acute disease and patients with more persistent infection. In an earlier study, we found that the sensitivity of the *Brucella* IgM/IgG LFA is 96% and that the specificity amounts to 99% (Irmak et al., 2004).

2. Materials and Methods

2.1 Samples and patient data

Samples were selected from the serum bank of the RSES and were provided without personal identifiers together with clinical and demographic information. A total of 471 single serum samples from 471 patients were selected and tested in the *Brucella* IgM/IgG LFA. The samples had been submitted to the RSES for laboratory confirmation of brucellosis because of suspicion of active brucellosis. For a subset of 76 patients, blood culture was performed and the culture results together with the serology data for all samples were retrieved from the data base of the RSES. Samples originated from hospitals in 5 regions. These regions are East Kazakhstan (64 samples), Kyzylorda (48 samples), South Kazakhstan (188 samples), Almaty (57 samples), and Zhambyl (114

samples). The mean age of the patients was 33 years (range, 13–83 years), and the male-to-female ratio was 2.16. Female patients (mean age, 38; range 18–75) were slightly older than male patients (mean age, 31; range 13–83). The majority ($n = 396$) of the patients suffered from acute disease (i.e., <6 months of illness), 30 were subacute (6–12 months of illness), and 43 were chronic (> 1 year of illness). The phase of disease was not recorded for 1 patient.

2.2. Serology

The RB and the SAT were performed according to routine laboratory procedures, and protocols were taken from the World Animal Health Organization (OIE) guidelines for the laboratory diagnosis of brucellosis. The Huddleson test is a modification of the RB (Černyševa et al., 1977). The RB is performed by dispending 30-μL drops of a 2-fold serial dilution of the serum sample onto a ceramic tile. Thirty microliters of the RB-stained *Brucella* antigen is then spotted alongside each serum drop, and the serum and the antigen are mixed using a disposable spreader. The tile is then gently rocked for 4 min after which the result is read. In the Huddleson test, *Brucella* antigen stained with gentian violet and brilliant green is used, the agglutination reaction is performed on a glass plate, agglutination is enhanced by heating the glass plate over a flame, and the result is read after 8 minutes.

The *Brucella* IgM/IgG LFA consists of 2 plastic cassettes, one for the detection of specific IgM antibodies and the other for the detection of specific IgG antibodies (Smits et al., 2003). Each of the assays is performed by spotting 5-μL serum into the sample port of the assay device followed by the addition of 130 μL running buffer supplied with the test. Test results are read after 10–15 min by visual inspection for a stained *Brucella* antigen line in the viewing port of the assay device. The test was scored negative when staining of the *Brucella* antigen line was not observed. Positive test results were subjectively rated 1+ when staining of the test line was weak, 2+ when moderately strong, 3+ when strong, and 4+ when very strong. The assay result is considered valid when a control line develops in the viewing port and invalid when the control line did not develop. The assay devices were obtained from Organon Teknika Ltd, Dublin, Ireland. The LFAs were performed at the RSES.

2.3. Blood culture

A 5-mL aliquot of venous blood was incubated at 37 °C in Castañeda biphasic medium consisting of brain heart infusion agar and broth, and the broth–blood mixtures were tilted over the agar phase each day for 40 days (Castañeda, 1954).

2.4. Statistical evaluation

The intermethod variation between tests was determined by calculating κ values. κ values express the agreement beyond chance. Generally, a κ value of >0.80 represents

almost perfect agreement beyond chance. κ values between 0.40 and 0.80 represent fair to good agreement, and values below 0.40 represent slight or no agreement.

3. Results

The results of the *Brucella* IgM and IgG LFAs obtained for samples collected from patients with clinical suspicion of brucellosis were compared with those obtained for the RB, the Huddleson test, and the SAT (Table 1). The percentage of samples that tested positive ranged from 52.4% in the RB to 73.9% in the LFA. The mean SAT titer of the samples agglutinating in this assay was relatively low (1:200; range 1:25–1:1600). In total, 344 (73.0%) samples agglutinated in any of the 3 agglutination tests. Samples originated from 5 districts. Notably, of the samples from one of the districts (Kyzylorda), only 6 (13.0%) revealed agglutination and just 1 (2.1%) reacted in the LFA.

