

## Original article

# Utility of a rapid immunochromatographic test (Enterocheck WB™) for the diagnosis of enteric fever in an endemic region

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### Abstract:

**Context:** The current diagnostic tests for enteric fever used either lack in sensitivity, specificity or turnaround time.

**Aims:** This study evaluates usefulness of the rapid immunochromatography test Enterocheck WB™ (ECWB) and antibiotic susceptibility profile of Salmonella typhi isolates.

**Settings and Design:** This cross sectional study was carried out in a tertiary care hospital in Mumbai over a period of one year.

**Material and Methods:** This study enrolled 518 consecutive suspected cases of enteric fever with duration of fever greater than 3 days and age  $\geq 3$  years. Cases were those in whom Salmonella typhi was isolated from blood. 64 cases (blood culture positive for S. typhi) and 50 controls (25 healthy volunteers and 25 with diagnosis other than enteric fever) were included. ECWB and Widal test were carried out on all cases and controls.

Statistical analysis used: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated through SPSS software for windows.

**Results:** When compared to blood culture, sensitivity, specificity, PPV & NPV of Widal test was 21.9% , 94%, 82.4% & 48.5% and of ECWB was 90.6% , 82.4%, 86.6%, 87.5% respectively. All strains were susceptible to chloramphenicol and co-trimoxazole. Susceptibility to other antimicrobials tested ranged from 21.9% (nalidixic acid) to 96.9% (cefepime and ceftriaxone).

**Conclusions:** Enterocheck WB™ demonstrated a better sensitivity in comparison to Widal test with a comparable specificity. It can be easily used in emergency and primary health care settings where adequate laboratory facilities are not available so that early appropriate therapy can be initiated.

**Key-words:** Enteric fever, Enterocheck test, Antimicrobial susceptibility test

**Key messages:** Laboratories should switch over from the old, tedious & time consuming serodiagnostic tests like Widal test to the more rapid, sensitive and specific, easy to perform IgM tests like Enterocheck WB™ for a rapid diagnosis in suspected enteric fever cases.

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### Introduction:

India is an endemic country for enteric fever with an annual incidence rate of 980 per 100000 population.<sup>1</sup> If not treated early, the disease has a mortality of 30% which decreases to <1% on appropriate diagnosis and treatment.<sup>2</sup> Bone marrow

culture is considered as the gold standard for the diagnosis of enteric fever. Blood culture is considered as a suitable alternative.<sup>3</sup> The turnaround time for the results of blood culture is prolonged with time delay of 2-3 days (commercial automated systems) or 3 – 7 days (conventional

blood culture). Due to this, clinicians rely more on the Widal test in most of the developing countries. Widal test is now considered as non-specific, poorly standardised giving inaccurate results and having limited diagnostic value with a sensitivity of 64% and specificity of 76% .<sup>1</sup>To overcome the above drawbacks, rapid serological tests for detecting specific IgM antibodies have been developed but none have been found to be optimal or are expensive and require some amount of expertise.<sup>3,4,5</sup>

#### **Aims and objectives:**

The primary objective of the present study was to evaluate the performance of Enterocheck WB™ (ECWB) test (Zephyr Biomedicals, India), which detects specific IgM antibodies and is based on the principle of immunochromatography (ICT). The parameters assessed include sensitivity, specificity, ease of use, turnaround time and cost. The secondary objective was to assess the antimicrobial susceptibility pattern of the *S. typhi* strains isolated.

#### **Materials and methods:**

This study was carried out at a tertiary care hospital in Mumbai over a period of one year between June 2012 to May 2013. Approval from the Institutional ethics committee was obtained before commencing the study.

This cross sectional study included consecutive suspected cases of enteric fever with duration of fever greater than 3 days and age  $\geq$  3 years. Subjects were enrolled in the study after providing an informed consent. For the purpose of this study, a confirmed case was considered as a patient with persistent fever, with laboratory-confirmed *Salmonella typhi* or *paratyphi* infection. Two groups of populations were used as controls, one were patients with fever who were culture negative for *Salmonella* species but had a confirmed diagnosis for other pathogen (C1) and the second were healthy volunteers (food handlers, C20)

referred for a routine check - up. Patients who had been vaccinated for enteric fever or who reported of a similar fever in the past one year were excluded from the study.

