Development of a Simple, Peripheral-Blood-Based Lateral-Flow Dipstick Assay for Accurate Detection of Patients with Enteric Fever

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Enteric fever is a systemic infection caused by typhoidal strains of Salmonella enterica and is a significant cause of mortality and morbidity in many parts of the world, especially in resource-limited areas. Unfortunately, currently available diagnostic tests for enteric fever lack sensitivity and/or specificity. No true clinically practical gold standard for diagnosing patients with enteric fever exists. Unfortunately, microbiologic culturing of blood is only 30 to 70% sensitive although 100% specific. Here, we report the development of a lateral-flow immunochromatographic dipstick assay based on the detection of Salmonella enterica serovar Typhi (S. Typhi) lipopolysaccharide (LPS)-specific IgG in lymphocyte culture secretion. We tested the assay using samples from 142 clinically suspected enteric fever patients, 28 healthy individuals residing in a zone where enteric fever is endemic, and 35 patients with other febrile illnesses. In our analysis, the dipstick detected all blood culture-confirmed S. Typhi cases (48/48) and 5 of 6 Salmonella enterica serovar Paratyphi A blood cultured-confirmed cases. The test was negative in all 35 individuals febrile with other illnesses and all 28 healthy controls from the zone of endemicity. The test was positive in 19 of 88 individuals with suspected enteric fever but with negative blood cultures. Thus, the dipstick had a sensitivity of 98% compared to blood culture results and a specificity that ranged from 78 to 100% (95% confidence interval [CI], 70 to 100%), depending on the definition of a true negative. These results suggest that this dipstick assay can be very useful for the detection of enteric fever patients especially in regions of endemicity.

Enteric fever can be due to typhoid and paratyphoid fever and is caused by infection with Salmonella enterica serovar Typhi (S. Typhi) or Salmonella enterica serovar Paratyphi (S. Paratyphi) (1, 2). Approximately 22 million cases of typhoid fever and 6 million cases of paratyphoid fever occur annually, resulting in over 100,000 deaths globally each year (3–5). The occurrence of enteric fever is mainly associated with the lack of proper sanitation and fecal contamination of water and food (2, 4). Rapid accurate diagnosis followed by early treatment with suitable antibiotics can reduce the rates of morbidity and mortality due to enteric fever. As the clinical features of enteric fever are nonspecific and overlap with other illnesses and all 28 healthy controls from the zone of endemicity. The test was positive in 19 of 88 individuals with suspected enteric fever but with negative blood cultures. Thus, the dipstick had a sensitivity of 98% compared to blood culture results and a specificity that ranged from 78 to 100% (95% confidence interval [CI], 70 to 100%), depending on the definition of a true negative. These results suggest that this dipstick assay can be very useful for the detection of enteric fever patients especially in regions of endemicity.

Although microbiologic culturing of bone marrow culture is often used as an alternative (11). Unfortunately, blood culture shows poor sensitivity, ranging from 30% to 70% depending on different factors, including blood volume and prior antibiotic treatment (1, 11–13); it may take 2 to 7 days to confirm the diagnosis and requires a well-equipped laboratory and expertise for microbiologic confirmation (11). The Widal test is the most widely used serological test for diagnosing individuals with enteric fever, but it lacks specificity, especially in areas where enteric fever is endemic (1, 2, 4, 10, 11). Additional serologic tests include Typhidot (Reszon Diagnostics, Malaysia) that detects IgM and IgG antibodies in peripheral blood to a 50-kDa outer membrane protein of S. Typhi and the Tubex assay (IDL Biotech AB, Sweden) that detects IgM responses in blood to S. Typhi O9 lipopolysaccharide (LPS). These assays have been associated with sensitivities and specificities of 56 to 95% and with specificities of 31 to 97% in field tests (1, 8, 14–16). Molecularly based methods, including nucleic acid amplification tests, have been hampered in field tests by the low organism load and presence of inhibitors in peripheral blood, reagent and equipment expense, and lack of technical expertise although such assays may have higher sensitivity than blood culture (17, 18). We have previously reported development of an enzyme-linked immunosorbent assay (ELISA)-based immunodiagnostic assay, the TPTest, for diagnosing individuals with typhoid and paratyphoid fever. This ELISA-based platform has a sensitivity of 100% compared to blood culture results and a specificity of 78 to 97% (95% confidence interval [CI], 73 to 100%), depending on the definition of a true negative (1, 10, 19).

