Validation of a new serology-based dipstick test for rapid diagnosis of typhoid fever

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Abstract

Currently, no reliable diagnostic test is available for typhoid fever. One serology-based dipstick test, developed indigenously, was validated in this study. Preserved sera from 336 fever patients with known culture results for Salmonella Typhi were blindly tested by the Widal test and the new assay. Analytical sensitivities, specificities, and efficiencies for the new assay versus the Widal test were 68.8% versus 62.5%, 71.1% versus 37.1%, and 70.5% versus 43.2%, respectively (p < 0.001), considering S. Typhi–positive samples as gold standards. Thereafter, fresh sera from 102 hospital-attending children with clinical typhoid fever (including 20 confirmed nontyphoidal cases as control) were tested by both methods and analyzed statistically. The diagnostic sensitivity, specificity, and efficiency were 51.2%, 85%, and 57.8% for the new assay, and 43.9%, 65%, and 48% for the Widal test, respectively. Overall performance ability of the new assay was not better than the Widal test (p > 0.5). Further improvement of the new point-of-care typhoid assay is recommended before implementation in the field setup.

Keywords:
Typhoid fever
Diagnostic test
Salmonella Typhi
Serology

1. Introduction

Typhoid fever has emerged as one of the major public health problems across the globe. It is estimated that around 21.7 million cases of typhoid fever with more than 217,000 deaths occur annually in Southeast Asian countries (Crump et al., 2004). However, this estimation seems to be underestimated due to poor disease surveillance and lack of reliable point-of-care diagnostic tests (Baker et al., 2010; Crump and Mintz, 2010). Accurate and rapid laboratory diagnosis of the disease is mandatory for early initiation of antibiotic treatment, thus reducing the risk of adverse outcome and mortality (Bhan et al., 2005; Bhutta and Mansurali, 1999).

Isolation of Salmonella enterica serovar Typhi (S. Typhi) in blood culture confirms the diagnosis of typhoid fever and is generally used as a gold standard for validation of new diagnostic assays. But it suffers from poor sensitivity which ranges from approximately 40% to 60% (Farooqui et al., 1991; Vallenas et al., 1985). Studies in Vietnam have reported the presence of lower numbers of the organisms (median, 1 CFU/ml; range, <0.3 to 387 CFU/ml) in peripheral blood due to frequent intake of newer generation of antibiotics by patients prior to attending hospitals (Wain et al., 1998). Bone marrow culture is more sensitive (~80%) than blood culture, and it has been documented that there are high numbers of organisms (median, 9 CFU/ml; range, 0.1 to 1580 CFU/ml) in bone marrow aspirates even with prior antimicrobial therapy, but it is rarely used due to the invasive nature of this technique (Kundu et al., 2006; Vallenas et al., 1985; Wain et al., 2001). Isolation of S. Typhi from stool, urine, or duodenal string cultures is another alternative test, but the results should be interpreted cautiously because it might reflect the chronic carrier state rather than acute infection (Vallenas et al., 1985).

The Widal test is the most commonly used serologic test, which measures the agglutinating antibody titers against S. Typhi lipopolysaccharide (LPS) “O” and flagellar “H” antigen. Test results have only 30% positive correlation with culture-confirmed typhoid fever. False positivity has been reported in fever cases other than typhoid like malaria, dengue, typhus fever, etc., which has negative influence on the reliability of the test (WHO, 2003). Determination of proper baseline cut-off titer in site-specific healthy population and examination of paired sera samples for interpretation further restricted its practical use in endemic areas (Clegg et al., 1994; Parry et al., 1999). Using molecular methods (polymerase chain reaction, DNA microarray) on direct samples might improve the case detection rate by increasing the sensitivity, but lack of standardization and expertise, and increased cost of reagents and equipment have made these methods less attractive in resource-poor settings. Therefore the search for a rapid, simple, and affordable point-of-care diagnostic test for typhoid that can be used in remote fields of endemic areas as a bedside test without the need for sophisticated equipment continues.