The results of the LFA compared well with those of the SAT at a cutoff value of 1:25. Of the 471 samples that were tested, 310 (65.8%) tested positive in the LFA and in the SAT, 109 (23.1%) tested negative in the LFA and in the SAT, 38 (8.1%) tested positive in the LFA and negative in the SAT, and 14 (3.0%) tested negative in the LFA and positive in the SAT. The observed agreement between the LFA and the SAT for the total groups of samples tested was 89.0% and the κ coefficient was 0.73 (SE of κ, 0.045; 95% confidence interval, 0.66–0.80). SAT correlated less well with the RB (observed agreement, 80.0%; κ coefficient, 0.58). The observed agreement of the SAT with the Huddleson test was 89.4% and the κ coefficient 0.75.

Blood culture was performed for a subset of samples from 2 districts, Almaty and Zhambyl, and the percentage of patients with a positive blood culture was 82.9%. A positive culture was obtained in 50 (96.2%) acute patients, in 2 (66.7%) subacute patients, and in 11 (52.4%) patients with chronic disease. The mean age of the patients with culture-confirmed brucellosis was 33 years (range, 21–83), and the male-to-female ratio was 2.71. The patients with culture-confirmed chronic brucellosis were on average older (mean

age, 46.5 years; range, 24–83) than the patients with culture-confirmed acute brucellosis (mean age, 32 years; range, 21–63). The sensitivity of the LFA assay was 100% for the groups of patients with culture-confirmed brucellosis and was higher than the sensitivity calculated for the RB and Huddleson tests (Table 2). Notably, the RB had a low sensitivity when testing samples from patients with culture-confirmed chronic brucellosis. All patients with culture-confirmed brucellosis tested positive in the SAT at a titer of 1:25 (mean titer, 1:150; range 1:25–1:1600). Mean SAT titers were one dilution step higher for patients with culture-confirmed acute or subacute brucellosis (mean, 1:200; range, 1:25–1:1600) than for patients with culture-confirmed chronic brucellosis (mean, 1:100; range, 1:25–1:400). Specific IgM antibodies measured in the LFA were detected in the samples from 90.4% of the combined group of culture-confirmed patients with acute or subacute brucellosis and in the samples from 72.7% of the culture-confirmed patients with chronic brucellosis. Specific IgG antibodies measured in this assay were detected in 75% and 100% of these 2 groups of patients. Of the 13 patients with a negative culture result, 10 had developed chronic disease, 2 were acute, and 1 was subacute. Only one of these patients agglutinated in the SAT (titer, 1:25), all were RB and Huddleson test negative, 3 tested positive in the *Brucella* IgM LFA, and 1 tested positive in the *Brucella* IgG LFA.

The majority (92.1%) of the samples from the patients with culture-confirmed brucellosis that tested positive in the LFA gave a moderately strong (2+) to very strong (4+) signal for either IgM or IgG (Table 3). The staining intensity was somewhat less strong for patients with chronic brucellosis than for patients with acute or subacute brucellosis. A ≥2+ staining intensity in either the IgM or the IgG test was obtained for 94% of the samples from culture-confirmed patients with acute or subacute disease and for 84.6% of the samples from culture-confirmed patients with chronic disease.

The mean (1:200; range, 1:25–1:1600) SAT titer measured for the SAT-positive samples from the groups of patients with a negative culture result or for whom culture not performed was slightly higher than the mean SAT titer

Table 1
Results of the *Brucella* IgM and IgG flow assays for the patients from the different districts and comparison with the RB, the Huddleson test, and the SAT

Region	No. of samples positive (%) in the following assays								
	RB	Huddleson	SAT ≥1:25	SAT ≥1:50	SAT ≥1:100	SAT ≥1:200	LFA IgM and/or LFA IgG	LFA IgM	LFA IgG
East Kazakhstan (n = 64)	51 (83.6)[a]	54 (85.7)[b]	50 (78.1)	45 (70.3)	37 (57.8)	30 (46.9)	57 (89.1)	55 (85.9)	47 (73.4)
Kyzylorda (n = 48)	0 (0)	6 (13.0)[c]	4 (8.3)	4 (8.3)	0 (0)	0 (0)	1 (2.1)	1 (2.1)	0 (0)
South Kazakhstan (n = 188)	126 (67.0)	135 (71.8)	138 (73.4)	123 (65.4)	115 (61.2)	91 (48.4)	146 (77.7)	126 (67.0)	118 (62.8)
Almaty (n = 57)	31 (54.4)	36 (63.2)	45 (78.9)	39 (68.4)	33 (57.9)	22 (38.6)	48 (84.2)	41 (71.9)	40 (70.2)
Zhambyl (n = 114)	39 (34.2)	78 (68.4)	87 (76.3)	76 (66.7)	46 (40.4)	25 (21.9)	96 (84.2)	81 (71.1)	61 (53.5)
Total (n = 471)	247 (52.4)	309 (65.6)	324 (68.8)	287 (60.9)	231 (49.0)	168 (35.7)	348 (73.9)	304 (64.5)	266 (56.5)