The TPTest ELISA detects S. Typhi membrane preparation (MP)-specific IgA responses in lymphocyte secretion prepared by
isolating peripheral mononuclear cells (PBMCs) separated using Ficoll Isopaque density gradient centrifugation (1, 10). We have previously reported pilot analysis of simplified methods for cell separation, cell incubation, and dot blot analysis for the detection of the MP-IgA response (1). We therefore undertook to further develop an accurate and simple assay for diagnosing individuals with enteric fever using an immunochromatographic dipstick assay to detect specific antibody responses in specimens prepared by erythrocyte lysis and room air (no supplemental CO₂) incubation at 37°C.

**MATERIALS AND METHODS**

**Ethics statement.** The study and all sample collections and analyses were approved by the research review and the ethical review committees of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), and the Institutional Review Board of the Massachusetts General Hospital. Written informed consent was obtained from all participants residing in Dhaka (Table 1 and Table 2). We collected a sample of venous blood from study participants.

**Study participants and specimen collection.** We enrolled participants presenting to the ICDDR,B with clinically suspected enteric fever (n = 142), defined as a systemic febrile illness of ≥38°C for 3 to 7 days' duration without another obvious source. The median age of the enrolled patients was 7 years (25th and 75th percentiles, 3 and 11 years, respectively). We also enrolled 35 study participants presenting to the ICDDR,B with a febrile illness confirmed not to be enteric fever and 28 adult healthy controls (median age, 25 years; 25th and 75th percentiles, 25 and 28 years, respectively) residing in Dhaka (Table 1 and Table 2). We collected a sample of venous blood from study participants.

**Diagnosis of enteric fever by blood culture.** Using a 3- to 5-ml sample of peripheral blood, we performed microbiological cultures for all suspected enteric fever patients using a BacT/Alert automated system, subculturing positive bottles on MacConkey agar, blood agar, and chocolate agar plates and identifying colonies using standard biochemical tests and reaction with Salmonella-specific antisera (1). Blood culture was carried out in patients with other illnesses except those with tuberculosis or kala-azar.

**Preparation of the coating antigen for strips.** We used membrane preparation (MP) and lipopolysaccharide (LPS) as coating antigens prepared from the Ty21a vaccine strain and S. Typhi wild-type strain (ST-004), respectively, for making immunochromatographic strips. MP antigen was prepared according to a previously described method (1). The bacterial strain was cultivated on horse blood agar plates, and bacteria were harvested in buffer (5 mM MgCl₂, 10 mM Tris, pH 8.0). The bacterial suspension was sonicated five times at 60% amplitude and centrifuged at 1,400 × g for 10 min. The supernatant was then transferred to fresh tubes and centrifuged at 14,900 × g for 30 min. The pellet was dissolved in harvest buffer, and the protein content was determined by a Bio-Rad protein assay. LPS antigen was prepared from a wild-type clinical isolate of S. Typhi isolated from a patient, using a phenol-water extraction procedure, followed by enzyme treatment with proteinase K, DNase, and RNase and ultracentrifugation as previously described (10).

**Gold preparation and conjugation.** To prepare colloidal gold, we mixed 0.01% HAuCl₄ (Sigma-Aldrich) with 0.024% sodium citrate (Sigma-Aldrich) in water for injection (WFI) and boiled the solution until it became the color of red wine. The colloidal gold was then filtered through a 0.2-μm-pore-size filter. We adjusted the pH of the gold solution to 8.0 (optimum pH for conjugation) and tested a range of goat anti-human IgG (Jackson ImmunoResearch) and goat anti-human IgA (Jackson ImmunoResearch) to conjugate 1 ml of colloidal gold, eventually choosing 12 μg and 16 μg, respectively, based on the data below. We blocked the conjugated antigen-gold using 20% bovine serum albumin (BSA) and then centrifuged at 10,000 rpm for 45 min at 4°C. We discarded the supernatant and resuspended the pellet in 0.02 M Tris buffer containing 1% BSA. This solution was passed through a 0.2-μm-pore-size filter and used as the detection conjugate.

