USE OF THE *Brucella* IgM AND IgG FLOW ASSAYS IN THE SERODIAGNOSIS OF HUMAN BRUCELLOSIS IN AN AREA ENDEMIC FOR BRUCELLOSIS

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Abstract. The clinical utility of two complementary tests for brucellosis, the *Brucella* IgM and IgG flow assays, was evaluated in a hospital in eastern Turkey. The results show that the flow assays are convenient diagnostic tests for use in endemic areas. A positive result in the flow assays was obtained in 91% and 97% of the admission sera from adult and pediatric patients with brucellosis, respectively, and the sensitivity at admission was 100% for culture-confirmed brucellosis. The assay system performed equally well in diagnosing patients at different stages of illness including patients with acute, subacute, or chronic disease and with relapse. The results of the flow assays correlated well with those of a serum agglutination test at a cut-off ≥1:160. The agreement was 92%. Application of the flow assays on serum samples collected during a village survey for brucellosis after an outbreak demonstrated their diagnostic potential as field tests.

INTRODUCTION

Brucellosis is a multi-system disease that may present with a broad spectrum of clinical manifestations and that requires laboratory testing for diagnosis. The isolation of the organism from blood samples or other clinical specimens is the confirmatory test for the disease. However, the sensitivity of this technique depends on the stage of illness as well as on other factors. Furthermore, culture does not provide a rapid result and many laboratories in endemic areas do not have culture facilities. Therefore, the diagnosis often relies on serologic testing. Not all physicians in areas that are endemic for brucellosis have access to laboratory facilities, and in these problematic situations a rapid field test with a high sensitivity and specificity is urgently needed. The Rose Bengal (RB) test is widely used as a rapid and simple screening test but requires confirmation by other tests because false-positive results may occur in endemic areas. For this the classic Wright tube agglutination test or the standard serum agglutination test (SAT) may be used. These agglutination tests are relatively complicated and time-consuming to perform. The recently developed immunochromatographic lateral flow assays, the *Brucella* IgM and IgG flow assays, for the detection of *Brucella*-specific IgM and IgG antibodies, respectively, are much easier and quicker to perform and are well standardized. The flow assay is simply performed by the addition of a drop of serum followed by some running fluid to the sample well of a plastic assay device. The test result is read after 10–15 minutes by visual inspection for staining of the antigen line in the test zone of the assay device. In a laboratory-based evaluation study, we demonstrated a sensitivity for the flow assays of 96% for admission sera from patients with culture-confirmed brucellosis, and showed that the sensitivity is equally high for patients with acute and with chronic disease. No significant degree of cross-reactivity when testing samples from patients with illnesses other than brucellosis was observed. The *Brucella* IgM and IgG flow assays thus would be ideal for use in areas that are endemic for brucellosis and in developing countries.

Brucellosis is still endemic in Turkey and presents a major public health, clinical, and diagnostic problem among the largely rural population, in particular in more remote parts of the country. The incidence of human brucellosis in Turkey is estimated to be 0.59 per 100,000 per year, but can be much higher among specific groups. The high incidence of brucellosis in Turkey is due to the widespread infection among domestic animals and the frequent contacts with livestock and the consumption of raw milk and traditionally prepared soft cheeses by the rural population. The presents study was undertaken to confirm the clinical utility of the *Brucella* IgM and IgG flow assays when applied in a clinical setting in an area that is endemic for brucellosis. The study was performed at a university hospital in eastern Turkey and demonstrates that the assay system will be very useful for the confirmation of brucellosis in situations where brucellosis is common.

