Development and Evaluation of a Rapid Dipstick Assay for Serodiagnosis of Acute Human Brucellosis

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A dipstick assay for the detection of brucella-specific immunoglobulin M antibodies was evaluated with 707 sera from 247 laboratory-confirmed brucellosis patients and 342 control sera from brucellosis-free individuals. These sera were collected from six different countries. The assay was found to be highly sensitive and specific. In addition, the test is easy to use and does not require specialized training or equipment, and the components are stable without a requirement for refrigeration. All of these factors make the test ideal for developing countries and rural settings.

Brucellosis is an important but often neglected cause of morbidity in many regions of the world (1, 2, 4, 9, 17, 19, 25). The disease is most common in rural areas and among those involved in animal husbandry. Brucellosis also occurs in urban settings when animals are kept in compounds around houses and among meat-packers, dairy workers, and veterinarians. Brucella abortus, Brucella suis, and Brucella melitensis are the causative agents, which have, respectively, cattle, swine, and goats and sheep as their main hosts. The disease is transmitted from infected animals by direct contact with blood, fetuses and fetal membranes, uterine secretions, and aborted material or through consumption of infected, raw animal products, of which milk and milk products are the most important (26).

The treatment of chronic brucellosis is complicated and requires prolonged medication compared to that for acute brucellosis; the disease should be diagnosed and treated promptly. Typical severe acute brucellosis in its early stages cannot be diagnosed on clinical grounds alone (11). Symptoms and signs are nonspecific, and several other febrile illnesses may be simulated, as for example glandular fever, influenza, malaria, and enteric infections. Also, when an unusual complication is suspected, brucellosis may be overlooked. Laboratory tests such as culture and serological tests including the serum agglutination test (SAT) (7, 24), the anti-human globulin test (Coombs test (21), the complement fixation test (12), and enzyme-linked immunosorbent assay (ELISA) (5, 13, 20), therefore, are indispensable for an accurate diagnosis.

The detection of Brucella-specific immunoglobulin M (IgM) antibodies allows the diagnosis of patients with brucellosis at an early stage or acute disease and also may help to discriminate between patients in the early phase of brucellosis and those with chronic brucellosis. In countries where the disease is highly endemic, a large proportion of the population may have persistent Brucella-specific IgG antibodies. Under such conditions, the detection of specific IgM antibodies is important to make the laboratory diagnosis of brucellosis in the early phase of the disease. Specific IgM antibodies can be detected by SAT performed in the presence of either 2-mercaptoethanol (2-ME test) or dithiothreitol (SAT-DTT) (3, 10, 18, 22) and by ELISA (5, 13, 20). The SATs and ELISA can be performed only by relatively skilled personnel in well-equipped laboratories, and these tests are too elaborate for widespread application under field conditions. In situations where appropriate diagnostic facilities are lacking, a colorimetric test, with a simplified format, giving a positive or negative result could serve as a confirmatory test for human brucellosis in the acute phase of the disease. For this reason, we developed a simple dipstick assay for the detection of Brucella-specific IgM antibodies in human serum samples, which is evaluated in this paper.

