SIMPLE DIPSTICK ASSAY FOR THE DETECTION OF SALMONELLA TYPHI-SPECIFIC IgM ANTIBODIES AND THE EVOLUTION OF THE IMMUNE RESPONSE IN PATIENTS WITH TYPHOID FEVER

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Abstract. Application of a dipstick assay for the detection of Salmonella typhi-specific IgM antibodies on samples collected from S. typhi or S. paratyphi culture-positive patients at the day of admission to the hospital revealed the presence of specific IgM antibodies in 43.5%, 92.9%, and 100% for samples collected 4–6 days, 6–9 days, and > 9 days after the onset of fever, respectively. The mean sensitivity for samples collected at an average of 6.6 days after the onset of fever was 65.3%. Culture was positive in 65.9% of the cases with a final clinical diagnosis of typhoid fever. Testing of paired serum samples from culture negative patients with a final clinical diagnosis of typhoid fever resulted in staining of the dipstick in 4.3% of the samples collected at the day of admission to the hospital and in 76.6% of the samples collected one week later, thereby provided strong supporting evidence of typhoid fever by demonstrating seroconversion in a large proportion of the patients. The dipstick assay may thus also be useful for the serodiagnosis of culture-negative patients with clinical signs and symptoms consistent with typhoid fever. The advantages of the dipstick assay are that the result can be obtained on the same day allowing a prompt treatment, that only a small volume of serum is needed, and that no special laboratory equipment is needed to perform the assay. The stability of the reagents of the dipstick and the simplicity of the assay allows its use in places that lack laboratory facilities.

INTRODUCTION

Typhoid fever is still an important health problem in many developing countries. Worldwide, an estimated 17 million cases occur annually. Most of this burden occurs among citizens of low-income countries, particular those in South East Asia, Africa, and Latin America. The clinical diagnosis of this condition is considered to be unreliable. A definite diagnosis is obtained when the etiologic agent, Salmonella typhi, is isolated from bone marrow or blood. Facilities to perform this condition is considered to be unreliable. A definite diagnosis is obtained when the etiologic agent, Salmonella typhi, is isolated from bone marrow or blood. Facilities to perform this complicated and time-consuming procedure are usually not available in endemic areas. In these problematic situations the Widal test can be used to aid the clinical diagnosis. However, many limitations lead to difficulties in the interpretation of the Widal test. Results of the Widal test have demonstrated to vary between different areas and in time, due to variation in background levels as well as a result of variation in the quality of the antigen. The need for a rapid and inexpensive laboratory test for early and accurate diagnosis of patients with typhoid fever has prompted the exploration of a variety of serologic and antigen detection methods, including counter immunoelectrophoresis, enzyme-linked immunosorbent assay (ELISA), dot immunoassay, hemagglutination, and coagglutination. However, these assays are not very easy to perform, not rapid, require special equipment or skills, or depend on electricity and on refrigeration for storage of components. None of these assays has yet reached widespread use.

To fulfill the need for a simple and rapid laboratory test we have developed a dipstick assay for the detection of IgM antibodies for S. typhi. The dipstick assay is a simplified version of the ELISA that can be used without the need for special equipment or electricity. The assay uses stabilized components that can be stored for more than two years outside the refrigerator. The ELISAs for typhoid fever have found to be superior to the Widal test. In this study, the clinical utility of the dipstick assay was evaluated in an endemic area in Indonesia and on a collection of serum samples from Kenya.

The dipstick assay is based on methodology also used in dipstick assays developed for the serodiagnosis of leptospirosis and brucellosis. The assay format has proven to be well suited for use in clinical settings that lack laboratory facilities to perform the more complicated standard laboratory tests.

MATERIALS AND METHODS

Patients. The following groups were included in the study. Patients from Indonesia with clinical suspicion of typhoid fever. Blood and serum samples were collected from clinically suspected typhoid patients on admission at the Hasanuddin University Hospital of Makassar, South Sulawesi, Indonesia at three primary health care centers in Makassar, and at a District Hospital in Gowa. Gowa district is located about 30 km south of Makassar. A total of 245 patients were included in the study. The mean age of the patients was 21.4 years (range = 7–50). They had a mean temperature on admission of 38.0°C (range = 37.0–40.5°C) and a mean duration of illness on admission of 6.6 days (range = 4–19). The main clinical symptoms were hepatomegaly (19.2%), confusion (17.6%), and splenomegaly (13.5%). Abdominal pain was reported in 3.3% of the patients. Gastrointestinal bleeding, jaundice, and rash were observed in less than 1%. Follow-up samples (n = 192) collected an average of one and two weeks after the initial sample could be collected from 86 (35.1%) patients.