MATERIALS AND METHODS

Patients and clinical specimens. Three groups were studied. Adult patients hospitalized with clinical suspicion of brucellosis. Adult patients (n = 172) admitted with clinical suspicion of brucellosis to different departments of the Yüzüncü Yıl University Medical School Hospital in Van, Turkey were entered into the study. Sixty-three adult patients were diagnosed with brucellosis based on clinical findings and laboratory testing and treated accordingly. A final diagnosis other than brucellosis was reached for 24 patients and this included 10 patients with (para) typhoid fever. The final diagnosis was debated in the remaining 85 patients because of inconsistent or incomplete clinical and or laboratory findings and these were designated as an unconfirmed diagnosis. Of the 63 confirmed cases of brucellosis, 26 had symptoms and signs for less than two months at the time of first diagnosis and were considered acute, 22 had been ill for 2–12 months (subacute), and 11 had been ill for more than one year (chronic). The four other patients had been previously treated for brucellosis and had a relapse. An admission sample was available from 54 patients with brucellosis, including the four patients with a relapse. Follow-up serum samples (n = 76) collected 1–30 weeks (median = 5 weeks) after the start of treatment were available from 30 of these patients with an admission sample. Admission sera were not collected from nine patients with brucellosis. Single serum samples collected during treatment were available from four of these nine patients and two or more samples were available from five of these patients (n = 16). Single serum samples collected at admission were avail-
lates were identified by conventional biochemical testing. Subcultured to chocolate and blood agar plates. Bottles giving a positive growth index were Gram stained and fluorescence emitted by the sensor at the bottom of the culture.

Flow assays have been described previously. The flow assays were performed by the addition of 5 \( \mu L \) of serum samples (30 \( \mu L \)) were mixed with an equal volume of antigen MM101 (Linear Chemicals, Barcelona, Spain). The mixtures were incubated for 24 hours at 37°C and read by visual inspection. A titer of 1:160 was considered consistent with brucellosis.

Hemoculture was performed by inoculation of 8–10 mL of freshly collected blood into each of a Plus aerobic/F BACTEC bottle and a Plus anaerobic/F BACTEC bottle and incubation for up to seven days in the BACTEC 9120 incubator instrument (Becton-Dickinson, Franklin Lakes, NJ). Bottles were examined for the presence of growth on a 10-minute cycle by the measurement of CO2-induced fluorescence emitted by the sensor at the bottom of the culture. Bottles giving a positive growth index were Gram stained and subcultured to chocolate and blood agar plates. Brucella isolates were identified by conventional biochemical testing.

The design and composition of the Brucella IgM and IgG flow assays have been described previously. The flow assays were obtained from Organon Teknika, Ltd. (Dublin, Ireland). The assay was performed by the addition of 5 \( \mu L \) of serum directly onto the sample application pad in the sample well of the plastic assay device, followed by the addition of 130 \( \mu L \) of running fluid. The test result was read by visual inspection for staining of the antigen and control lines in the test zone of the device. The assay was scored negative when no staining of the antigen line was observed and positive when a distinct staining of the antigen line was observed. The antigen line may stain at different intensities and was subjectively rated 1+ when staining was weak, 2+ when staining was moderate, 3+ when staining was strong, and 4+ when staining was very strong. Undetermined staining represented by very weak (+) staining was considered negative.

Statistical analysis. The observed agreement and the agreement beyond chance (kappa) between the results of the SAT and the flow tests was determined using Epi-Info version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA). Kappa values range from −1.0 for total disagreement to +1.0 for total agreement. Agreement was considered almost perfect for values >0.8.

RESULTS

Brucella was isolated in 39% (n = 11) and 30% (n = 10) of the hemocultures from the adult and pediatric patients with a final diagnosis of brucellosis, respectively. The SAT (titer ≥1:160) confirmed the clinical diagnosis of brucellosis in an additional 52 adult and 23 pediatric patients.

The results obtained in the Brucella IgM and IgG flow assays and SAT for four patients with brucellosis and one patient with a disease other than brucellosis are shown in Figure 1. The admission serum from all 11 (100%, 95% confidence interval [CI] = 68–100%) culture-positive adult patients tested positive in the IgM flow assay and 10 gave a positive result in the IgG flow assay. All 11 samples also tested positive in RB test and the SAT. The RB test, SAT, IgM flow assay, and IgG flow showed positive results in 98%, 98%, 65%, and 82%, respectively, of the total group of 54 admission sera collected from the adult patients with brucellosis (Table 1). The IgM and IgG flow assays combined gave a positive result in 49 (91%) admission sera. The IgM flow assay gave the highest number of positive results for the samples from the acute cases (83%), and this value was significant higher than that for patients with subacute (53%) and chronic (33%) disease. The IgG flow assay gave the highest number of positive results for the patients with subacute disease (94%), which was somewhat higher than that of acute (75%), chronic (78%), and relapsing (75%) cases. Clinical symptoms and signs were the same but generally were milder in patients with subacute disease than in patients with acute disease. Complications, mostly of the skeletal system, were seen in patients with chronic disease.