[a] n = 61.
[b] n = 63.
[c] n = 46.

Table 2
Sensitivity of the *Brucella* IgM and IgG flow assays for serum samples from patients with culture-confirmed brucellosis and comparison with other serological tests

Group	% Sensitivity (95% CI) of the following assays								
	RB	Huddleson	SAT ≥1:25	SAT ≥1:50	SAT ≥1:100	SAT ≥1:200	LFA IgM and/or LFA IgG	LFA IgM	LFA IgG
Culture-confirmed acute + subacute (n = 52)	94.2 (83.1–94.2)	88.5 (75.9–95.2)	100 (91.4–100.0)	90.4 (78.2–96.4)	73.1 (58.7–84.0)	50.0 (36.0–64.0)	100 (91.4–100.0)	90.4 (78.2–96.4)	75.0 (60.8–85.5)
Culture-confirmed chronic (n = 11)	54.5 (24.6–81.9)	72.7 (39.3–92.7)	100 (67.0–100.0)	90.9 (57.1–99.5)	72.7 (39.3–92.7)	54.5 (24.6–81.9)	100 (67.9–100.0)	72.7 (39.3–92.7)	100 (67.9–100.0)
Total culture-confirmed (n = 63)	71.4 (58.5–81.8)	85.7 (74.1–92.9)	100 (92.8–100.0)	90.5 (79.8–96.1)	73.0 (60.1–83.1)	50.8 (38.0–63.5)	100 (92.8–100.0)	87.3 (76.0–94.0)	79.4 (67.0–88.1)

measured for the samples from the patients with culture-confirmed brucellosis. The majority (82.1%) of the 291 samples with a positive result in the LFA from this group of patients gave a ≥2+ staining intensity for either IgM or IgG (Table 3). Again, the observed staining intensity was in general somewhat less strong for patients with chronic disease than for patients with acute disease. In this group a ≥2+ staining intensity was obtained for 73.3% of the positive samples from patients with chronic disease, for 68.8% of the positive samples from patients with subacute disease, and for 83.5% of the positive samples from patients with acute disease.

4. Discussion

In Kazakhstan, the laboratory diagnosis of brucellosis is traditionally based on a combination of results obtained in the RB, the Huddleson test, and the SAT. Only recently, blood culture was introduced allowing assessment of the diagnostic sensitivity of the employed serological tests. Our results confirm the high sensitivity of the SAT and demonstrate that the *Brucella* IgM/IgG LFA provides an attractive alternative for the more time consuming and laborious SAT. Like the SAT, the LFA tested positive in all serum samples collected from the patients with culture-confirmed brucellosis including those with chronic disease. The LFA has the additional advantage that the assay is easy to perform and to read, provides a rapid result, and does not require special equipment or electricity. The RB test and the Huddleson test prove to be less useful. They failed to agglutinate the samples from patients with culture-confirmed brucellosis in 14.3% and 28.6%, respectively, and as they gave negative results for in particular patients with chronic brucellosis, their diagnostic value appears to be low. Blood culture is an important diagnostic tool as it provides direct evidence for the presence of the pathogen. In this study, blood culture-confirmed brucellosis in 82.9% of the patients for whom culture was attempted, and this value ranged from 96.2% for patients with acute disease to 52.4% for chronic patients. This result compares favorably with literature reports, indicating that the sensitivity of culturing *Brucella* from blood varies between 15% and 70% compared to clinical evidence of infection and that positive culture rate serology depends on the patient population and culture method employed (Ruiz et al., 1997; Yagupsky, 1999).