**Aggregation testing.** To assess stability of the conjugate and to define the optimum pH and minimum concentration of antibody required for conjugating the colloidal gold, we used an aggregation assay (20–22). Specifically, we added 10% NaCl to the gold-protein suspension, incubated it for 10 min, and then assessed stability and polydispersity by measuring the absorbance at 520 nm, 580 nm, and 600 nm (20–22).

**Preparation of the lateral-flow strip.** We used a backing card containing a nitrocellulose membrane on which antigen and antibody were dispensed to create test control lines by using a rapid test dispenser (HM3030; Kin Biotech Co., China). The dispensed membrane was dried for 90 min. A conjugate pad was then made by soaking glass fibers (Kin Biotech Co., China) in the gold conjugate solution and drying the pad for 2 h. The pad was then pasted on the backing card in a way that overlapped the nitrocellulose membrane (High Flow Plus 120 Membrane Card; Millipore). A glass fiber sample pad was placed at the bottom of the backing pad.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value for the subject group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td>Value for the subject group</td>
</tr>
<tr>
<td>No. of male subjects (%)</td>
<td>71 (50)</td>
</tr>
<tr>
<td>No. of female subjects (%)</td>
<td>71 (50)</td>
</tr>
<tr>
<td><strong>Sample size (no.)</strong></td>
<td>53</td>
</tr>
<tr>
<td><strong>Median age (25th and 75th percentiles [yr])</strong></td>
<td>6 (3, 12)</td>
</tr>
<tr>
<td><strong>No. of female subjects (%)</strong></td>
<td>35 (66)</td>
</tr>
<tr>
<td><strong>Duration of fever (25th and 75th percentiles [days])</strong></td>
<td>5 (5, 6)</td>
</tr>
<tr>
<td><strong>No. of subjects with prior use of antibiotics (%)</strong></td>
<td>9 (17)</td>
</tr>
<tr>
<td><strong>Parameter</strong></td>
<td>Value for the subject group</td>
</tr>
<tr>
<td>Suspected enteric fever</td>
<td>7 (3, 11)</td>
</tr>
<tr>
<td>Other febrile illness</td>
<td>25 (23, 30)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>25 (25, 28)</td>
</tr>
<tr>
<td>Median age (25th and 75th percentiles [yr])</td>
<td>69 (19)</td>
</tr>
</tbody>
</table>

**TABLE 1** Characteristics of study participants

**TABLE 2** Characteristics of study participants with suspected enteric fever
card to overlap the conjugate pad to facilitate the flow of sample from a specimen vial to the strip. To accelerate migration of the samples through the strip, we used cellulose fiber as an absorbent pad pasted on the backing card opposite the conjugate pad. All pads were cut to make the desired strip shape by using a guillotine cutter (CT300 and ZQ2000; Kin Biotech Co., China).

Separation of peripheral blood cells by erythrocyte lysis. To recover peripheral lymphocytes, we lysed erythrocytes present in 1 to 2 ml of venous blood using lysis buffer (0.15 M ammonium chloride, 1 mM potassium bicarbonate, and 0.01 mM disodium EDTA) at a 1:5 dilution and mixed the sample by gently inverting the tube (BD Falcon) three to five times. The tube was then kept at room temperature for 10 min and centrifuged at 953 × g for 5 min at 20°C. We decanted the supernatant and resuspended the pellet in 150 μl of RPMI 1640 medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (HyClone), 1% penicillin-streptomycin (Gibco), 1% sodium pyruvate (Gibco), and 1% L-glutamine (Gibco). We cultured the suspended cells in culture vials (North China Pharmaceuticals Co., Ltd., China) without any antigenic stimulation at 37°C without 5% CO₂ for 48 h. We then harvested the culture suspension and centrifuged it at 11,600 × g at 20°C for 5 min to collect the supernatant.