Hospital control group from Indonesia. Acute-phase serum samples were collected from a group of 259 hospitalized patients from Makassar with a final diagnosis other than typhoid fever. The final diagnosis was hepatitis for 82 patients, leptospirosis for 35 patients, malaria for 74 patients, upper respiratory tract infection for 48 patients, and pyrexia of unknown origin for 20 patients. Their mean age was 32 years (range = 2–70). They had a mean temperature on admission of 38.2°C (37.1–40.1°C) and a mean duration of illness on admission of eight days (range = 4–21).

Healthy controls from Indonesia. Serum samples were collected from 194 healthy school children (mean age = 11.7
years, range = 10–14) from a village located in Gowa district, Sulawesi, Indonesia.

Hospital control group from a non-endemic area. Samples were collected from patients from the Netherlands with various diseases including infection with human immunodeficiency virus (n = 20), hepatitis A (n = 10), hepatitis B (n = 9), syphilis (n = 20), malaria (n = 20), toxoplasmosis (n = 11), meningitis (n = 10), meningococcal meningitis (n = 10), Lyme borreliosis (n = 20), hantavirus infection (n = 20), and an autoimmune disease (rheumatoid arthritis n = 10, systemic lupus erythematosus n = 20).

Case patients and controls from Kenya. Serum samples were collected at St. Mary’s Hospital, Mumias, Kenya from patients in the early stage of the disease with fever including 19 patients with a blood culture proven S. typhi infection, eight patients with a blood culture-proven S. enteritidis infection, six patients with a blood culture-proven S. typhimurium infection, and 185 patients with a final diagnosis other than enteric fever.

Ethical considerations. The project was approved by the review boards of the participating institutes and informed consent for participation in the study was obtained from all participants or their parents/guardians.

Culture. Blood culture was performed for each of the group of patients with clinically suspected typhoid fever from Indonesia and for all patients with fever from Kenya. The blood culture was performed by inoculation of 15 ml of bile broth (Merck, Rahway, NJ) with 5 ml of freshly collected blood. Cultures were incubated for 24 hr at 37°C. A 1 ml culture sample was then plated on Salmonella Shigella agar. After incubation for 24 hr at 37°C, colonies were examined by Gram staining and tested biochemically to identify S. typhi-positive cultures.

Widal test. The Widal test procedure using O antigen was performed according to the manufacturer’s protocol (Murex Biotech, Ltd., Dartford, UK). Briefly, two-fold serial dilutions (1:20–1:1,280) of the serum sample were prepared. One drop (~25 μl) of the O antigen suspension was added to each tube containing the diluted sample. Antigen and serum were mixed and incubated at 50°C. Tubes were checked for agglutination after 4 hr. According to routine diagnostic criteria, a titer ≥ 1:320 was considered positive for the samples tested in Indonesia. In Kenya, a titer ≥ 1:160 was considered positive.

Dipstick assay. The dipstick consists of a strip of nitrocel lulose membrane containing a 2 mm-wide line of immobilized antigen as detection band and a separate line of immobilized anti-human IgM antibody as reagent control that is adhered to a rigid backing. The antigen was prepared from a culture of a recent isolate of S. typhi from Indonesia. The culture was grown in LB broth and the antigen was prepared by heating a washed and 15× concentrated bacterial cell suspension of a three-day old culture for 30 min at 95°C. Cell debris was removed by centrifugation. The supernatant was diluted five times and blotted in 2 mm-wide lanes onto a nitrocellulose membrane by incubation for 2 hr at 40°C. At the end of the incubation the lanes of the blotting apparatus were rinsed with phosphate-buffered saline (PBS) to remove excess antigen. Blotted strips were rinsed with PBS, blocked with 3% skim milk, rinsed again, and allowed to dry. The non-enzymatic detection reagent consists of a monoclonal anti-human IgM antibody conjugated to a colloidal suspension of Palanyl red. Briefly, the monoclonal antibody was labeled with a washed suspension of Palanyl red in 10 mM phosphate buffer containing 2.7 mM NaCl. The concentration of the dye suspension was adjusted such that a 1:500 dilution had a spectrophotometric absorbance of 520 nm (A520) of 1. The conjugate was subsequently blocked with 30% bovine serum albumin in 5 mM NaCl. After blocking, the conjugate was suspended in 32.3 mM phosphate buffer containing 125 mM NaCl, 6% trehalose, and 1.67% bovine serum albumin. Finally, the suspension was lyophilized for preservation.