The flow assays showed negative results for the admission sera from five patients with brucellosis. Interestingly, one of these patients showed seroconversion in the IgG flow assay with serum samples collected 4 and 6 weeks after the start of treatment testing positive (Figure 2a). An admission sample was not available for nine patients. The first available follow-up sample from all except one of these patients tested positive in either of the two flow assays. Thus, 58 (92%) adult patients with brucellosis tested positive and five (8%) tested negative in the flow assays (Table 1).

The Brucella IgM and IgG flow assays showed positive results in 76% and 91%, respectively, of admission sera from the pediatric group of patients with brucellosis. A positive result in the two flow assays combined was obtained in 97%. The results obtained in the flow assays, as well as those of the
The serum from the pediatric patient that tested negative was strongly hemolyzed. All 10 (100%, 95% CI = 66–100%) samples from the culture-confirmed pediatric patients tested positive in the IgM flow assay as well as in the IgG flow assay.

The staining intensity of the antigen band in the flow assays recorded for the admission sera from the adult and pediatric patient groups are shown in Table 2. A moderate (2+) to very strong (4+) staining intensity in either of the two assays was observed in the majority of the samples that tested positive. This was 88% (n = 43) for the adult patients with brucellosis and 81% (n = 26) for the pediatric patients. For the other positive patients, a weak (1+) test result was recorded as strongest test result. The admission sera of all (100%) culture-confirmed patients gave a moderate to very strong staining intensity in either or both flow assays.

**TABLE 1**

<table>
<thead>
<tr>
<th>Serum group and stage (no. of samples)</th>
<th>RB</th>
<th>SAT 1:2,560</th>
<th>SAT 1:640</th>
<th>SAT 1:320</th>
<th>SAT 1:2,560 ≤1:120</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>IgM</td>
<td>IgG</td>
<td>IgM + IgG combined</td>
</tr>
<tr>
<td>Patients with brucellosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults (n = 63)*</td>
<td>62</td>
<td>62 (98, 90–100)</td>
<td>59 (94, 84–98)</td>
<td>45 (71, 59–82)</td>
<td>55 (87, 76–94)</td>
</tr>
<tr>
<td>Admission serum (n = 54)</td>
<td>53</td>
<td>53 (98, 89–100)</td>
<td>49 (91, 79–97)</td>
<td>35 (65, 51–77)</td>
<td>44 (82, 68–90)</td>
</tr>
<tr>
<td>Acute (n = 24)</td>
<td>24</td>
<td>24 (100, 83–100)</td>
<td>23 (96, 77–100)</td>
<td>20 (83, 62–95)</td>
<td>18 (75, 53–89)</td>
</tr>
<tr>
<td>Subacute (n = 17)</td>
<td>16</td>
<td>16 (94, 69–100)</td>
<td>16 (94, 69–100)</td>
<td>9 (53, 29–76)</td>
<td>16 (94, 69–100)</td>
</tr>
<tr>
<td>Chronic (n = 9)</td>
<td>9</td>
<td>9 (100, 63–100)</td>
<td>6 (67, 31–91)</td>
<td>3 (33, 9–49)</td>
<td>7 (78, 40–96)</td>
</tr>
<tr>
<td>Relapse (n = 4)</td>
<td>4</td>
<td>4 (100, 40–100)</td>
<td>3 (75, 57–88)</td>
<td>3 (75, 57–88)</td>
<td>3 (75, 57–88)</td>
</tr>
<tr>
<td>Pediatric (n = 33)</td>
<td>32</td>
<td>33 (100, 87–100)</td>
<td>33 (100, 87–100)</td>
<td>25 (76, 57–88)</td>
<td>30 (91, 75–98)</td>
</tr>
<tr>
<td>Non-brucellosis patients (n = 24)</td>
<td>6</td>
<td>0 (0, 0–17)</td>
<td>0 (0, 0–17)</td>
<td>0 (0, 0–17)</td>
<td>1 (4, 0–23)</td>
</tr>
<tr>
<td>Unconfirmed (n = 85)</td>
<td>14</td>
<td>7 (8, 4–17)</td>
<td>5 (6, 2–14)</td>
<td>12 (14, 8–24)</td>
<td>8 (9, 4–18)</td>
</tr>
</tbody>
</table>