Either whole blood culture or clot culture was performed. 7-10 ml of blood in adults and 5-7 ml in children was collected by venepuncture aseptically and added to blood culture bottles. In addition, 5ml blood was collected in a plain sterile test tube for Widal and ECWB tests. Serological tests were carried out for: 1) all culture positive patients, 2) 25 age and sex matched C1 and 3) 25 from C2 group. Blood and clot culture were processed as per standard protocol for isolation of bacteria. <sup>6</sup> Any growth was identified upto species level using standard biochemical tests and *Salmonella* serotyping antisera ( *Salmonella* O Antiserum Poly A – I & Vi, Groups 1 – 16, 19, 22 – 25, 34, Vi; BD Diagnostics). Antibiotic susceptibility testing was done by Kirby – Bauer disc diffusion as per CLSI standards.<sup>7</sup> Antibiotics that were tested were ampicillin (10  $\mu$ g), nalidixic acid (30  $\mu$ g), chloramphenicol (30  $\mu$ g), co-trimoxazole (1.25  $\mu$ g/ 23.75  $\mu$ g), ciprofloxacin (5  $\mu$ g), cefepime (30  $\mu$ g) and ceftriaxone (30  $\mu$ g).

Widal tube agglutination test (Typhocheck test by Tulip Diagnostics Ltd, India) was performed and interpreted as per manufacturer's instructions. Widal positive titre was taken as  $\geq$  1:160 for O & H agglutinins of *S. typhi*, *S. paratyphi* A and *S. paratyphi* B .<sup>8</sup>ECWB test utilizes the principle of sandwich immunoassay in immunochromatographic format for detection of *S.typhi* specific anti LPS IgM antibody. The conjugate pad has two components – anti human IgM antibody and rabbit globulin(control), both conjugated to colloidal gold. As the test specimen flows through the membrane test assembly, the anti-human IgM antibody – colloidal gold conjugate complexes with the *S.typhi* specific IgM antibodies in the specimen

and travels on the membrane due to capillary action. This complex advances further on the membrane to the test region (T) where it is immobilized by the S.typhi specific LPS antigen coated on the membrane leading to formation of a pink to pink-purple coloured band. This test is read within 15 minutes. The absence of this coloured band in the test region indicates a negative test result. This control band acts as a procedural control and serves to validate the results. Other tests that were carried out for confirmation of other illnesses were peripheral blood smear for malaria, sputum smear for acid fast bacilli, IgM antibody detection for leptospirosis, IgM/ IgG antibody for dengue & blood and urine culture for bacterial pathogens. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated through SPSS software for windows. Results of Widal and ECWB tests were compared with blood culture.

**Observations and Results:**

518 consecutive suspected enteric fever patients were enrolled in the study. Of these, 64 patients were culture positive for S.typhi. C1 group consisted of four with dengue, 10 patients with leptospirosis, six with malaria either vivax or falciparum or both, two with pulmonary tuberculosis, one with Klebsiella pneumoniae isolated from blood and two with E.coli from urine. 25 C2 controls were included. A total of 114

patients were thus enrolled in the study. Of these, 70 (61.4%) were adults and 44 (38.6%) were in the paediatric age group. 19 of the 64 culture positive cases were on antibiotic treatment at the time of collecting blood for culture. Among the culture positive patients, 42 (65.6%) were adults and 22 (34.4%) were children while in the culture negative group with other fever related diagnosis, 15 (60%) were adults and 10 (40%) were children. All the 25 healthy controls included were adults and all the tests for enteric fever carried out on them were negative.

Based on the duration of symptoms at the time of specimen collection (Table 1), it was found that Widal and ECWB test positivity was maximum in the second week of illness (41.2% & 76.1%) while culture positivity was higher in the first week. Taking the result of blood/ clot culture as gold standard, the sensitivity, specificity, PPV & NPV of Widal test was 21.9% , 94% , 82.4% & 48.5% and of ECWB was 90.6% , 82.4% , 86.6%, 87.5% respectively (Table 2). ECWB demonstrated a better sensitivity (90.6% v/s 21.9%) while Widal demonstrated a better specificity (94% v/s 82.4%). However, the PPV & NPV of ECWB was better than Widal test. All strains were susceptible to chloramphenicol & co-trimoxazole (Table 3). Susceptibility to other antimicrobials tested ranged from 21.9% (nalidixic acid) to 96.9% (cefepime and ceftriaxone).

