Prospective evaluation of a rapid diagnostic test Typhidot® for typhoid fever

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Background & objectives: Typhoid fever still continues to be a major public health problem around the world. A simple, reliable and affordable rapid diagnostic test has been a long felt need of the clinicians. We therefore prospectively evaluated the sensitivity and specificity of Typhidot® test.

Methods: The study was carried out between January 2002 and December 2003, on a total of 563 samples from patients clinically suspected to have typhoid fever; blood culture as well as serum for Typhidot® test were received.

Results: Of the 563 samples, Typhidot® test and blood culture were positive in 36 patients, both the tests were negative for 503 patients. Typhidot® test was positive for 9 patients with S. Paratyphi A infection. The sensitivity and specificity of the test using blood culture as gold standard were 92.3 and 98.8 per cent respectively for the typhoid fever.

Interpretation & conclusion: Typhidot® test is rapid, easy to perform and reliable test for diagnosing typhoid fever, useful for small less equipped laboratories as well as for those with better facilities.

Key words S. Typhi - Typhidot® test - typhoid fever

Typhoid fever continues to be prevalent in several countries around the world. These are mostly low- or middle-income countries with inadequate sanitation and hygiene, particularly regarding food, water and disposal of human excreta. In such places there are also other causes of febrile illnesses, such as vector-borne malaria, dengue fever and rickettsiosis as well as environmentally transmitted leptospirosis and melioidosis. During the first week of fever these illnesses are not easily distinguished from each other. Therefore, reliable laboratory tests are essential to establish aetiologic diagnosis so that appropriate treatment can be given. Delay or error in diagnosis prolongs the misery of the patient and increases the cost of treatment and other expenses for the family. The current standard diagnostic test for typhoid fever is blood culture, which may not be available or done properly in many clinics or small hospitals. Among the many thousands of laboratories in India, about 200 have joined the available
microbiology external quality assessment scheme run under the auspices of Indian Association of Medical Microbiologists. The availability of a simple and reliable rapid diagnostic test has been a long-felt need of clinicians in many parts of the world. Recently such a test has become commercially available under the proprietary name Typhidot® (Malaysian Biodiagnostics Research Sdn. Bhd, Selangor Darul Ehsan, Malaysia). It is an enzyme linked immunosorbant assay (ELISA) in the dot test format, which detects IgM and IgG antibodies against Salmonella Typhi. One or another version of this test has been evaluated in Malaysia, Singapore, Pakistan and India and the results have been quite satisfactory. In Singapore and Pakistan, it was evaluated by using a variety of case definitions; the sensitivity ranged from 84 to 93 per cent and specificity from 77 to 89 per cent. Our own previous retrospective assessment of Typhidot test on stored samples showed specificity of 80 per cent, but sensitivity of 100 per cent.

In the light of such encouraging results, we undertook this study to conduct a large scale prospective evaluation of Typhidot test.

**Material & Methods**

This study was carried out in the Department of Clinical Microbiology, Christian Medical College & Hospital, Vellore, south India during two years (from January 2002 to December 2003). Blood samples from patients clinically suspected to have typhoid fever were collected for culture and often, a parallel clotted blood sample as well. Blood culture was done according to standard procedures using the BacT/ALERT automation system (Biomerieux, Lyon, France). Any isolated bacteria were identified according to the recommended standard protocol.

The Typhidot test was done on serum samples, according to the procedure stipulated by the manufacturer. Each time the test was done, positive and negative controls supplied in the kit were also included. Each serum sample was diluted to 1:100 with the supplied diluent and one nitrocellulose acetate disc each for IgM and IgG was incubated in it, for 20 min at room temperature, with continuous slow rotation. The two discs were then washed with the supplied buffer solution and again incubated as before, one with anti-human IgM and the other with anti-IgG. After 15 min, they were washed again and treated with freshly prepared substrate solution for color development. The positive control formed duplicate dark spots of 2 mm diameter each. Any test sample showing similar or darker spots was defined positive. The absence of visible spot indicated a negative test result. If the spots were fainter than the control, that sample was also considered negative, according to the directions given in the kit. In case of discrepant appearance of the duplicate spots, the test was repeated and only if both dots were darker than control, the sample was taken as positive.