A number of new-generation serology-based rapid diagnostic tests for typhoid have been commercially available like Typhidot (Malaysian Bio–Diagnostics Research SDN BHD, Malaysia), TUBEX (IDL Biotech, Sollentuna, Sweden), Multi-Test Dip-S-Tics (PanBio Indx, ...
Baltimore, MD, USA), whose performances have been evaluated worldwide. But none of these tests was time tested and yielded sustainable results when validated in different endemic setups (Parry et al., 2011).

A new serologic assay, based on the principles of immunochromatography as a lateral flow dip stick test (IC-LFT), was developed indigenously by SPAN Diagnostics, Surat, India. IC-LFT qualitatively detects both IgM and IgG antibodies, specific to lipopolysaccharide (LPS) and flagellin of S. Typhi, in human sera/plasma. The test kit was standardized and found appropriate for specific diagnosis of typhoid by the in-house test (data not published). The new assay system has undergone further external validation at the Bacteriology Division of the National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India. The result of the validation is presented in this article.

Briefly, sera samples (collected from fever cases with known culture result for S. Typhi), obtained from the serum bank of NICED, Kolkata, were tested in a blinded manner to determine the analytical sensitivity and specificity of the new assay. Determination of diagnostic efficiency and evaluation of performance ability of the new diagnostic test were carried out in an actual field setting by testing prospective sera samples from hospital-attending children with clinical diagnosis of typhoid fever following standard published methods (OIE, 2012).

2. Materials and methods

2.1. Determination of analytical sensitivity and specificity

Stored sera samples from NICED serum bank collected from fever patients for other studies were used for determining the analytical sensitivity and specificity in the validation assay. A number of sera samples with known S. Typhi culture result were selected and tested blindly by the new kit following the manufacturer’s instructions by 2 separate technicians. Samples were coded for blinding by a separate person who was not involved in the study. A sample was read positive if it was found positive by both workers. All blinded study samples were also examined by the Widal test (Span Diagnostics, Surat, India), and a titer of TO ≥1:80 was considered as positive (Dutta et al., 2006). After decoding all the results of blood culture, the Widal test and the new diagnostic test were made available to a statistician for statistical analysis and comparison between two serology-based tests (new kit versus Widal) considering positive blood culture as the gold standard.

2.2. Determination of diagnostic performances of the new kit by testing prospective sera samples from clinically diagnosed typhoid fever cases in real-life situation

2.2.1. Study population

To determine the diagnostic efficiency of the new kit in a field setup, prospective sampling of blood was carried out at Dr. B. C. Roy Post Graduate Institute of Pediatric Sciences from febrile children of 2–12 years of age, attending the outpatient department of the hospital for seeking treatment from Monday through Friday from 9 am to 1 pm during the period between April 2009 to September 2010. The children, who presented with high fever (>39 °C) and other signs and symptoms suggestive of typhoid fever, were selected for blood sample collection and separation of sera irrespective of history of antibiotic intake and severity/duration of the disease. Dr. B. C. Roy Post Graduate Institute of Pediatric Sciences is the largest referral pediatric hospital in Kolkata and patients from all socioeconomic status come to the hospital either directly or after being referred from other state hospitals.

2.2.2. Sample collection and processing

Blood samples (5 mL) were collected aseptically from febrile children clinically diagnosed as having typhoid fever and were immediately inoculated (4 mL) into a Bactec Peds Plus bottle (BD Bactec System, Franklin Lakes, NJ, USA) for isolation and identification of S. Typhi at the bacteriology laboratory of NICED following standard microbiological techniques (WHO, 1983). Sera were separated from 1-mL blood samples and tested by commercially available Widal test kit (Span Diagnostics) and by the new assay kit. The sera samples were also tested using the Dengue IgM capture ELISA kit (Omega Diagnostics, Scotland, UK) for the diagnosis of dengue fever. Two drops of blood samples were taken on a single glass slide for a thick and thin blood film which was stained by Leishman’s stain and examined microscopically for detection of malaria parasites.

2.3. Ethical consideration

The present study was reviewed and approved by the institutional ethics committee. Blood samples were collected from the febrile children after receiving informed consent from their parents or guardians.