Consistent with the notion that low SAT titers are important in the serodiagnosis of brucellosis, almost half (49.2%) of the serum samples agglutinated at relatively low titers of 1:25 to 1:100. In a study performed in Spain, the sensitivity of the SAT at a cutoff value of ≥1:160 was calculated to be 65.8% for the initial sera from patients with confirmed brucellosis, and the specificity at this cutoff value was 100% (Orduña et al., 2000). Most patients with SAT titers below this cutoff value were diagnosed with acute brucellosis, but this group also included patients with

Table 3
Staining intensity of the *Brucella* IgM/IgG flow assay

Group and subgroup	No. of samples with the following staining intensity in the *Brucella* IgM flow assay					No. of samples with the following staining intensity in the *Brucella* IgG flow assay				
	Negative	1+	2+	3+	4+	Negative	1+	2+	3+	4+
Culture-confirmed brucellosis										
Total (*n* = 63)	8	7	22	11	16	13	19	16	8	8
Acute (*n* = 50)	5	6	16	8	16	12	12	13	7	7
Subacute (*n* = 2)	0	0	0	2	0	1	0	1	0	0
Chronic (*n* = 11)	3	1	6	1	0	0	7	2	1	1
Culture negative and culture not performed										
Total (*n* = 409)	155	66	76	39	73	194	76	84	31	18
Acute (*n* = 348)	120	56	63	38	71	155	66	79	24	17
Subacute (*n* = 28)	16	5	6	0	1	15	6	2	5	0
Chronic (*n* = 33)	19	5	7	1	1	24	4	3	2	1

chronic brucellosis and relapsing patients. At a cutoff value of ≥1:20, the sensitivity increased to 91.5%, whereas the specificity dropped slightly to 99.5%. In a study performed in the United States, the sensitivity of the SAT at a cutoff value of ≥1:160 was calculated to be 87.7% (Young, 1991). In that study, SAT titers ranging from 1:20 to 1:1280 (mean titer, 1:80) were reported for a group of patients with evidence of prior brucellosis or previous professional exposure to either animals or the vaccine strain S19 and in whom active brucellosis was considered unlikely because of the absence of clinical signs and symptoms. Therefore, when employed in areas endemic for brucellosis, the specificity of the SAT could be affected. A survey for brucellosis performed in Turkey showed seroprevalence rates in different provinces and villages ranging from zero to 8.5% in the RB and from zero to 5.6% in the SAT at a cutoff titer of 1:100 (Kose et al., 2006). Seroprevalence of *Brucella* antibodies in healthy individuals from Saudi Arabia was 4.4% at a cutoff titer of 1:320 and 11% at a cutoff titer of 1:160 (Al Sekait, 1999; Cooper, 1992). Combined, these data show the importance of considering low agglutination antibody titers in patients with clinical suspicion of brucellosis not only in patients with persistent disease or relapsing brucellosis but also in patients with acute brucellosis. However, these data also demonstrate the limited diagnostic value of low agglutinating antibody titers in patients residing in an endemic area and in those with a history of brucellosis or repeatedly exposed to the pathogen. Therefore, normal diagnostic values should be established for tests applied in endemic areas such as Kazakhstan to address the diagnostic specificity and to assess the positive predictive value of low titers.

The result obtained for the *Brucella* IgM/IgG LFA compares favorably with an earlier study performed in Turkey showing that the sensitivity of the LFA for patients with acute, subacute, chronic, and relapsing brucellosis amounts to 92%, 100%, 78%, and 75%, respectively (Irmak et al., 2004). However, a more complete study is required to determine the diagnostic value of the LFA for the later 2 groups of patients as the numbers of such patients tested is

still low. One of the objectives of this study was to determine whether the application of the *Brucella* IgM/IgG LFA could help to distinguish patients with chronic brucellosis. In an earlier study, we showed that specific IgM antibodies dominated in samples from patients with acute brucellosis, whereas specific IgG antibodies dominated in samples from patients subacute, chronic, and relapsing brucellosis (Irmak et al., 2004). In the current study group, patients with culture-confirmed acute brucellosis slightly more often had specific IgM antibodies compared with patients with culture-confirmed chronic brucellosis who more often had specific IgG antibodies. However, this tendency was not seen for the group of culture-negative patients or for whom culture was not performed. However, not all patients may have accurately reported the onset of illness, and a more complete study is required to examine the role of serology in chronic disease.