*Test results given for group of 63 adult patients are for any sample, admission and or follow-up.
Follow-up sera had been collected from 35 adult patients with brucellosis. Seroconversion or an increase in staining intensity in either the IgM or the IgG flow assay for samples collected 2–4 weeks after the start of treatment was observed in 12 (34%) patients. As mentioned earlier, one patient showing seroconversion had a negative result in both assays for the admission sample. Two other patients showing seroconversion in one of the flow assays already tested positive in the other flow assay for the admission sample. Three patients showing an increase in staining intensity during follow-up gave a weak positive result for the admission sample (Figure 2b–d). For the other six patients, an increase in staining intensity from 2+ to 3+ or 4+ was observed. In spite of the relatively short duration of follow-up (median = 5 weeks) a decrease in staining intensity during follow-up was seen in 13 patients.

A negative result in the flow assays was obtained for all samples including the follow-up from 23 (95.8%, 95% CI = 77–100%) of 24 adult patients admitted with clinical suspicion of brucellosis, but with a different final diagnosis (Table 1). The samples from all 10 patients with typhoid and paratyphoid fever tested negative in the flow assays. The RB test showed positive results in six (25%) patients and one of these gave a weak positive result in the IgG flow assay (Table 2). The SAT did not agglutinate or showed only an insignificant titer (1:80).

The RB test showed a positive result in the admission sera from 14 patients for whom the final diagnosis was debated (Table 1). The flow assay reacted in 13 of these RB test-positive samples, with five of them giving a moderate to very strong staining intensity. The flow assays also reacted in one RB test-negative sample. The SAT agglutinated (≥1:160) in seven RB test positive samples, five of which gave a ≥2+ staining intensity in the flow assays (Table 2).

The strength of the staining intensity of the antigen band in the flow assay showed a strong correspondence with the titer in the SAT (Table 3). The agreement between SAT (≥1:160) and the flow assays was 92% (kappa = 0.84, SE = 0.07) for the combined results obtained for the admission sera from the adult and pediatric patients with brucellosis, non-brucellosis patients, and the patients with an undetermined diagnosis.

Of the 27 serum samples collected during the village survey for brucellosis following an outbreak in animals and transmission to humans, 12 (44%) tested positive in the RB test, six (22%) in the SAT, and nine (33%) in the Brucella IgM and or IgG flow assay (Table 4). The flow assays confirmed the results of the RB test in eight cases. The SAT confirmed the result of the RB test in six cases. All six SAT-positive cases also were positive in the flow test. Two RB test-positive samples that did not agglutinate in the SAT tested positive in the flow assays. One of these tested 1+ in the IgM flow assay and the other tested 3+ in the IgG flow assay. One RB test-negative, SAT-negative sample showed a 1+ staining intensity in the IgG flow assay and likely presents a false-positive result.