The dipstick assay was performed by incubation of a wetted dipstick in a mixture of 5 μl of serum and 250 μl of detection reagent for 3 hr at room temperature. At the end of the incubation the dipsticks were thoroughly rinsed with water, and dried. The staining intensity of the antigen band was then graded by comparison with a colored reference strip. The test result is scored as negative when no staining was observed, 1+ when a weak staining was observed, and 2+, 3+, or 4+ when a moderate-to-strong staining was observed.

Statistical analysis. Difference in the immune response between groups for age, fever, and duration of illness at time of sampling, and for result of culture were analyzed by linear regression analysis using the SPSS (SPSS, Inc., Chicago, IL) computer package.

RESULTS

A final diagnosis of enteric fever due to either S. typhi or S. paratyphi was made for 179 of the 245 suspected patients from Indonesia. The final diagnosis was based on a positive blood culture for 118 (65.9%) patients and on clinical symptoms and signs consistent with typhoid or paratyphoid fever for 61 (34.1%) patients. Salmonella typhi was isolated from the cultures of 112 patients and S. paratyphi from six of them. An alternative final clinical diagnosis was made for 64 patients. The final diagnosis for the latter group of patients was malaria (23 cases), hepatitis (20 cases), upper respiratory tract infection (11 cases), dengue hemorrhagic fever (1 case), and pyrexia of unknown origin (9 cases). Three patients died before a final diagnosis could be made.

A positive result in the dipstick assay for samples collected at the time of hospitalization was obtained for 73 (65.2%) of the S. typhi culture-positive patients and for four (66.6%) of the S. paratyphi culture-positive patients (Table 1). A positive result in the dipstick assay was also obtained for some (13.1%) of the blood culture negative patients with a final clinical diagnosis of typhoid fever. None of the samples from the patients with a final diagnosis other than enteric fever gave a positive result. These results indicate a sensitivity for the dipstick assay of 65.3% for samples collected at the time of admission to the hospital from the S. typhi and S. paratyphi culture-positive patients (Table 2). The sensitivity for samples collected at admission for the total group of culture-positive and -negative patients with a final diagnosis of enteric fever was 47.5%.

The sensitivity of the dipstick increased with the duration of fever and was, as calculated for the group of culture-proven patients, 43.5% for patients with 4–6 days of fever prior to admission to the hospital and laboratory testing, 92.9% for patients with 7–9 days of fever, and 100% for patients with > 9 days of fever (Table 2). For the combined group of culture-positive and culture-negative patients with a final diagnosis of typhoid or paratyphoid fever, the sensitivity was lower in par-
Results of the dipstick assay and Widal test for samples collected at
clinically suspected typhoid and paratyphoid patients (Kenya)
Hospital controls (The Netherlands)
School children (Indonesia)
Hospital controls (The Netherlands)
Clinically suspected typhoid and paratyphoid patients (Kenya)
<table>
<thead>
<tr>
<th></th>
<th>No. of patients with a positive result (%)</th>
<th>total no. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipstick assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture positive</td>
<td>85 (47.5)/179</td>
<td>85 (47.5)/179</td>
</tr>
<tr>
<td>Culture negative</td>
<td>73 (65.2)/112</td>
<td>68 (60.7)/112</td>
</tr>
<tr>
<td>Culture positive</td>
<td>4 (66.6)/6</td>
<td>5 (83.3)/6</td>
</tr>
<tr>
<td>Culture negative</td>
<td>8 (13.1)/61</td>
<td>12 (19.7)/61</td>
</tr>
<tr>
<td>Final diagnostic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture positive</td>
<td>0 (0)/64</td>
<td>10 (15.6)/64</td>
</tr>
<tr>
<td>Culture positive</td>
<td>0 (0)/259</td>
<td>NT*</td>
</tr>
<tr>
<td>Culture positive</td>
<td>2 (1)/194</td>
<td>NT</td>
</tr>
<tr>
<td>Culture negative</td>
<td>0 (0)/180</td>
<td>NT</td>
</tr>
<tr>
<td>Clinically suspected typhoid and paratyphoid patients (Kenya)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. typhi culture positive</td>
<td>14 (73.7)/19</td>
<td>16 (84.2)/19</td>
</tr>
<tr>
<td>S. enteritidis culture positive</td>
<td>5 (62.5)/8</td>
<td>6 (75.0)/8</td>
</tr>
<tr>
<td>S. typhimurium culture positive</td>
<td>0 (0)/6</td>
<td>0 (0)/6</td>
</tr>
<tr>
<td>Culture negative</td>
<td>7 (3.8)/185</td>
<td>33 (33.0)/100</td>
</tr>
</tbody>
</table>