2.4. Microbiological culture of blood

The inoculated Bactec bottles were incubated at 37 °C for 7 days in a Bactec 9120 system (Becton Dickinson, Franklin lakes, NJ, USA), and subcultures were made on MacConkey and nutrient agars (Difco, Sparks, MD, USA) when there was any alarm signal during the incubation period. Non-lactose-fermenting smooth colonies were checked for Salmonella by Gram stain and other biochemical tests following a standard protocol (WHO, 1983). Confirmation of identification of the isolates was done by serotyping in slide and tube agglutination test using Salmonella O, H, and Vi antisera (Denka Seiken, Tokyo, Japan). If there was no growth after 7 days of incubation, blood culture was read as negative.

2.5. Serologic test: the Widal test

Widal test was performed using a quantitative tube agglutination test kit (Span Diagnostics). Serum was serially diluted in physiological saline (0.85%) for titers ranging from 1:4 to 1:64. To each set of diluted serum S. Typhi O and H, antigen suspensions were added separately, so that the final titers became 1:40 to 1:640. The tubes were incubated overnight at 37 °C. The results were read as highest serum dilution giving a visible agglutination with a cut-off titer of TO ≥80 (Dutta et al., 2006). Widal test–positive controls were run in each batch of test.

2.6. New assay system: IC-LFT

The new test kit was used according to the manufacturer’s recommendations. The kit contained antigen (S. Typhi LPS and flagelline) impregnated dipstick and chase buffer. A total of 200 μL of chase buffer was mixed with 10 μL of serum in a test tube. Dipstick was placed vertically inside the test tube until the mixture liquid front reached the arrow mark. The conjugate releasing pad due to capillary action, the anti-human IgM and/or IgG-impregnated colloidal gold conjugate formed complex with S. Typhi–specific IgM and/or IgG antibodies present in the sample. The complex moved on the nitrocellulose membrane and bound to the immobilized LPS and flagellin antigens. Development of one or two pinkish-red color bands on the dipstick after 20 min was considered as a positive result. Presence of an in-built immobilized control ensured the validity of the test. The test device was stable at 4–30 °C until the expiry date.
2.7. Statistical analysis

Experimental data were decoded and entered into a personal computer for statistical analysis by using the computer package SPSS for Windows (SPSS Benelux, Gorinchem, The Netherlands). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and efficiency of the new diagnostic assay were calculated using standard formulae (Hatta et al., 2002b). Blood culture was considered as the gold standard for determination of analytical sensitivity and specificity of the new kit. However, for determination of diagnostic efficiency and performance evaluation of the kit in a field setting, clinically diagnosed typhoid fever cases were considered as standard. McNemer ($\chi^2$) test was used to determine the significance of difference between the test systems. A p value of <0.05 was considered as statistically significant (Hatta et al., 2002b).

3. Results

3.1. Determination of analytical sensitivity and specificity

A total of 336 preserved sera samples (with known S. Typhi blood culture result) were tested blindly by both the Widal test and the new kit test. After decoding the data of culture results, 80 (23.8%) samples were found positive for S. Typhi, 211 (62.8%) were positive by the Widal test (TO $\geq$1:80), and 129 (38.4%) were positive by the new kit test (Table 1). Performance indicators such as analytical sensitivity, specificity, PPV, and NPV of the 2 serology-based tests (Widal and new test) were determined (Table 2) considering culture positive samples as the gold standard. Briefly, in analytical validation assay, efficiency of the new kit (70.5%) was found to be significantly better than that of the Widal test (43.2%) (p < 0.001).

3.2. Determination of performance (diagnostic) ability of the new kit in a field setting

### 3.2.1. Clinical profiles of the prospective study population

For the evaluation assay, a total of 102 hospital-attending febrile children with clinical diagnosis of typhoid fever were included. Median age of the patients was 5 years (range, 1–12 years) with a median duration of fever of 8 days (range, 3–21 days). According to the information provided by accompanying parents, 67 (65.7%) children had a history of prior antibiotic intake, 16 (15.7%) did not take any antibiotics, and 19 (18.6%) failed to confirm their status of antibiotic intake.

Twenty (19.6%) of 102 study children were confirmed as nontyphoidal cases by laboratory tests which were included in the study as negative controls. Of the 20, 10 suffered from dengue fever (positive for dengue IgM), 8 were positive for malaria parasites (blood smear showed *Plasmodium vivax*), and 2 were confirmed paratyphoid fever (S. Paratyphi A was grown in culture) cases.