The dipstick heat-resistant antigen was prepared from a liquid culture of B. abortus 1119-2 by heating washed cells at 95°C followed by removal of cell debris by centrifugation, and this preparation was then applied as a line to a nitrocellulose strip (16). To obtain an internal control, an antihuman IgM antibody was applied as a coating to the nitrocellulose as a separate line (16). The coated strips were blocked with skimmed milk and dried, making it adhere to a plastic backing with double-sided tape, cut into 2.5-mm-wide sticks, and shipped with a strip of wetting agent. A nonenzymatic detection reagent was prepared by conjugation of a monoclonal anti-human IgM antibody to colloidal dye particles (palanyl red) according to a patented method (14, 15, 23). To increase stability, the stained antibody suspension was lyophilized and air dried.
Coombs test. Thirty-nine (26%) patients had positive blood
brucellosis patients based on the results of culture, SAT, and
Netherlands. Patients were considered laboratory-confirmed
patients with 49 samples from Spain, and 2 patients from The
with 71 samples from Portugal, 90 patients from Russia, 19
tested in the United States, and in Yemen, a group of samples
Furthermore, samples from an outbreak of brucellosis were
the disease and the results of these studies were combined.
the sensitivity and specificity of the assay at different stages of
brucellosis-free individuals were tested in order to determine
samples from laboratory-confirmed brucellosis patients and
accompanying protocol. In the laboratories in Portugal, Rus-
ary tubes and were asked to perform the assay according to an
accompanying protocol. In the laboratories in Portugal, Rus-
United States were provided with dipsticks, test reagents, and
ratories in Portugal, Russia, Spain, The Netherlands, and the
reference strip; when no coloring is observed, the test is neg-
cates a positive reaction. The staining of the antigen band can
ambient temperature. A reddish-stained antigen band indi-
cates a positive reaction. The staining of the antigen band can
be scored as 1+ through 4+ by comparison with a colored
reference strip; when no coloring is observed, the test is neg-
In order to assess the clinical utility of the assay, labora-
tories in Portugal, Russia, Spain, The Netherlands, and the
United States were provided with dipsticks, test reagents, and
test tubes and were asked to perform the assay according to an
accompanying protocol. In the laboratories in Portugal, Russia,
Spain, and The Netherlands, randomly selected serum
samples from laboratory-confirmed brucellosis patients and
brucellosis-free individuals were tested in order to determine
and the sensitivity of the assay at different stages of the
disease and the results of these studies were combined.
Furthermore, samples from an outbreak of brucellosis
were tested in the United States, and in Yemen, a group of samples
from culture-proven patients was tested.
The first study group of 150 patients included 39 patients
with 71 samples from Portugal, 90 patients from Russia, 19
patients with 49 samples from Spain, and 2 patients from The
Netherlands. Patients were considered laboratory-confirmed
brucellosis patients based on the results of culture, SAT, and
Coombs test. Thirty-nine (26%) patients had positive blood
cultures, 38 of which were positive for B. melitensis and 1 of
which was positive for B. suis. The control group (342 samples)
included 94 patients with clinical suspicion of brucellosis but
negative results in the Rose Bengal test. The remaining mem-
bers of the control group were patients with the following
conditions (number of patients): autoimmune disease (28),
bartonellosis (Bartonella henselae) (2), hantavirus infection
(11), hepatitis A (5), hepatitis B (7), human immunodeficiency
virus infection (20), legionellosis (11), leptospirosis (8), Lyme
borreliosis (20), malaria (20), meningitis (7), meningococcal
meningitis (8), Mycoplasma pneumoniae infection (7),
ochrobacteriosis (2), syphilis (20), toxoplasmosis (9), tularemia
(Francisella tularensis) (12), Yersinia pseudotuberculosis II
infection (1), Yersinia enterocolitica 03 infection (1), and Y.
enterocolitica 09 infection (4). Forty-five serum samples from
healthy donors were also included.
To calculate the sensitivity of the dipstick assay at different
stages of brucellosis, the serum samples from the patients were
stratified according to the duration of the disease: 73 samples
collected within 2 months, 77 samples for the period of 2 to 4
months, 52 samples for the period of 4 to 6 months and 77
samples collected after >6 months of treatment (Table 1). The
sensitivity of the dipstick assay was 89.0% for the samples collected
within 2 months after the onset of the disease and
83.1% for the samples collected 2 to 4 months after the onset
of the disease (Table 2). The sensitivity dropped to 32.6 and
29.8% for the two groups of samples collected after 4 and 6
months of treatment, respectively (Table 2). Furthermore, the
sensitivity of the dipstick assay was higher than the sensitivity
of the SAT performed in the presence of reducing agent, an
assay often used to assess the presence of specific IgM anti-
obodies, for all four groups of samples. The difference in sen-
sitivity between these two assays was largest for the samples
collected 2 to 4 months after the onset of the disease. Samples
collected from 5 of 89 patients during the first 6 months of the
disease were negative in the dipstick assay. These samples
were also negative in the 2-ME test. The clinical symptoms of these
five patients were consistent with chronic brucellosis rather
than acute or recent-onset brucellosis; the final diagnosis was
Brucella arthritis, spondylitis, and neurobrucellosis for one pa-
tient each, and Brucella was isolated from bone marrow from
two patients. Only 4 of 297 samples from the noncase patients
gave a positive result in the dipstick assay, giving a specificity of
98.6%. These four patients included one suspected brucellosis
patient with a negative result in the Rose Bengal test, one
patient with syphilis, and two patients with yersiniosis. The
sensitivity intensity of all four samples was rated 2+. None of the
45 blood bank sera gave a positive result.
Of the case patients from Portugal, Russia, and Spain, 39
patients had a positive blood culture. Serum samples from 36
(92.3%) of these 39 blood culture-positive patients gave a
positive result in the dipstick assay. Of the patients with a
positive result in the dipstick assay, one was culture positive for
B. suis and the others were positive for B. melitensis. To dem-
strate the reactivity of the dipstick for patients infected with
B. abortus, a group of single serum samples from 60 culture-
proven patients from Yemen was tested. Of these patients, 42
had a positive culture for B. abortus and 18 were positive for
B. melitensis. The sensitivity of the dipstick assay for these two
groups of patients was 95.2 and 83.3%, respectively. The
majority (53 and 80%, respectively) of the dipstick-positive sam-