### DISCUSSION

A simple and rapid diagnostic test for the confirmation of brucellosis in particular is needed in endemic areas where not all health workers have access to laboratory facilities that offer laboratory testing for brucellosis. Brucellosis is very common in eastern Turkey. The present study confirms the high sensitivity and specificity of the Brucella IgM and IgG flow assays and shows that these rapid and simple diagnostic tests may be used for the confirmation of patients with clinical suspicion of brucellosis and living in an area endemic for this disease. A positive result in the flow assays was obtained in 100% of the admission sera from culture-confirmed adult and pediatric patients with brucellosis. We previously tested admission sera collected from patients with confirmed brucellosis from Spain and calculated a sensitivity of 96%. Hemo-culture was not used or showed negative results for some of the patients included in the present study. The diagnosis was confirmed in these patients by serologic testing with the SAT. The sensitivity of the Brucella IgM and or IgG flow assays was 91% for the admission sera from the total group of adult patients with brucellosis and 98% for the admission sera from the total group of pediatric patients with brucellosis. We also confirmed the high specificity of the flow assays. We previously calculated a specificity >99% for samples collected in a non-endemic area and the present results indicate a specificity of 95.8%.
Elevated levels of specific antibodies may remain present in patients treated for brucellosis for a long time and also may be detected in individuals exposed to the pathogen or with an inactive infection. For this reason, a higher cut-off value for the SAT is sometimes used in areas that are endemic for brucellosis.13,14 We previously showed that reactivity in the flow assays decreases during and after treatment and that most samples collected six months or more after the start of treatment gave a negative result or a weak staining intensity.7 Reactivity at a low-staining intensity in the flow assays may also be observed in veterinarians exposed to the pathogen.7 Thus, some reactivity at low-staining intensity can be expected in the flow assay in non-brucellosis patients when using the assays in an area where brucellosis is common. A study performed among the inhabitants of five villages in Van Province demonstrated a high seroprevalence of Brucella-specific antibodies of 27% in the RB test and 27% in the SAT (titer 1/11350 1:20, 8% for SAT titer 1/11350 1:160).15 The flow assays reacted with a moderate (2+) to very strong (4+) staining intensity in all admission sera from the culture-confirmed adult and pediatric patients. Also, the majority of the admission sera from the culture-negative patients with brucellosis confirmed by the SAT showed a ≥2+ staining intensity in the flow assays, which clearly confirmed an active infection with Brucella in these patients. However a weak (1+) positive test result in the flow assays was obtained for a number of the admission sera from the culture-negative patients with brucellosis confirmed by the SAT, and a few others showed a negative test result. Testing of follow-up samples could be

### Table 3
Correlation of staining intensity in the *Brucella* IgM and IgG flow assays with the serum agglutination test (SAT) titer

<table>
<thead>
<tr>
<th>Admission sera from adult and pediatric patients with brucellosis*</th>
<th>Staining intensity in flow assay†</th>
<th>Reciprocal SAT titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3+</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Follow-up samples from patients with brucellosis

<table>
<thead>
<tr>
<th>Staining intensity in flow assay†</th>
<th>Reciprocal SAT titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>1 (1)</td>
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<td></td>
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</tr>
</tbody>
</table>

Non-brucellosis patients and patients with an unconfirmed final diagnosis‡

<table>
<thead>
<tr>
<th>Staining intensity in flow assay†</th>
<th>Reciprocal SAT titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values are for both adult patients. Values in parentheses are for pediatric patients.
† The staining intensity for either the IgM or IgG flow assay is given, whichever was highest. The number of sera with the indicated staining intensity in the flow assay and the corresponding titer in the SAT are given.
‡ Values are for patients with a clinical suspicion of brucellosis at admission but with a final diagnosis other than brucellosis. Values in parentheses are for patients with an undetermined final diagnosis.

### Table 4
Serologic test results of samples collected during a village survey for brucellosis after an outbreak*

<table>
<thead>
<tr>
<th>Test result in the following assays</th>
<th>Serum no.</th>
<th>RBT</th>
<th>SAT titer</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3, 5, 7-10, 15, 20, 22 and 23</td>
<td>0</td>
<td>Neg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1</td>
<td>1:40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0</td>
<td>Neg</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>2</td>
<td>Neg</td>
<td>0</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>2 and 6</td>
<td>1</td>
<td>1:40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2</td>
<td>1:40</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>3</td>
<td>1:80</td>
<td>+/-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>3</td>
<td>1:320</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>4</td>
<td>1:320</td>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>4</td>
<td>1:320</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>4</td>
<td>1:320</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4</td>
<td>1:320</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>4</td>
<td>1:640</td>
<td>1+</td>
<td>2+</td>
</tr>
</tbody>
</table>

* RBT = Rose Bengal test; SAT = serum agglutination test; Neg. = negative.
useful in such cases, and we were able to demonstrate sero-
conversion or an increase in staining intensity in the flow
assays in a number of these patients. Demonstration of sero-
conversion of an increase in titer is generally accepted as
strong supportive evidence for active disease. We found that
34% of the patients diagnosed with brucellosis seroconverted
or showed an increase in staining intensity in the flow assays
for samples collected 2–4 weeks after first diagnosis.

