

Evaluation of Enterocheck WB - a rapid test for diagnosis of typhoid fever

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Abstract: Blood culture although continues to be the gold standard for diagnosis of typhoid fever, its applications for routine diagnosis of typhoid fever is restricted because of several limitations. Widal test has been used extensively as a laboratory tool for diagnosis of typhoid fever in most laboratories, but it is laborious, time consuming and may not be positive in early stages and is to be interpreted judiciously. In the recent times, several other serological tests other than Widal test have been described and evaluated. In the present study, we evaluated Enterocheck WB, an immunochromatographic rapid test for diagnosis of typhoid fever, in Widal positive cases. This test detected IgM antibodies and was found to have a sensitivity of 90% and a specificity of 94.6%. The positive and negative predictive values of Enterocheck WB was 84.9% and 96.5% respectively. The test serves as an useful alternative to other conventional tests for rapid diagnosis of typhoid fever.

Key words: Enteric fever, Enterocheck, Rapid test, Typhoid fever

I. Introduction

Typhoid fever continues to be prevalent in several countries around the world. It poses an important public health problem in many parts of India where it is endemic, accounting for considerable morbidity and mortality [1]. Incidence of typhoid fever has been estimated as approximately 17 million cases; with 600000 associated deaths occurring annually [2-3]. The disease presents a clinical dilemma with varied manifestations, closely resembling several other clinical conditions presenting with febrile illnesses, such as vector borne malaria, dengue fever and rickettsiosis as well as environmentally transmitted leptospirosis and melioidosis [4-8]. According to World Health Organization (WHO) a confirmed case of typhoid fever is defined as a patient with fever ($>38^{\circ}$) that has lasted for at least three days, with a laboratory confirmed positive culture of *S. typhi* [9]. Probable case of typhoid fever is a patient with fever ($>38^{\circ}$) that has lasted for 3 days, with positive serodiagnosis or antigen detection test but without *S. typhi* isolation [9]. A chronic carrier is determined by excretion of *S. typhi* in stools or urine for longer than one year after the onset of acute typhoid fever.

Laboratory diagnosis of typhoid fever is based on isolation of bacilli from patient's blood and demonstration of antibodies in the serum. Blood culture and Widal test are the routinely employed investigations for diagnosis of typhoid fever in clinical settings. Blood culture although confirmatory, requires cultural processing and identification of the causative agent, which is labor intensive and relatively costly. Widal test has been used in diagnosis of typhoid illness for a long time in the country, but it remains a serological test with moderate sensitivity and specificity [10]. There is a need for rapid, reliable and easy to perform test with high degree of sensitivity and specificity, which will assist the clinicians in early diagnosis and prompt treatment.

With this objective, we evaluated the efficiency of Enterocheck WB immunochromatographic card test as a predictor of typhoid fever and compared its performance with standard culture and Widal test.

II. Materials and methods

This study was conducted in the Department of Microbiology, Goa Medical College, Bambolim Goa. Widal tube agglutination test was done using standard stained antigens supplied by Tulip Diagnostics (P) Ltd Bambolim Goa, on all clinically suspected cases of enteric fever. The clinical response to specific therapy for typhoid fever in all cases was also noted.

Fifty suspected cases of typhoid fever which were Widal positive were subjected to immunochromatographic card test (Enterocheck WB) supplied by Zephyr Biomedicals, Verna Goa.

Enterocheck WB is a rapid qualitative immunochromatographic sandwich immunoassay for detection of IgM antibodies to *S. typhi* in serum / plasma / whole blood. It detects antibodies specifically directed against lipopolysaccharide (LPS) of *S. typhi*. The test employs nitrocellulose membrane conjugate pad containing anti human IgM conjugated to colloid gold and rabbit IgG conjugated to colloid gold. In positive samples the *S. typhi* specific serum IgM forms complex with anti IgM colloid gold and then moves along the membrane to the test region where it is immobilized by the *S. typhi* specific LPS antigen coated on the membrane, giving a pink

purple band. In negative cases only the rabbit IgG gold conjugate moves further on the membrane to the control region where it is immobilized by anti rabbit antibodies, giving a pink purple band. (see figure 1)

This test was performed as per the technical instructions provided by the manufacturer, using 5 μ l of patient's serum and charging it into the sample port 'A' with the loop provided with the kit. 5 drops of sample running buffer was later added to the port 'B'. The test was readable after 15 minutes.

Blood cultures were also done on all the 50 cases, simultaneously using Glucose Broth and Hartley's broth employing standard culture techniques.

Samples from hundred antenatal women attending the antenatal clinics of this Institution and fifty healthy blood donors were taken as controls and their serum samples were subjected to Enterocheck WB and Widal test.

III. Results

Amongst 50 suspected cases of typhoid fever, blood culture was positive in only 8 (16%) cases. Widal test was found to be positive in all 50 cases, while Enterocheck WB was positive in 45 cases (90%) only. Widal test detected five more positive cases as compared to Enterocheck WB, but none of the Widal test negative cases were positive by Enterocheck WB. In the control groups, Enterocheck WB was positive in 5 (5%) antenatal samples and 3 (6%) blood donors' samples. Similarly, Widal test was positive in 5 (5%) antenatal and 3 (6%) blood donors' samples (Refer Table 1).

Considering Widal positivity and clinical response to therapy in suspected cases as a marker of typhoid fever, all Widal positive cases that responded to therapy, were considered as probable cases of typhoid fever. We evaluated usefulness of Enterocheck WB for diagnosis of typhoid fever based on this parameter.