<p>| TABLE 1. Staining intensity of dipstick assay for samples collected at different stages of the disease |</p>
<table>
<thead>
<tr>
<th>Staining intensity of dipstick assay</th>
<th>No. of samples with indicated staining intensity at mo after onset of disease:</th>
<th>0–2</th>
<th>2–4</th>
<th>4–6</th>
<th>&gt;6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td></td>
<td>8</td>
<td>13</td>
<td>35</td>
<td>54</td>
</tr>
<tr>
<td>1+</td>
<td></td>
<td>5</td>
<td>19</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>2+</td>
<td></td>
<td>17</td>
<td>14</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>3+</td>
<td></td>
<td>16</td>
<td>15</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>4+</td>
<td></td>
<td>27</td>
<td>16</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>73</td>
<td>77</td>
<td>52</td>
<td>77</td>
</tr>
</tbody>
</table>

| TABLE 2. Sensitivity of dipstick assay and other serological tests in relation to duration of the disease |
| Test** | Sensitivity (%) at mo after onset of disease: | 0–2 | 2–4 | 4–6 | >6 |
| Dipstick assay |                                   | 89.0 | 83.1 | 32.6 | 29.8 |
| SAT-DTT and 2-ME** |                               | 79.5 | 64.4 | 76.9 | 50.6 |
| SAT-DTT and 2-ME |                               | 67.1 | 19.1 | 13.5 | 6.0 |
| SAT-DTT and 2-ME |                               | 39.7 | 88.1 | 92.3 | 81.8 |

* The dipstick assay was considered positive when a staining intensity of 1+ was observed. The SAT was considered positive when a titer of ≥1:160 was obtained. The 2-ME test and the SAT-DTT were considered positive when a ≥4-fold reduction in titer compared with that of the SAT was observed. The Coombs test was considered positive when a ≥4-fold increase in titer was measured.

** The SAT-DTT was performed on the samples from Spain. The 2-ME test was performed on the samples from Portugal. The SAT-DTT or the 2-ME test was not done for the samples from Russia and The Netherlands.
TABLE 3. Dipstick results and sensitivity for serum samples collected from brucellosis patients during a B. suis slaughterhouse outbreak in the United States (1976)