The low sensitivity of the RB and the Huddleson tests was somewhat surprising. In a study performed in Spain, the sensitivity of the RB ranged from 91.7% for *Brucella* patients repeatedly exposed to the pathogen to 93.8% for *Brucella* patients with no history of or exposure to *Brucella* and to 96.1% for *Brucella* patients with a previous history of brucellosis (Ruiz-Mesa et al., 2005). These values are higher than the sensitivity of 85.7% calculated for the RB in our study for patients with culture-confirmed brucellosis. Differences in reactivity of the RB antigen could be due to differences in the source or preparation of the reagent. The low sensitivity calculated for the RB test in our study was in part attributable to a low reactivity in samples from patients with chronic brucellosis. Reactivity of the RB antigen with *Brucella*-specific IgG antibodies is known to be sensitive to pH (Rubio et al., 2002). Perhaps a suboptimal quality of the locally produced antigen may explain the failure to agglutinate the serum of a number of patients with culture-confirmed brucellosis. According to literature data, the Huddleson test has a slightly lower sensitivity compared with the RB (Černyševa et al., 1977).

Blood culture was negative in 11 patients with chronic disease and in 3 with acute or subacute disease. The diagnosis of chronic brucellosis remains cumbersome. Blood

culture has a low sensitivity in particular if the patient has taken antibiotics. The absence of specific antibodies in most of these culture-negative patients may suggest that these patients suffered from an illness other than brucellosis. The use of the more sensitive Coombs test or molecular detection using the polymerase chain reaction could be useful in such cases (Al Dahouk et al., 2003; Elfaki et al., 2005). However, these methods are complex and not suitable for routine application in most diagnostic laboratories. The lysis centrifugation method could improve the sensitivity of blood culture also in patients with chronic brucellosis, but this method increases the risk of laboratory acquired infection (Espinosa et al., 2009).

The results of the SAT and the *Brucella* IgM/IgG LFA suggest that a high proportion of the patients from 4 (East Kazakhstan, South Kazakhstan, Almaty, and Zambyl) of the 5 districts with a sample submitted for confirmation of brucellosis indeed suffered from this disease. The SAT agglutinated in 73.4–78.9% of these samples and the LFA tested positive in 77.7–89.1% of them. Apparently, brucellosis was uncommon among the patients from Kyzylorda the 5th district, where patients were recruited. Of the sera collected in this district, only 8.3% reacted in the SAT and 2.1% in the LFA. Although blood culture was not performed for a large proportion of the patients and hence the serologic evidence for brucellosis could not be confirmed, we believe that most of these seropositive patients suffered from active brucellosis. Firstly, blood culture was performed for a random selection of the patients from 2 districts and the extrapolation of the culture results indicates that about 80% of the patients suffered from brucellosis, and this figure correlates well with the observed seroreactivity. Secondly, the mean SAT titer for sample from patients for whom blood culture was not performed was slightly higher to that for the samples from the culture-confirmed group of *Brucella* patients. Thirdly, we previously demonstrated that the specificity of the LFA is high and showed that in endemic areas also, the seroprevalence among the general population as measured in the LFA is relatively low (Irmak et al., 2004; Smits et al., 2003). Fourthly, although some patients recalled brucellosis in a family member, very few patients had previously suffered from brucellosis. In rural areas in Kazakhstan, the population may be exposed to brucellosis either because of the consumption of contaminated fresh dairy products or because of contact with infected livestock. Seroprevalence studies among the general population in areas where brucellosis is endemic in Kazakhstan may help to determine the significance of positive test results in the SAT and the LFA. The relatively low number of seropositive patients from Kyzylorda is noteworthy. This could indicate that brucellosis is uncommon in this district. However, the possibility that staff at the participating hospital used a different diagnostic norm for referral of patients for laboratory testing needs further investigation.

In conclusion, serological evidence combined with blood culture results provides strong evidence that in rural areas of Kazakhstan, brucellosis is an important cause of illness.

Because of its user-friendliness and favorable diagnostic characteristics, the *Brucella* IgM/IgG LFA could be a very useful diagnostic tool for patients with chronic disease in hospitals in Kazakhstan and other countries where brucellosis is common (Mendoza-Núñez et al., 2008; Roushan et al., 2005; Zeytinoğlu et al., 2006).

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