The sensitivity and specificity of Enterocheck was found to be 90% and 94.6% respectively. Further, the positive predictive value for Enterocheck WB was 84.9%, while negative predictive value was 96.5%. The Enterocheck WB showed an acceptable positive likelihood ratio of 16.6 (acceptable limit > 10), and an acceptable negative likelihood ratio of 0.10 (acceptable limit < 1).

IV. Discussion

Typhoid fever remains an important public health problem in developing countries. The definitive diagnosis is based on the isolation of the causative agent *Salmonella typhi* from blood, faeces, urine or other clinical samples. Although blood culture remains the gold standard test in diagnosis of typhoid fever, its utility in diagnosis is restricted to early phase of illness and is not useful for rapid diagnosis [10].

Although blood culture is considered as gold standard for diagnosis of typhoid fever, in the present study, only 8 out of 50 (16%) suspected cases were culture positive. This could be attributed to the fact that most of these patients were presenting after first week of illness and had received some antibiotic therapy before being referred to tertiary care centre.

In developing countries, facilities for isolation and culture are often not available, especially in smaller hospitals and diagnosis relies upon the clinical features of the disease and agglutinating antibodies to *S. typhi* [11]. Widal test has been used very extensively in serodiagnosis of typhoid fever and in developing countries particularly, remains the only practical test available [11]. However interpretation of Widal test is difficult in areas where *S. typhi* is endemic when basal titre of the population is not known. Further, in areas where fever due to infection is a common occurrence, the possibility exists that false positive reactions may occur as a result of non-typhoidal fevers [11].

There is a need for a rapid and reliable diagnostic test to overcome these problems. Recently, numerous tests other than culture isolation and Widal test are made available to support the diagnosis of typhoid fever. These include amongst others, Multi-test Dip-S-Ticks, TyphiDot and TUBEX to detect IgG, IgG and IgM and IgM, respectively [12, 13]. TyphiDot, an ELISA based assay in dot test format has been tried in Malaysia, Singapore, Pakistan and India with satisfactory results [14]. However, some of them are either expensive or labor intensive.

In the present study, we evaluated the usefulness of Enterocheck WB in cases where presumptive diagnosis of typhoid fever was based on clinical presentation, a positive Widal test and response to therapy. The test had a high positive predictive value of 84.9% and a negative predictive value of 96.5%, besides having sensitivity and specificity of 90% and 94.6% respectively. The test was comparable to Widal test, but had a superior interpretative value, as it detected IgM antibodies denoting recency of infection. The other advantage of this test was that it is less laborious, rapid and results are obtained within 15 minutes. This will help the clinicians to initiate antimicrobial therapy at an early stage of the disease, thereby decreasing morbidity.

The test is however designed to detect only cases of enteric fever caused by *S. typhi* and not those caused by *S. paratyphi* A, B or C. The test can well serve as a substitute to Widal test, which is time consuming and laborious. Further evaluations need to be done to ascertain its comparative value with other rapid tests available commercially.

V. Figures and tables

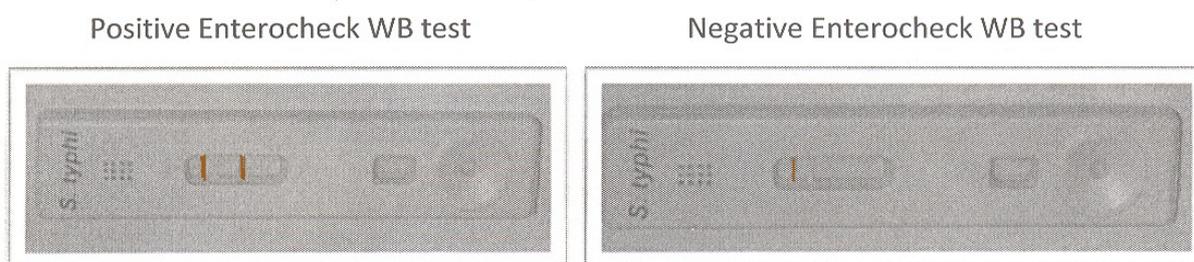


Figure1. Positive and negative Enterocheck WB test

Table 1:
Result of Enterocheck WB, Widal and Blood Culture

Category	Enterocheck WB Positive	Enterocheck WB Negative	Widal Positive	Widal Negative	Blood Culture Positive	Blood Culture Negative
Suspected typhoid cases (n= 50)	45	5	50	-	8	42
Blood Bank donors (n=50)	3	47	3	47	-	-
Antenatal cases (n=100)	5	95	5	95	-	-
Total (n=200)	53	147	58	142	8	42

VI. Conclusion

Diagnosis of enteric fever often comes under scrutiny, as blood cultures may not be rewarding specially when patient is partially treated or when the patient presents a week or later after onset of acute illness. Widal test which is commonly done for diagnosis of enteric fever serves as the widely used test especially in developing countries, although it has a moderate sensitivity. Hence there is a need to opt for any better test that would give reliable test results.

In the past many rapid tests like Multi-test Dip-S-Ticks, TyphiDot an ELISA based assay in dot test format and TUBEX to detect IgG, IgG and IgM and IgM, respectively have been tried with fairly satisfactory results. In the current, we compared Enterocheck WB rapid test for diagnosis of typhoid fever with blood cultures and widal test and found it to be comparable to widal test. More over this test detects IgM antibodies and is indigenously prepared. This test should be subjected to extensive use to further ascertain its diagnostic utility.

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