### 3.2.2. Determination of diagnostic sensitivity and specificity of the new assay

Of 82 clinically diagnosed typhoid fever cases, blood culture for S. Typhi, Widal test, and the new kit assay was positive in 9 (11.0%), 36 (43.9%), and 42 (51.2%) cases, respectively (Table 3). False-positive result of the new kit was observed in 3 (15.0%) confirmed nontyphoidal cases of which 1 had dengue fever and another 2 suffered from paratyphoid fever. Widal test was positive in 7 (35.0%) confirmed nontyphoidal cases (3 dengue, 2 malaria, and 2 paratyphoid cases).

Since the percentage positivity of blood culture (11.0%) was lowest among the 3 methods due to frequent antibiotic intake by the study subjects, culture-confirmed cases could not be used as gold standard. Instead, clinically diagnosed typhoid fever cases were considered as standard for analysis and the diagnostic sensitivity, specificity, and predictive values of the new kit assay were found to be higher when compared with blood culture and the Widal test (Table 4). In the case of the Widal test, the sensitivity and specificity were calculated as 43.9% and 65.0%, respectively, with a diagnostic efficiency of 48.0%. The new kit performed marginally better than the Widal test with 51.2% sensitivity, 85.0% specificity, and 57.8% diagnostic efficiency. The overall performance indicators of the new kit were statistically significant when compared with blood culture (p < 0.001), whereas the difference in diagnostic efficiency was not statistically significant when compared with the Widal test (p > 0.5).

We analyzed the percentage positivity data of the 3 methods (blood culture, Widal test, and IC-LFT) at the end of every week based

### Table 1

<table>
<thead>
<tr>
<th>Sample categories</th>
<th>Test result and total no. (%) with a positive result in the respective tests</th>
<th>No. (%) of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td>Widal ($\geq$1:80)</td>
<td>IC-LFT</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>−</td>
<td>−</td>
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<tr>
<td>5</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>6</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

| 80 (23.8) | 211 (62.8) | 129 (38.4) | 336 (100) |

+ = Positive; − = negative.

### Table 2

<table>
<thead>
<tr>
<th>Tests</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widal ($\geq$1:80)</td>
<td>50/80, 62.5</td>
<td>95/256, 37.1</td>
<td>50/211, 23.7</td>
<td>95/125, 76.0</td>
<td>145/336, 43.2</td>
</tr>
<tr>
<td>IC-LFT</td>
<td>55/80, 68.8</td>
<td>182/256, 71.1</td>
<td>55/129, 42.6</td>
<td>182/207, 87.9</td>
<td>237/336, 70.5</td>
</tr>
</tbody>
</table>

CI = Confidence interval; PPV = positive predictive value; NPV = negative predictive value.

### Table 3

<table>
<thead>
<tr>
<th>Sample categories</th>
<th>Test result and total no. (%) with a positive result in the respective tests</th>
<th>Patient group/no. of cases (%) with suspected typhoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td>Widal ($\geq$1:80)</td>
<td>IC-LFT</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
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<tr>
<td>3</td>
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<td>7</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>9 (11.0)</td>
<td>36 (43.9)</td>
<td>42 (51.2)</td>
</tr>
</tbody>
</table>

Nontyphoid:

| 1                | −                  | +       | 3 (15.0) |
| 2                | −                  | −       | 0 (0.0)  |
| 3                | −                  | −       | 13 (65.0) |
| 4                | 0 (0.0)            | 7 (35.0) | 3 (15.0) |
| 5                | −                  | −       | 20 (100) |

* Confirmed dengue fever (10 cases), malaria (8 cases), and culture-positive paratyphoid (2 cases) were considered as controls.
on the duration of fever. Higher sensitivity of the IC-LFT test was observed at the end of the first, second, and third week of fever in comparison with the blood culture and the Widal test (Fig. 1).