**Table 1:**

**Comparison of test positivity to disease duration:**

( n = 114 )

Days of fever (Total no. of patients)	n	1 <sup>st</sup> week	%	2 <sup>nd</sup> week	%	3 <sup>rd</sup> week	%	>3 weeks	%
Culture positive for S.typhi	64	26	40.6	22	34.4	10	15.6	6	9.4
Widal positive	17	3	17.6	7	41.2	5	29.4	2	3
Enterocheck positive	67	6	9	51	76.1	8	11.9	2	3

**Table 2:**

n=114		Blood/clot culture				
<b>Widal test</b>	Positive	Negative	Total			
Positive	14	3	17	Sensitivity	21.90%	
Negative	50	47	97	Specificity	94%	
Total	64	50	114	PPV	82.40%	
				NPV	48.50%	
<b>Enterocheck WB</b>	Positive	Negative	Total			
Positive	58	9	67	Sensitivity	90.60%	
Negative	6	41	48	Specificity	82.40%	
Total	64	51	114	PPV	86.60%	
				NPV	87.50%	

**Table 3:**

**Antibiotic susceptibility to commonly used antibiotics:**

Antibiotic n = 64	Sensitive		Resistant	
	Nos.	%	Nos.	%
Nalidixic acid	14	21.9	50	78.1
Chloramphenicol	64	100	0	0
Co-trimoxazole	64	100	0	0
Ciprofloxacin	54	84.4	10	15.6
Cefepime	62	96.9	2	3.1
Ceftriaxone	62	96.9	2	3.1
Ampicillin	54	84.4	10	15.6

**Table 4:**

**Comparison of performance characteristics of serodiagnostic tests:**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<b>Typhidot</b>				
Olsen et al, 2004	79	89	96	59
Prasad et al, 2015	97.29	97.4	98.18	96.15
<b>Tubex</b>				
Olsen et al, 2004	78	94	98	59
Siba et al, 2012	52	90.6	46.4	92.3
<b>Widal test</b>				
<b>Present study</b>	<b>21.9</b>	<b>94</b>	<b>82.4</b>	<b>48.5</b>
Olsen et al, 2004	61-64	76-100	88-100	43-48
Siba et al, 2012	40	97.5	71.4	91.2
Anagha et al, 2012	63.15	62.50%		
<b>Enterocheck WB™</b>				
<b>Present study</b>	<b>90.6</b>	<b>82.4</b>	<b>86.6</b>	<b>87.5</b>
Anagha et al, 2012	89.47	96.87		
Jose et al, 2013	90	94.6	84.9	96.5
Anusha et al, 2011	85.5	88.6	97.7	51.1
Hamdy et al, 2014	86	89	56	98

**Table 5:****Comparison between some of the commercially available assays:**

Test	Cost (approx.) Rs.	Time for test	Specimen	Quantity of serum required ( $\mu$ l)	Storage temperature ( $^{\circ}$ C)	Indigenous/ Imported
Widal	Cheap	Overnight	Serum	300	2 $^{\circ}$ -8 $^{\circ}$ C	Indigenous
Enterocheck WB	Cheap	15 mins	Serum/ plasma/ whole blood	5	Room temperature	Indigenous
Typhidot	Expensive	60 mins	Serum	2.5	2 $^{\circ}$ -8 $^{\circ}$ C	Imported
Tubex	Expensive	20 mins	Serum	35-40	2 $^{\circ}$ -8 $^{\circ}$ C	Imported

**Discussion:**

Enteric fever remains an endemic disease in a developing country like India. Blood culture and Widal test remain the mainstay in diagnosis till today though both have their limitations. An IgM based assay is considered more accurate.<sup>9</sup> In the present study which compared two sero-diagnostic assays, Widal and ECWB, ECWB showed a high sensitivity, good PPV & NPV .