<table>
<thead>
<tr>
<th>Sample group (n)</th>
<th>No. of samples with the indicated staining intensity:</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (37)</td>
<td>0 1+ 2+ 3+ 4+</td>
<td>100</td>
</tr>
<tr>
<td>12 mo (36)</td>
<td>14 14 4 4</td>
<td>61.1</td>
</tr>
</tbody>
</table>

amples of these two groups gave a moderate to strong staining intensity. To validate the dipstick assay further and to test the reactivity of the dipstick assay for patients with a B. suis infection, the assay was applied on 93 paired serum samples derived from 37 laboratory-confirmed brucellosis patients infected with B. suis during a localized outbreak in a slaughterhouse in the United States during 1976. All initial serum samples tested positive in the dipstick, giving a sensitivity of 100% (Table 3). Of the samples taken 12 months on average after the first sample, 61% still tested positive, while the staining intensity of the initial samples was ≥2+ for all but one sample, most of the subsequent samples gave a 1+ staining intensity.

The results of the present study show that the dipstick assay is highly sensitive and specific for the serodiagnosis of human brucellosis in the early phase of the disease. For the combined group of samples from Portugal, Russia, Spain, and The Netherlands, the sensitivity was 89.0% for samples collected during the first 2 months of the disease and 83.1% for samples collected 2 to 4 months after the onset of the disease. The sensitivity for the group of samples from the United States collected early in the disease during an outbreak of brucellosis was even higher (100%). Infection with B. melitensis was the most common cause of disease among the first study group. All patients in the outbreak were infected with B. suis. The somewhat lower sensitivity of the dipstick assay calculated for the results of the first study, however, most likely was not related to the difference in causative agent. Some of the patients with a negative result in the dipstick assay from the first study group likely suffered from chronic disease despite the reported recent onset of the disease, and this may well have accounted for the lower sensitivity. The dipstick assay performed equally well for the confirmation of suspected brucellosis patients with B. abortus, B. melitensis, or B. suis infections. The sensitivity was high irrespective of whether it was calculated based on the results obtained for patients confirmed by serological methods or by culture. The sensitivity was 93.5% when only blood culture-proven brucellosis patients were considered.

The specificity of the dipstick assay was calculated to be 98.6%. The selectivity for sera of patients with Y. enterocolitica or cholera infection needs further investigation, as these organisms share antigenic structures with brucellas (6, 8). However, the symptoms of patients with yersiniosis or cholera are distinct from those of patients with brucellosis.

The high sensitivity and specificity of the dipstick assay for samples collected in the acute phase of the disease demonstrated that the assay is highly suitable for use in serodiagnosis of patients with acute disease. Compared with SAT-DTT or the 2-ME test, the dipstick assay has a higher sensitivity and is easier to use: the dipstick assay requires only a few minutes of handling time to perform, does not need special equipment or electricity, and can be performed by modestly trained personnel with a minimum of instructions. The dipstick assay also is easier to apply than ELISA. The dipstick assay is suited for use in the field and in laboratories that are not equipped to perform the more complicated tests. The assay also has potential to replace the other methods for distinguishing patients with acute brucellosis from those with a chronic infection or with persisting IgG antibodies due to a previous infection. It may be noted, though, that consistent with the results of ELISA (7, 11) our results show that specific IgM antibodies may remain detectable for several months and sometimes even much longer after the onset of the disease. However, while most serum samples collected early in the disease gave a ≥2+ staining, most of the sera collected 4 to 6 months after treatment had been initiated, tested as 1+.

The five laboratories participating in the study found the test easy to perform and were satisfied with the result. It was also noted that the development of color on the dipstick could be maintained as a permanent record in a folder or attached to the patient record.

In conclusion, the dipstick assay described here is an easy-to-perform method for the quick serodiagnosis of acute human brucellosis. Due to its robustness and simplicity, the assay is highly suitable for application under field conditions. Ideally, application of two dipsticks, one for the detection of specific IgM antibodies and another for the detection of specific IgG antibodies, would be needed to cover the possibility of both acute or recent and chronic brucellosis. The development of an IgG-specific rapid test for brucellosis is now in progress. A further prospective study will be required to demonstrate the clinical utility of the assay and to calculate the sensitivity, specificity, and predictive value for patients living in an area where brucellosis is endemic.

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REFERENCES