* NT = not tested.

ticular for samples collected during the first days with fever
(Table 2). The increase in sensitivity of the dipstick assay with
the duration of fever correlated with an increase in the staining
intensity of the antigen band of the dipstick. The staining
intensity was graded moderate (2+) to strong (4+) for 13.3%,
68%, and 85.7% of the positive samples collected from the
patients with fever ranging from 4–6, 7–9, and > 9 days of fever, respectively. A
1+ staining intensity was recorded for the remaining dipstick-positive
samples.

To further investigate the antibody response, follow-up
samples collected an average of one and two weeks after
culture-proven positive patients. A positive result in the dipstick assay was obtained in
76.9% of the initially collected samples and seroconversion was observed for eight
(20.5%) patients at subsequent collections. In contrast, the
initially collected samples from patients with disease were collected in the early stage of the disease from culture-proven
patients with a negative blood culture. The samples from the
culture-negative patients with typhoid fever were collected an
average of two days earlier than the samples from the culture-positive
patients. Regression analysis indicated that this difference in time of sample collection contributed to the lower
percentage of dipstick positive result for the initially collected
samples of the culture-negative group and thus to the higher
seroconversion rate observed for this group. Age and the
degrees of fever were not related to the difference in results
of the dipstick assay for the two groups.

The specificity of the dipstick assay as calculated for the
group of suspected patients with a final diagnosis other than
paratyphoid fever was 100% (Table 1). The high
specificity was confirmed by testing samples collected in an
endemic area from a hospital population with a final diagnosis
other than typhoid fever and by testing samples from healthy
schoolchildren from Indonesia. None of the 258 sera from the
hospital control group tested positive in the dipstick assay.
Two samples from the school children survey tested weakly
positive. The other 192 (99%) samples were negative. Cross-
reactivity also was not observed for the 180 samples from
patients with various diseases from a non-endemic area.

The sensitivity of the Widal test at the routinely used cut-
off level of ≥ 1:320 for samples collected at hospital admission
was 60.7% for the S. typhi culture-positive patients and 83.3%
for the S. paratyphi culture-positive patients (Table 1). The
specificity was 88.4%.

In a separate study performed in Kenya on serum samples
collected in the early stage of the disease from culture-proven
patients, a positive result in the dipstick assay was obtained in
samples from 14 (73.7%) of 19 patients with an S. typhi
infection, and from five (62.5%) of eight patients with an S.
enteritidis infection. Samples from six patients with an S.
typhimurium infection gave a negative result. Staining was also
observed for seven of 185 patients with a final diagnosis other
than typhoid fever, indicating a specificity of 96.2%. In the
study performed on the samples collected in Kenya, a strong
cross-reactivity was observed for a sample from a patient with
a positive blood culture for Escherichia coli. The Widal test
was performed on part of the samples from Kenya and gave
a relatively low sensitivity (66.6%) and specificity (67.0%) at
the routinely used cut-off level of ≥ 1:160.