In the present study, ECWB (the new test under evaluation) and Widal (an existing test) were evaluated against blood culture (table 4). ECWB demonstrated a high sensitivity (90.6%) & specificity (82.4%). Similar results have been reported in literature with sensitivities varying from 86 % to 90% and specificity varying from 89% to 96.9% (table 4).<sup>8,10,11</sup> Anagha et al have reported a sensitivity of 89.5%.<sup>8</sup> Anusha et al in Chennai have reported a sensitivity of 85.5% and specificity of 88.6%.<sup>9</sup> Jose et al have reported a sensitivity of 90%, specificity of 94.6% with a PPV of 84.9% & NPV of 96.5%.<sup>10</sup> Since ECWB has a better PPV and NPV as compared to the Widal test, it is a better test to incorporate. Also, because it's a rapid user friendly test, it is especially useful during monsoon when the patient load is expected to be high and empirical treatment results in unnecessary initiation of antibiotics in all patients with fever.

In this study, it was observed that blood culture was maximally positive in first week of illness as

compared to sero-diagnostic tests which demonstrated a higher positivity in second week of illness which was an expected finding. 12 There were 26 cases that were culture positive in first week of which ECWB was negative in 20. Of these 20 cases, 16 had a duration of fever <7 days and one was a case of myeloproliferative disorder on immunosuppression since one year which might explain seronegativity. 28 patients in second week of illness were positive for ECWB where blood culture was negative. This may be attributed to the decreasing sensitivity of blood culture as the duration of illness progresses.

Blood culture being less sensitive than bone marrow culture, there may be chances of missing positive cases and in such cases the rapid antibody test may in fact be diagnosing the missed true positives. But this needs further evaluation. This is one limitation of this study but it is not feasible to do a bone marrow culture in all patients.

Various other serological tests have been evaluated for the diagnosis of enteric fever such as Typhidot IgM & IgG and Tubex test with good sensitivity, specificity, NPV & PPV. But these tests are expensive, time consuming and need some amount of expertise.<sup>3,4,5</sup> Since Widal test detects both IgG & IgM, in order to be of diagnostic value, paired serum samples taken at least two weeks apart should demonstrate a rising titre of agglutinins. This is not practical as follow up of patients is

difficult. In areas of high endemicity, a single Widal test can lead to false positive and false negative results.<sup>12</sup> Another limitation of this study is that an IgM antibody assay is compared with culture positivity which may not be optimal since the former is likely to be positive after five days of illness while the latter is positive at the start of illness itself.

In this study, the isolation rate of *S.typhi* from clinically suspected cases was relatively low (8.1%). Similar results have been reported in studies by Anusha et al in India (12%) and Hamdy et al (13.6%) in Egypt.<sup>9,11</sup> Automated blood culture systems have demonstrated better yields as reported by Fadeel et al in 2012 with an isolation rate of 22.89%.<sup>13</sup> Increasing the volume of blood cultured also improves sensitivity.<sup>14</sup> Laboratories should look into upgrading to automation to get better yield. The slightly lower culture positivity in the present study may be due to the nature of referring (tertiary care) where patients are likely to be on antibiotics before availing services of a tertiary care centre.

Over time, the drug of choice for therapy has moved from chloramphenicol to ciprofloxacin to third generation cephalosporins. Sensitivity to these three antibiotics was 100%, 97% and 84% respectively. Resistance to nalidixic acid may be considered as a marker for ciprofloxacin treatment failure even if the strain demonstrates in-vitro susceptibility to ciprofloxacin.<sup>15</sup> 78% of the strains were resistant to nalidixic acid. Of these, 15.6% were also resistant to ciprofloxacin. In a study by Singhal et al, resistance to nalidixic acid was reported as 100%.<sup>2</sup> Though a low level of resistance to third generation cephalosporins has been observed in this study, it is a growing problem and underscores the significance of judicious antibiotic use.<sup>2</sup> Studies in neighbouring countries have

reported resistance rate which was not seen earlier (Capoor et al).<sup>16</sup> In the study by Singhal et al, trends in susceptibility of *S. typhi* to various antibiotics over a period of twelve years was carried out, a downward trend in multi-drug resistant cases is noted.<sup>2</sup> This is a good indication for clinicians since the drugs which had become ineffective earlier could again be recycled & used for treatment.

ECWB meets the criteria of an ideal diagnostic test as it has a good sensitivity, specificity, NPV and PPV and is simple, sensitive, rapid, and economical test requiring minimal operator training, no specific storage conditions and therefore can be used in field applications. The turnaround time for the test is fifteen minutes and can be carried out on serum or plasma or on whole blood (Table 5) and therefore can be useful in peripheral hospitals, medical centres & camps where laboratory facilities for blood culture are not available.