4. Discussion

A serology-based rapid diagnostic test (IC-LFT) for typhoid fever was validated by blindly testing sera samples collected from fever patients with known blood culture results for S. Typhi. Performance indicators of the new test kit were also determined by testing prospective sera samples collected from children with fever having a clinical diagnosis of typhoid fever, who attended the outpatient department of a tertiary-care referral hospital for children at Kolkata, India. Stored sera with known culture results were randomly selected for culture positivity (23.8%; 80/336 true typhoid cases), and sample with positive blood culture was considered as the gold standard in the validation assay. In contrast, for prospective sampling, culture positivity rate was low (11.0%; 9/82) due to prior intake of antibiotics, which restricted the use of only positive blood culture cases as true positives. Instead, fever cases with clinical diagnosis of typhoid were considered as the standard. However, high culture positivity despite antibiotic therapy was also reported from Southern Vietnam during 2000–2002 (Olsen et al., 2004).

In the analytical validation assay, the new kit (70.5%) was found to be significantly more efficient than the popular Widal test (43.2%) when compared by McNemer test (p < 0.001) based on results of 336 stored sera samples considering blood culture positive cases as the gold standard (Table 2). These encouraging results led us to determine the diagnostic performance of the new kit in a field setup. To mimic a real-life scenario, blood samples of hospital-attending febrile children with clinical diagnosis of typhoid fever were randomly collected irrespective of their status of antibiotic intake and duration/severity of the disease, and were processed as stated earlier for prospective evaluation of the new assay kit and for comparison of diagnostic performances with other available serologic tests. Blood culture was found to be 100% specific, but insufficiently sensitive (11.0%) and efficient (28.4%) in prospectively collected samples, indicating that conventional blood culture no longer reflected the true burden of the disease and therefore should not be used as reference or standard test for typhoid diagnosis. In this study, we have considered clinically diagnosed typhoid fever cases, followed by exclusion of nontyphoidal cases by laboratory confirmation, as true positives to determine the diagnostic performances of the new test assay.

A total of 102 prospective febrile children were screened and 20 of them were laboratory-confirmed nontyphoidal cases like dengue, malaria, and paratyphoid fever, which were considered as negative controls for the analysis. This may be due to simulation of the clinical signs and symptoms of typhoid fever with other febrile illnesses (Ross and Abraham, 1987; Vollaard et al., 2005). A diagnostic sensitivity of 51.2%, specificity of 85.0%, and efficiency of 57.8% were calculated for the new dipstick kit (Table 4). Widal test was found to be less sensitive (43.9%), specific (68.9%), and efficient (48.0%) when compared with the new kit; however, the difference was not statistically significant (p > 0.5). Among prospective study children, 65.7% received antibiotics before attending the hospital, which might contribute to the lower sensitivity of both the serodiagnostic tests (Widal and the new kit) (Table 4) due to weak immune response following antibiotic intake due to probable S. Typhi infection (Robertson and Abdel Wahab, 1970; Shukla et al., 1997). Of the 82 clinically diagnosed typhoid fever patients, diagnosis could not be confirmed in 39.0% (32 patients) of cases, who were negative by all laboratory tests for typhoid, paratyphoid, dengue, and malaria.

Two different gold standards needed to be chosen for testing 2 different sets of samples (retrospective versus prospective), which provided justification for statistical analysis between two serodiagnostic tests (the new kit versus the Widal) (Parry et al., 2011). A significant difference in overall efficiency of the new kit was observed in the analytical validation assay (p < 0.001), whereas the performance ability of the kit was not so significant in the diagnostic evaluation assay (p > 0.5).

Performance characteristics of serologic kits like Typhidot and TUBEX have been evaluated and reviewed worldwide (Parry et al., 2011). Variable results with respect to sensitivity and specificity were observed for Typhidot which ranged from 45% to 65% (Dutta et al., 2006; Naheed et al., 2008) to as high as 85% to 98% (Gopalakrishnan et al., 2002; Jesudason and Sivakumar, 2006; Kawano et al., 2007; Olsen et al., 2004). Similarly, TUBEX, another serodiagnostic test, also showed inconsistent result with respect to sensitivity and specificity (56–94%) (Dong et al., 2007; Dutta et al., 2006; House et al., 2001; Kawano et al., 2007; Naheed et al., 2008; Olsen et al., 2004, Rahman et al., 2007). This variation in results of available serodiagnostic tests complicated the situation of typhoid diagnosis and thereby delayed the initiation of appropriate antibiotics for treatment (Bhutta and Mansurali, 1999; Crump et al., 2010).