![Table 2](image)

<table>
<thead>
<tr>
<th>Duration of fever (days)</th>
<th>No. of patients with a positive dipstick result (%)</th>
<th>total no. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients with a final clinical diagnosis of typhoid or paratyphoid fever</td>
<td></td>
</tr>
<tr>
<td>Dipstick assay</td>
<td>Culture positive</td>
<td>Culture positive and negative</td>
</tr>
<tr>
<td>4–6</td>
<td>30 (43.5)/69</td>
<td>33 (28.9)/114</td>
</tr>
<tr>
<td>7–9</td>
<td>26 (92.9)/28</td>
<td>28 (70.0)/40</td>
</tr>
<tr>
<td>&gt;9</td>
<td>21 (100)/21</td>
<td>24 (96.0)/25</td>
</tr>
<tr>
<td>Total</td>
<td>77 (65.3)/118</td>
<td>85 (47.5)/179</td>
</tr>
<tr>
<td>Widal test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–6</td>
<td>33 (47.8)/69</td>
<td>39 (34.2)/114</td>
</tr>
<tr>
<td>7–9</td>
<td>21 (75.0)/28</td>
<td>25 (62.5)/40</td>
</tr>
<tr>
<td>&gt;9</td>
<td>19 (90.4)/21</td>
<td>21 (84.0)/25</td>
</tr>
<tr>
<td>Total</td>
<td>73 (61.8)/118</td>
<td>85 (47.5)/179</td>
</tr>
</tbody>
</table>
Dipstick results for follow-up samples collected from Salmonella typhi culture-positive patients and from culture-negative patients with a final diagnosis of typhoid fever

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean no. of days with fever (25th–75th percentiles)</th>
<th>No. of patients with a positive dipstick result (%)</th>
<th>Staining intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st collection</td>
<td>30 (76.9)/39</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>2nd collection</td>
<td>32 (82.1)/39</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>3rd collection</td>
<td>38 (97.4)/39</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Culture-negative patients

| 1st collection    | 2 (4.3)/47                                           | 45                                                 | 1                 |
| 2nd collection    | 36 (76.6)/47                                         | 11                                                 | 2                 |
| 3rd collection    | 39 (83.0)/47                                         | 8                                                  | 4                 |

Total

| 1st collection    | 32 (37.2)/86                                         | 54                                                 | 1                 |
| 2nd collection    | 68 (79.1)/86                                         | 18                                                 | 2                 |
| 3rd collection    | 77 (89.5)/86                                         | 9                                                  | 3                 |

DISCUSSION

In our study in Indonesia, blood culture confirmed the final diagnosis of typhoid fever in 112 patients with clinical suspected typhoid fever. Culture demonstrated an infection with S. paratyphi in six other patients. A final diagnosis of typhoid fever also was made on clinical signs and symptoms for 61 suspected patients despite of a negative culture result. A final diagnosis other than typhoid fever was made for 64 suspects based on the evolution of the disease and additional laboratory testing. These results demonstrate that the early clinical symptoms and signs of typhoid fever are not very specific and that further clinical and laboratory investigation is needed to come to a final diagnosis.

Culture is an accurate method for the diagnosis of typhoid fever for blood samples drawn early in the disease. More than 70% of the cases will be confirmed when multiple blood samples are tested. However, the use of antibiotics and the often low amount of bacteria present in the blood may hamper the result. The amount of blood used to inoculate the culture also may affect the detection rate of culture. Ideally, at least 10 ml of blood is needed to inoculate the culture. Reluctance to donate a sufficiently large blood volume is common in many countries. For this reason, 5 ml of blood was used to inoculate the cultures. Culture has a number of other limitations as well. Culture takes a minimum of 2–3 days to get a result, is expensive, and requires specific laboratory facilities and trained staff to perform the culture and the serologic and biochemical testing that is needed for identification. Based on the final clinical diagnosis, the detection rate of the culture was 65.9% for single blood samples drawn an average of six days after the onset of fever. The use of antibiotics before hospital admission could well have contributed to the relatively low sensitivity of the culture procedure.