**Results**

Of the 563 febrile patients concurrently tested by blood culture and Typhidot test, 39 were positive for S. Typhi and 18 for S. Paratyphi A. The results were analysed after excluding the 18 subjects with paratyphoid fever. Among the remaining 545 patients, Typhidot test was positive in 42 (Table). The test sensitivity was 92.3 per cent and specificity 98.8 per cent. The positive predictive value was 85.7 per cent and negative predictive value was 99.4 per cent. Among the 18 subjects with S. Paratyphi A in blood culture, 9 were positive in Typhidot test.

The case histories of the six patients with apparent false positive Typhidot test result were re-evaluated. Five of them had fever more than 7 days without evidence of other febrile illnesses, and were presumptively diagnosed to have typhoid fever and treated with ciprofloxacin. They made full recovery. However, their blood cultures had grown α- Streptococci (2 subjects), obvious contaminants (2) and Proteus mirabilis (1). The two patients with α- Streptococci were also treated with doxycycline covering for bacteraemia from cryptic source. The sixth patient was diagnosed with tuberculosis lymphadenitis and showed marked clinical improvement on anti-tuberculosis treatment.
In three patients the Typhidot test result was falsely negative. On review of case records it was found that one subject was tested on day 2 of fever as her son had just been diagnosed to have typhoid fever. Another patient had typhoid fever two months earlier and this time, suspecting relapsing typhoid fever, blood was taken for culture on day 3 of fever. The third patient had one month of fever and grew S. Typhi in blood culture.

Discussion

The Typhidot® test was easy and rapid to perform and to read. The turn around time for the test was about one hour, whereas blood culture results were available one day later in most instances, but occasionally 2-4 days later.

Since S. Paratyphi A is closely related to S. Typhi 18 subjects with the former were excluded. However, in spite of the fact that the test is meant to diagnose only typhoid fever, we found it useful in paratyphoid A fever also, with 50 per cent sensitivity and 98.8 per cent specificity. If we were to use Typhidot test to test for “enteric fever” (typhoid and paratyphoid A fevers), there were 51 positive as against 57 positive by culture. Thus, if the entire study population was included (563), the sensitivity and specificity of the test for enteric fever diagnosis were 78.9 and 98.9 per cent, respectively. The corresponding positive and negative predictive values were 88.2 and 97.6 per cent, respectively.

There were six false positive serum samples in the rapid test, by definition. Five were indeed diagnosed clinically as typhoid fever, and treated with ciprofloxacin and all had recovered uneventfully. In one subject, the final diagnosis was tuberculosis (lymphadenitis, laboratory proven). This subject indeed had false positive result, but it is possible that an anamnestic response could have been the real reason.

There were three false negative results. In two subjects blood had been collected as early as two or three days from the onset of fever, clearly too early to detect antibody in the test. One of them had relapse of illness which could probably be due to antibody deficiency. Ordinarily typhoid fever would be suspected and blood collected for serology only after one week of continuous fever. The third subject had fever for a month, the blood culture grew S. Typhi and yet the rapid test was negative. On account of the high accuracy indices of the test, Typhidot requests were frequent and the department received request for Typidot test (without blood culture) in 1324 patients and among them 280 (21%) were positive. During the study period (January 2002 to December 2003) the actual proportion positive was 7.5 per cent. One drawback of this rapid test is that one does not have the organism to check antimicrobial sensitivity.

In our earlier study10, using a retrospective approach by testing stored samples, the sensitivity and specificity were 100 and 80 per cent, respectively.

<table>
<thead>
<tr>
<th>Typhidot test</th>
<th>Blood culture for S. Typhi</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
</tr>
<tr>
<td>No. positive</td>
<td>36</td>
</tr>
<tr>
<td>(True positive)</td>
<td></td>
</tr>
<tr>
<td>No. negative</td>
<td>3</td>
</tr>
<tr>
<td>(False negative)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
</tr>
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</table>

*18 subjects with S. Paratyphi A in blood culture were excluded in this analysis

Sensitivity = 92.3%, Specificity = 98.8%
Positive predictive value = 85.7%, Negative predictive value = 99.4%
respectively. The present prospective study showed superior results.

In summary, Typhidot® is a rapid, reliable and affordable test for typhoid fever appropriate in small institutions without laboratory equipment or trained technicians as well as in better equipped and staffed laboratories.

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References


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