The adult patients with brucellosis included cases with
acute, subacute, and chronic disease, and with relapse, and
the flow assays performed equally well at each of these stages.
However, in accordance with the notion that specific IgM
antibodies are predominant during the early stages of the
illness and specific IgG antibodies predominate at later
stages, the sensitivity of the Brucella IgM flow assay was high-
est for sera collected during the acute stage and the sensitivity of the Brucella IgG flow assay was higher and a stronger
staining intensity was obtained for sera collected from pa-
tients in the more progressed stages of illness. Nine percent of
the admission sera from the adult patients with brucellosis
tested positive in the IgM assay and negative in the IgG assay,
56% tested positive in both tests, 26% tested positive only in
the IgG assay, and 9% tested negative in both tests. For the
pediatric patients, these values were 9%, 67%, 21%, and 3%,
respectively. Thus, although the majority of the patients may
present with specific IgM as well as IgG antibodies, others
may have either specific IgM or IgG antibodies. In clinical
practice, the two assays should be used as complementary
tests. To control costs, either of the two assays may be applied
first as guided by the stage of illness. Cases with acute disease
more likely are to present with a strong IgM response than
cases presenting at a more progressed stage or with a relapse
who are more likely to have a strong IgG reactivity.

The RB test is a very simple test that often is used as a first
screening step for patients with clinical suspicion of brucellosis. The RB test-positive samples may then be tested further
in the Wright agglutination test or the SAT. Screening by the RB
test is also a useful strategy when the flow assays are used
for confirmation. Whereas 25% of the non-brucellosis pa-
ients tested positive in the RB test, only one tested positive (1+)
in the flow assays. Also, 17% of the admission sera from
the patients for whom the final diagnosis was subject to de-
bate tested positive in the RB test and of the 14 RB test-
positive samples, 5 had a ≥2+ test result in the flow assays.
Only one of the RB negative samples gave a positive result in
the flow assay. This sample tested 3+ and may represent a
false-positive result.

The Brucella IgM and IgG flow assays are very simple and
easy to use and read. Thus, they are an attractive diagnostic
alternative for field use. Following the hospitalization of se-
cveral cases with severe brucellosis from a single village and the
reporting of disease consistent with brucellosis in animals
kept in the village, a survey team visited the village to find any
additional cases of clinical or subclinical brucellosis. No clini-
cal cases were found. Blood samples were collected from 27
villagers and tested for specific antibodies in the RB test and
the SAT. Antibiotics were given to SAT-positive individuals.
Laboratory testing required the transportation of the speci-
mens to the laboratory in Van and a second visit was required
to provide the antibiotics. The use of the flow assays during
the survey would have allowed on-site testing and immediate
treatment of positive individuals. The components of the flow
assay are stable and do not need refrigeration, and the flow
assay easily can be applied during a field study. The sero-
prevalence was high, 44% in the RB test, 22% in the SAT,
and 33% in the flow assays. It is plausible that the seropositive
patients had subclinical disease at the time that the survey was
held. Another explanation is that these individuals had spe-
cific antibodies due to recent exposure to the pathogen but
did not develop disease. Adults living in an area endemic for
brucellosis and belonging to a high risk group may well have
developed a certain degree of immunity. A more detailed
follow-up study would be needed to distinguish between these
possibilities. Interestingly, the flow assays detected the same
six individuals that tested positive in the SAT plus one indi-
vidual with a low (1:40) SAT titer and two individuals whose
serum samples that did not agglutinate in the SAT. Of the
latter two individuals, one tested strongly (3+) positive in the
IgG flow assay. We previously found several culture-
confirmed patients with serum samples with low or negative
SAT titers that were clearly (1+ to 4+) positive in the flow
assay.7

The patients with brucellosis included in this study had a
wide variety of non-specific clinical symptoms and signs ne-
cessitating the need for laboratory testing.16 This study con-
irms that the Brucella IgM and IgG flow assays present a
highly convenient diagnostic alternative for culture and sero-
logic testing by the Wright test or the SAT. The flow assays
are intended for the confirmation of patients with clinical
symptoms and signs consistent with brucellosis and may be
used as a rapid diagnostic test in combination with the Rose
Bengal test as a simple screening test. A moderate to strong
positive result is highly consistent with active brucellosis.
A negative or weak positive result may be confirmed by testing
of a follow-up sample. The observation of reactivity in the
flow assays as well as in the agglutination test in samples
collected after an outbreak from individuals with no clinical
illness at the time of sampling shows that some caution in the
interpretation of a positive test result is needed when testing
samples from high risk groups.

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