Testing the strip. The strip contained two lines on the nitrocellulose membrane: one was the test line containing MP or LPS antigen, and the other was the control line containing rabbit anti-goat IgG (Jackson ImmunoResearch). The conjugate pad contained goat anti-human IgG or goat anti-human IgA conjugated to colloidal gold. We diluted 75 μl of the lymphocyte culture supernatant with 0.02 M Tris–1% BSA–3% Tween at a 1:1 dilution in a microcentrifuge tube and dripped the strip into the tube for 15 min. The test line and/or control line would appear as a red line. The presence of both the control line and the test line indicated that the sample was positive for the test undertaken. The presence of only the control line but no test line indicated a negative result for the test.

Detection of S. Typhi LPS-specific antibodies in specimens by ELISA. We compared strip results to those obtained using an anti-S. Typhi LPS ELISA format, as previously described (10). Briefly, we coated microtiter plates (Nunc F; Nunc) with 100 μl of LPS (2.5 μg/ml) and blocked the samples with 1% BSA in phosphate (PBS). To detect antigen-specific responses, we added 100 μl of lymphocyte culture supernatant (1:2 dilution in 0.1% BSA–PBS–Tween) to coated plates, which were then incubated for 90 min at 37°C. After plates were washed with 0.05% PBS–Tween, we added rabbit anti-human IgG (1:1,000 dilution; Jackson ImmunoResearch) conjugated to horseradish peroxidase and incubated the plates for 90 min at 37°C. We then washed the plates with phosphate (Sigma) in 0.1 M sodium citrate buffer and 0.1% hydrogen peroxide after the plates were washed with 0.05% PBS–Tween. We then read the plates kinetically at 450 nm for 5 min at 19-s intervals. The maximal rate of optical density (OD) change was expressed as milliabsorbance per minute. For normalization of the data, the results were divided by readings of in-house pooled convalescent-phase standard sera of blood culture-confirmed typhoid patients and multiplied by 100. Results were expressed as ELISA units (EU).

Statistical analysis. We used GraphPad Prism, version 4, and OpenEpi, version 3, for data management, analysis, and graphical presentation.

RESULTS
Characterization of colloidal gold. After making colloidal gold, we determined the size of the gold nanoparticles by differential light scatterings using a Zetasizer Nano ZS90 instrument (Malvern Instrument, Ltd.). The measurements were carried out at 25°C with a count rate of 193.7 kcps at a scattering angle of 173°. The average diameter of the prepared gold nanoparticles was 20 nm, as determined from the dynamic light scattering (DLS) spectrum (Fig. 1).

 Determination of optimum pH and minimum concentration of detection antibody for conjugation. We performed aggregation testing at different pHs and with increasing amounts of goat anti-human IgG or goat anti-human IgA to produce conjugated gold. After the addition of NaCl, we measured the optical density (OD) at 520 nm, 580 nm, and 600 nm. We used the ratio of the OD at 520 nm to that at 580 nm to assess stability and the ratio of the OD at 600 nm to that at 520 nm to assess polydispersity (20–22). We found the highest stability and lowest polydispersity when colloidal gold was conjugated to both anti-human IgG and IgA at pH 8.0 (Fig. 2). We also found that a minimum of 12 μg of goat anti-human IgG or 16 μg of goat anti-human IgA was required to stabilize 1 ml of colloidal gold solution (Fig. 3).

Results of blood culture. We enrolled total 142 suspected enteric fever patients; among them, 54 patients were blood culture-confirmed enteric fever patients. Among 54 bacteremic patients, 48 were S. Typhi-positive and 6 were S. Paratyphi A-positive patients.

Results of the strip test. We tested the immunochromatographic strips detecting S. Typhi LPS-specific or MP-specific IgA or IgG in lymphocyte culture supernatant of subsets of our cohorts. The strip detecting S. Typhi LPS-specific IgG in lymphocyte culture supernatant was positive in all (n = 48) S. Typhi bacteremic patients and in 5 of 6 S. Paratyphi A bacteremic patients. We also tested the LPS-specific IgG assay using lymphocyte culture supernatant prepared from venous blood of 88 blood culture-negative patients (clinically suspected to have enteric fever), as well as from 28 healthy controls and 35 patients with other febrile illness (tuberculosis, kala-azar, dengue, and non-Salmonella bacteria). We found that the dipstick was positive for 19 blood culture-negative patients and negative for all healthy controls as well as for all the patients with other febrile illness (Fig. 4 and Table 3). The strip that detected S. Typhi MP-specific IgA responses showed variable intensities of test and control lines in culture-confirmed patients, and the strip detecting MP-specific IgG responses, although detecting all blood culture-confirmed patients, was also positive in patients with other febrile illnesses.