The dipstick assay was demonstrated to have a high specificity. The specificity was 100% for patients with clinical suspicion of typhoid fever from an endemic area in Indonesia. The fact that it usually takes about 5–7 days after the onset of fever before detectable antibody levels are present contributes to the limited sensitivity of this serologic detection method in that period of the disease. The sensitivity was 65.3% for samples drawn at hospitalization from blood culture-positive patients with an average 6.6 days of fever and clearly varied with the duration of the disease from 43.5% for patients with 4–6 days of fever to 92.9% for patients with 7–9 days of fever and to 100% for patients with more than nine days of fever. Ideally, serologic testing for infectious diseases should be performed on paired serum samples as demonstration of seroconversion or an increase in titer provides stronger evidence of the infection than demonstration of an elevated antibody level in a single serum sample. Testing of paired samples increased the sensitivity of the dipstick. Indeed testing of a second serum sample collected on average after one week of hospitalization increased the sensitivity from 76.9% for samples collected at the time of hospitalization to 83.1% for the culture-positive patients. Seroconversion also was observed for the majority of the blood culture-negative patients with clinical typhoid fever. The sensitivity for this group of patients was 4.3% for samples collected at the time of hospitalization and 76.6% for samples collected one week later. Possibly the group of culture-negative patients with a final clinical diagnosis of typhoid fever included a number of patients who did not have typhoid fever, explaining the somewhat lower response in the dipstick assay as well as in the Widal test. One also could speculate that antibiotic use before hospitalization or the dose of infection might have affected the result of culture and contributed to a slower development of the immune response in the culture-negative group. It also is possible that they were cases who presented earlier in the course of their illness.

Cross-reactivity in the dipstick assay could be expected in case of septicemia with other salmonellae species sharing antigen determinants with S. typhi. Salmonella typhi shares the somatic or O antigen type 9 with a number of other salmonellae species of Salmonella group D1 organisms including S. enteritidis. In addition S. typhi shares the O antigen type 12 with group A and B organisms including S. paratyphi A and S. paratyphi B and S. typhimurium, respectively. The results of the present study demonstrated cross-reactivity in the dipstick assay in patients with a positive blood culture for S. paratyphi or S. enteritidis. A positive result in the dipstick assay for patients with an S. paratyphi or S. enteritidis infection in the blood would still result in an appropriate treatment. No reactivity was observed when testing samples from six patients with an S. typhimurium infection. Despite the fact...
that *S. typhi* and *S. typhimurium* share some antigens, these results may indicate that these common antigens either are not present in the antigen preparation used in the dipstick assay or that these common antigens do not give rise to a strong detectable IgM antibody response. It should be noted although that the samples from the *S. typhimurium*-positive patients were all collected during the first week of the illness, possibly before the appearance of specific antibodies. Cross-reactivity potentially also could be expected in patients with previous typhoid vaccination. However, vaccination is rare in Indonesia and in Kenya.

The sensitivity of the dipstick assay and of the Widal test were similar, but the specificity of the dipstick assay was higher. In this study, results of the Widal test were based on routinely applied cut-off values. Although the specificity of the Widal test could be improved by using a one-titer step higher cut-off value, this was unacceptable because it resulted in a sensitivity that was too low. The value of the Widal test recently was re-evaluated in a study performed in Vietnam. 37

The major advantages of the dipstick assay are that it assay is easy to use, does not require special equipment or training, and uses stabilized components. It therefore has a potential high degree of acceptability. Culture is clearly the method of choice in situations where laboratory facilities to perform the assay and the required typing procedure are available. Disadvantages of culture are that it can be done only by few specialized laboratories, it requires a relatively large volume of blood collected by venipuncture, and the result is obtained only after 2–3 days. A further disadvantage is that multiblood cultures must be performed to ensure a high sensitivity, and to exclude contamination. Performance of the dipstick assay does not require laboratory facilities and the result of the dipstick can be obtained in about 3 hr. Thus, application of the dipstick clearly offers the opportunity to start the appropriate treatment at the same day the patient is admitted to the hospital. For this reason, the dipstick assay could be of high value even in situations where culture facilities are available. Testing of follow-up samples improves sensitivity. Testing of follow-up samples in the dipstick assay proved to be useful in culture-negative patients who had clinical signs and symptoms consistent with typhoid fever since seroconversion was observed in 72.3% of these patients at a average of seven days after admission to the hospital. Therefore, the dipstick assay not only can be useful for diagnosing typhoid fever in health care facilities with no laboratory support capable of performing cultures, but also can provide a meaningful addition to culture. The clinical utility of the dipstick assay was recently also demonstrated in a study performed in Vietnam. 38 A sensitivity of 77% and a specificity of 95% was calculated for the dipstick assay in that study.

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