One assay (LPS-based dipstick IgM assay) showed a sensitivity of between 65% and 88%, and a specificity of between 95% and 100%, and the result was available after 2–3 h (Gasem et al., 2002; Hatta et al., 2002a,b; House et al., 2001; Ismail et al., 2002). Later, the assay was modified for lateral flow test, which required only 10 min for reading the result with a sensitivity of 62% and a specificity of 98% (Pastoor et al., 2008). The detection of IgM antibodies was suggestive of current infection and helped to estimate the disease burden in any endemic regions, whereas presence of IgG antibody indicated either a current

<table>
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<tr>
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<th>Sensitivity (95% CI)</th>
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<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td>9/82, 11.0 (5.88–19.6)</td>
<td>20/20, 100.0 (NA)</td>
<td>9/9, 100.0</td>
<td>20/3, 68.0</td>
<td>99/102, 98.4 b</td>
</tr>
<tr>
<td>Widal (≥1:80)</td>
<td>36/82, 43.9 (13.2–80.5)</td>
<td>36/82, 43.9 (13.2–80.5)</td>
<td>13/20, 65.0</td>
<td>20/3, 68.0</td>
<td>80/102, 80.8 c</td>
</tr>
<tr>
<td>IC-LFT</td>
<td>42/82, 51.2 (17/20, 80.0)</td>
<td>42/82, 51.2 (17/20, 80.0)</td>
<td>42/45, 93.3 (17/20, 80.0)</td>
<td>29/8, 35.4</td>
<td>59/102, 57.8 b,c</td>
</tr>
</tbody>
</table>

a Considering 82 clinically diagnosed typhoid cases as true positive and 20 laboratory-confirmed nontyphoidal cases as controls.

b p < 0.001 using McNemer test.

c p > 0.5 using McNemer test.

Fig. 1. Percentage positivity of the 3 methods (blood culture, Widal, and the new assay) by weeks after fever onset in clinically diagnosed typhoid fever cases.

Table 4

Determination of diagnostic sensitivity, specificity, and efficiency of various assays in a field setting including prospective blood samples from hospital-attending clinically diagnosed typhoid fever cases (n = 102) a.
or a past infection (Parry et al., 2011). The disadvantage of the IgM-based kits was that these may show negative results during the early stage of current infection or re-exposure in endemic areas, due to the transient and relatively lower production of IgM antibodies (Rahman et al., 2007). Another kit, the Multi-Test Dip-S-Ticks IgG assay, was developed which detected 5 pathogens including S. Typhi and was found to be moderately sensitive (89.0%), but poor in specificity (50.0%) (Olsen et al., 2004).

The new dipstick assay has been designed to detect both IgM and IgG at a time against dual antigens (LPS and flagellin) of S. Typhi to enhance the sensitivity and specificity of the test when used in endemic areas. To our knowledge of modern development in typhoid fever diagnosis, this was the first rapid diagnostic test in the form of an immunochromatographic lateral flow technique (IC-LFT) which could detect both the antibodies simultaneously against dual antigens specific for S. Typhi. The new assay was found positive in 3 nontyphoidal cases also: 1 dengue and 2 paratyphoid fever patients. This may be due to the cross-reactivity of S. Typhi antigens with other pathogens.

Weekly analysis revealed that the positivity of IC-LFT was better than those of the other 2 tests (blood culture and Widal) from the first through the third week of fever. The positivity of the new assay increased from 37.8% in the first week to 75.0% in the third week. The kit required neither specialized instruments nor expertise for its execution. Refrigeration was not necessary for storage of components, which supported its intended use in remote areas of developing countries. Both sera and plasma samples could be used. The kit was cost-effective (~$1 per kit) and readily available at a reasonable rate. This was a rapid test in the true sense as the time required for colored bands development in positive samples was only 15–20 min.

In view of the result, it may be concluded that the new kit was an efficient and alternative test to the age-old Widal test without much significant difference ($p > 0.5$) in diagnostic ability. The new kit was cheap, user-friendly, and rapid, thus fulfilling the criteria for any ideal rapid diagnostic test (RDT). Further improvisation of the kit is recommended which would meet the challenging demand of typhoid fever diagnosis leading to its useful and justified applications in resource-limited endemic areas.

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