Assessing LPS-specific antibody responses in lymphocyte culture supernatant using ELISA. We next assessed IgG antibody responses using an ELISA format and lymphocyte culture supernatant collected from our different categories of patients. Among
them, 22 patients were positive by blood culture and IgG LPS-specific strip test, 9 patients were negative by blood culture and positive by strip test, and 35 patients were negative for both blood culture and strip test. We also measured LPS-IgG responses in 16 healthy individuals and 16 other febrile-illness patients. The patients who had LPS-IgG responses of ≥16 EU were positive by the strip (Fig. 5).

**Determination of sensitivity, specificity, PPV, and negative predictive value for strip test detecting S. Typhi LPS-IgG.** We calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the IgG LPS-specific lateral-flow dipstick (LFD) using OpenEpi, version 3, an open source calculator for the evaluation of diagnostic tests. The test had a sensitivity of 98% compared to blood culture results and specificity, PPV, and NPV that ranged from 78 to 100% (95% CI, 68 to 100%), 73 to 100% (95% CI, 62 to 100%), and 98 to 99% (95% CI, 91 to 99), respectively, depending on the definition of a true negative (Table 4).

**DISCUSSION**

Enteric fever remains an important public health concern in many developing countries. There is a very real need for a low-tech, reliable, and affordable diagnostic test that shows high sensitivity and specificity. In our study, we have reported development of such a test. The test can be performed using 1 ml of venous blood, erythrocyte lysis buffer, a tabletop centrifuge, and a 37°C incubator without CO₂. Responses are detected visually with a simple dipstick. Although such a test cannot be used at the bedside, results with excellent precision and reliability and minimal laboratory capacity are available at 48 h. Our previous data suggest that a reading at 24 h may also be informative (1).

The current immunochromatographic assay and the previously reported ELISA-based TPTest are both based on detection of antibodies secreted ex vivo by activated lymphocytes recovered from the peripheral circulation during acute infection (1, 10, 19). These lymphocytes have been stimulated by the recent infection and require no ex vivo stimulation. Removing the plasma component of blood limits the confounding influence of preexisting circulating antibodies that reflect prior exposure. These circulating antibodies can affect assay specificity and have markedly limited the utility of plasma antibody-based assays in areas of the world where enteric fever and salmonellosis are endemic.

We evaluated our strips using specimens from patients clini-
Healthy controls 28 0 28
S. Paratyphi bacteremia 6 5 1
S. Typhi bacteremia 48 48 0

Study participant group

TABLE 3 Results of strip test detecting LPS-specific IgG

<table>
<thead>
<tr>
<th>Study participant group</th>
<th>Total</th>
<th>Positive strip test</th>
<th>Negative strip test</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhi bacteremia</td>
<td>48</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>S. Paratyphi A bacteremia</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Clinically suspected for enteric fever</td>
<td>88</td>
<td>19</td>
<td>69</td>
</tr>
<tr>
<td>but blood culture negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Febrile illness other than enteric fever</td>
<td>35</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>28</td>
<td>0</td>
<td>28</td>
</tr>
</tbody>
</table>

Clinically suspected to have enteric fever, as well as specimens from healthy individuals and patients with other febrile illnesses. We defined participants whose blood cultures were positive for S. Typhi or S. Paratyphi A as definitive cases of enteric fever. The LPS-specific IgG lymphocyte supernatant-based dipstick had a sensitivity of 98% using this definition. We defined participants with other febrile illnesses and healthy controls as definitive negatives. The LPS-specific dipstick test had a specificity of 100% using this definition. Since there is no clinically practical gold standard for enteric fever and since microbiologic culturing of blood is known to have a sensitivity of 30 to 70% (3, 12, 13), the interpretation of enteric fever and since microbiologic culturing of blood is problematic. Presumably, a subset of these individuals actually do have enteric fever. However, we could estimate a lower specificity value: if we assume that all blood culture-negative patients indeed did not have enteric fever, the LPS-specific IgG dipstick test would still have a specificity of 87% when all negative controls are included. These results are very promising, and considering that some of the culture-negative patients probably did indeed have enteric fever, they are very likely an underestimate of true specificity.