#### **Limitations:**

Since the study site is a tertiary care hospital, patients were already on antibiotics before being tested. This may interfere with the growth of *S. typhi* in culture and also titres of antibodies in the serological tests are low giving a lower positivity rate. Another limitation of the study is that blood culture was taken as the gold standard for enteric fever diagnosis instead of bone marrow culture as this test is not feasible in all patients.

#### **Conclusions:**

In a resource constrained setting, along with diagnostic accuracy, cost, ease of use and storage conditions are important factors to be considered especially taking into account the endemicity of the disease and high patient load. ECWB meets these criteria. It can be performed on serum, plasma or whole blood and therefore suitable for use even in field applications.

**References:**

1. Beig F, Ahmad F, Ekram M, Shukla I. Typhidot M and Diazo test vis-À-vis blood culture and Widal test in the early diagnosis of typhoid fever in children in a resource poor setting. *The Brazilian Journal of Infectious Diseases*. 2010;14:589-593.
2. Singhal L, Gautam V, Gupta P, Kale P, Ray P. Trends in antimicrobial susceptibility of Salmonella Typhi from North India (2001-2012). *Indian Journal of Medical Microbiology*. 2014;32:149.
3. Keddy K, Sooka A, Letsoalo M, Hoyland G, Chaignat C, Morrissey A et al. Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub-Saharan African sites. *Bulletin of the World Health Organization*. 2011;89:640-647.
4. Olsen S, Pruckler J, Bibb W, Thanh N, Trinh T, Minh N et al. Evaluation of Rapid Diagnostic Tests for Typhoid Fever. *Journal of Clinical Microbiology*. 2004;42:1885-1889.
5. Prasad KJ, Oberoi JK, Goel N, Wattal C. Comparative evaluation of two rapid Salmonella IgM tests and blood culture in the diagnosis of typhoid fever. *Indian Journal of Medical Microbiology*. 2015; 33:237.
6. Mathew R, Jobin SR. A comparative study on methods for diagnosis of enteric fever. *Cur Res Rev*. 2013;5:88-95.
7. CLSI. Performance standards for antimicrobial susceptibility testing; twenty fifth information supplement. CLSI document M100-S25. Wayne, PA: Clinical and laboratory standards Institute; 2015.
8. Kinikar A, Bhalerao D, Shahriar R, Kulkarni S. The Easy and Early Diagnosis of Typhoid fever. *Journal of Clinical and Diagnostic Research*. 2012;6:198-199.
9. Anusha R, Ganesh R, Lalitha J. Comparison of a rapid commercial test, Enterocheck WB ® , with automated blood culture for diagnosis of enteric fever. *Annals of Tropical Paediatrics*. 2011;31(3):231-234.
10. Jose W and Rodrigues S. Evaluation of Enterocheck WB - a rapid test for diagnosis of typhoid fever. *IOSR-JDMS*. 2013;11:48-51.
11. Hamdy M, Abdel-Rahman S, Abdel-Mgeed M, Hameed S. Evaluation of Enterocheck WB test in diagnosis of Typhoid fever among Egyptian adults. *Egyptian Journal of Medical Microbiology*. 2014;23: 47-52.
12. Olopoenia L, King AL. Classic methods revisited: Widal agglutination test - 100 years later: still plagued by controversy. *Postgraduate Medical Journal*. 2000;76:80-84.
13. Fadeel M, House B, Wasfy M, Klena J, Habashy E et al. Evaluation of a newly developed ELISA against Widal, TUBEX-TF and Typhidot for enteric fever surveillance. *J Infect dev countries*. 2012; 5: 169-75.
14. Connell TG, Rele M, Cowley D, Buttery JP, Curtis N. How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. *Pediatrics*. 2007; 119:891.
15. Ray P, Sharma J, Marak RS, Garg RK. Predictive efficacy of nalidixic acid resistance as a marker of fluoroquinolone resistance in Salmonella enterica var Typhi. *Indian J Med Res* 2006; 124: 105-8.
16. Capoor MR, Nair D, Hasan AS, Aggarwal P, Gupta B. Enteric fever: Narrowing therapeutic options in India. *Southeast Asian J Trop Med Public Health*. 2006; 37: 1170-4.