Our assay is based on detecting LPS derived from S. Typhi. Interestingly, our test also detected patients with S. Paratyphi A bacteremia. Paratyphoid fever caused by S. Paratyphi A accounts for up to 1 in 5 cases of enteric fever in some areas of Asia, including Bangladesh (4), and paratyphoid and typhoid fevers can be clinically indistinguishable (4, 23). The S. Typhi LPS serotype is defined by the O antigen, determined by the O-specific oligonucleotide and polysaccharides associated with the LPS. S. Typhi O antigens include serotypes 9 and 12, often expressed on the same organism. S. Paratyphi A antigens include serotypes 1, 2, and 12. The identification of S. Paratyphi A-infected patients by the dipstick assay presumably rests upon the detection of circulating lymphocytes expressing anti-serotype 12 O-antigen antibodies in these individuals.

Invasive salmonellosis is a distinct clinical entity from enteric fever that is caused by traditionally nontyphoidal strains of Salmonella enterica, especially S. Typhimurium and S. enterica serovar Enteritidis. Such invasive nontyphoidal salmonellosis (iNTS) is a significant cause of mortality in malnourished and immunocompromised children, especially HIV-infected individuals in sub-Saharan Africa (24). Although we did not assess our dipstick assay in patients with iNTS (who are rare in Dhaka, Bangladesh), we are encouraged to note that both S. Typhimurium and S. Enteritidis can express O antigen 12, suggesting that the current dipstick assay might be able to detect at least a subset of individuals with iNTS.

Our dipstick assay has a number of limitations. It is not point of care, it requires electricity (centrifuge and incubator), it requires 24 to 48 h until results are available to the clinician, and it does not discern antimicrobial susceptibility profiles. We could not collect a large volume of blood for culture, which may be a reason for the low sensitivity of the blood culture. We enrolled adult healthy controls although suspected enteric fever patients were largely children. However, the dipstick assay is extremely simple, requires only a small volume of peripheral blood and rudimentary laboratory equipment and training, can be read with the naked eye, is as sensitive as blood culture, may be more sensitive than blood culture, and is estimated to be more specific than currently available serology assays. This dipstick assay thus may be quite useful in detecting patients with enteric fever in resource-limited settings with limited facilities, identifying those who could most benefit from appropriate treatment to prevent subsequent complications of enteric fever and minimizing the use of inappropriate antimicrobial agents that
TABLE 4 Sensitivity, specificity, positive predictive value, and negative predictive value of strip test detecting LPS-specific IgG

<table>
<thead>
<tr>
<th>Characterization of participant status</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture-positive patients considered positive; healthy individuals and patients with other febrile illnesses considered negative</td>
<td>98 (90–99)</td>
<td>100 (94–100)</td>
<td>100 (93–100)</td>
<td>98 (91–99)</td>
</tr>
<tr>
<td>Blood culture-positive patients considered positive; blood culture-negative patients, healthy individuals, and other febrile illnesses considered negative</td>
<td>98 (90–99)</td>
<td>87 (81–91)</td>
<td>73 (62–82)</td>
<td>99 (95–99)</td>
</tr>
<tr>
<td>Blood culture-positive patients considered positive; only blood culture-negative patients considered negative</td>
<td>98 (90–99)</td>
<td>78 (68–85)</td>
<td>73 (62–82)</td>
<td>98 (92–99)</td>
</tr>
</tbody>
</table>

*Values in parentheses are 95% CIs.*

drive drug resistance and can cause unnecessary adverse events. This assay could also assist in defining the burden of enteric fever in resource-limited regions and could assist in judging the impact of control programs.

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This test was developed in collaboration with a commercial company.

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