

# Typhoid Fever in Kuala Lumpur and a Comparative Evaluation of Two Commercial Diagnostic Kits for the Detection of Antibodies to *Salmonella typhi*

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## ABSTRACTS

The aim of this study was to compare two commercial kits, the Typhidot and the PanBio ELISA with the present Widal test. Demographic data for all serodiagnosed cases for the years 1991-1998 were collected. From this data, 144 were selected as samples for comparative evaluation of the commercial kits. Fifty sera were culture positive for *Salmonella typhi*, 50 were culture negative but clinically diagnosed as typhoid fever and Widal positive and 44 were serodiagnosed as enteric of which 21 were culture positive for other *Salmonella* species, 20 were serodiagnosed for other febrile illnesses and three sera culture positive for other species of enterobacteriaceae. The specificity, sensitivity and efficiency of the tests were calculated with the positive culture for *S. typhi* as the gold standard. Sensitivity, specificity and efficiency of test for Typhidot and Typhidot M kits were 98%, 76.6% and 84.0% and PanBio ELISA were 78%, 80% and 79.9%. The two commercial kits evaluated were found to be less time consuming and easier to perform than Widal. The Typhidot M seems to be a practical alternative in the field and in small hospitals with lesser facilities.

**Keywords:** *Salmonella typhi*, ELISA, immunodot blot, Widal

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## INTRODUCTION

Typhoid fever is a systemic prolonged febrile illness caused by certain *Salmonella* serotypes including *Salmonella typhi*, *S. paratyphi A*, *S. paratyphi B* and *S. sendai*. It emerged as an important infectious disease in the early 19<sup>th</sup> century. The illness begins with mounting fever, headache, vague abdominal pain and constipation, which may be followed by appearance of rashes. During the third week, the patient reaches a state of prolonged apathy, toxemia, delirium, disorientation and/or coma followed by diarrhoea. If left untreated, it can lead to complications affecting various organ

systems<sup>(1)</sup>. Infection occurs in all age groups with a higher incidence and more variable clinical presentation in children. Since the late 1940s typhoid fever was successfully treated with one of the several antibiotics, chloramphenicol, ampicillin and trimethoprim-sulphamethoxazole. However, from 1990, multidrug resistant strains to the previously useful antibiotics have emerged, and treatment for such strains requires the use of more expensive quinolone antibiotics such as oral ciprofloxacin or third generation cephalosporins such as ceftriaxone<sup>(2)</sup>. Human beings are the only reservoir and host for typhoid fever and is transmitted by faecally contaminated water and food in endemic areas especially by carriers handling food. Estimates from the WHO suggest that the worldwide incidence of typhoid fever is approximately 16 million cases annually, with >600,000 deaths, of which seven million cases occur annually in South East Asia. Typhoid fever has important socioeconomic impact because, most of the time, several months are necessary for a patient to recover and be able to work again.

Accurate diagnosis of typhoid fever at an early stage is important not only for etiological diagnosis, but also to identify individuals that may serve as a potential carriers, who may be responsible for acute typhoid fever outbreaks<sup>(3)</sup>. During the course of infection, the causative bacilli can be isolated from faeces, urine, bone marrow and blood. Specific agglutinins appear during the course of most of the attacks during the second week of infection. Detectable levels of IgM antibodies against *S. typhi* appear and persist for four months. IgG antibodies are detected thereafter and remain in blood for two years. The gold standard in the diagnosis of typhoid fever is the isolation of the organism. Serological diagnosis can be made by the Widal test, indirect haemagglutination, counter-immunoelectrophoresis, solid phase radioimmunoassay and ELISA<sup>(4)</sup>. The limitations of the above traditional methods have prompted other novel tests to be developed. In the past two decades, various immunoassays to detect *S. typhi* antigens or antibodies<sup>(5)</sup> and nucleic acid based assays to detect *S. typhi* genes have attempted to be more sensitive, practical,

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economical and rapid than bacteriological culture. Unfortunately, no assay has adequately accomplished all these aims, and a satisfactory test to replace bacteriological culture remains a desirable, but elusive goal<sup>(2)</sup>.

At the University hospital, diagnosis is made by culture from blood and faeces, and serologically by the Widal test. Although it is cheap and easy to perform, the Widal test lacks specificity. Also paired sera is required for accurate interpretation. The current study aims to compare the existing diagnostic methods with two other commercially available serological tests, the Typhidot ELISA (MBDR Sdn Bhd) and PanBio IgG and IgM ELISA.

## MATERIALS AND METHODS

### Patients Sera

The study was carried out at the University Hospital, Kuala Lumpur. Serological data collected from requested tests (Widal and blood culture) for the years 1991-1998 were screened for positive cases. Demographic data including age, sex and race were recorded. All samples used were serum samples stored in 0.02% sodium azide. From these samples, 100 single serum samples positive for Widal (greater than or equal to 640) were selected based on two criteria, *S. typhi* culture positive (50) and clinically typhoid but culture negative (50). A third group of controls were included which consisted of non-typhoid (positive culture for other *Salmonella*) (21) and a panel of sera collected from patients with other PUOs (23). A Widal agglutination titre of  $\geq 640$  is considered positive, while a titre of 320 was noted as borderline.

### Widal Test

The Widal test measures serum agglutinins against somatic and flagellar antigens. The method of Pang et al<sup>(6)</sup> was followed. Briefly 25  $\mu$ l of serum added to microtitre plates for the O antigen assay and 100  $\mu$ l of serum to Dryers tubes for the H antigen assay. Similar quantities of O and H antigen of *S. typhi*, *S. paratyphi A* and *S. Paratyphi B* were then added at a fixed concentration (which was optimised initially). The antigens were procured from Murex Biotech Ltd., England. The Dryers tubes were incubated at 52°C for two hours and left at room temperature overnight. The microtitre plates were incubated overnight at 37°C. Plates were left at 4°C for one hour before reading.

### Typhidot

The Typhidot is a qualitative dot enzyme immunoassay designed to detect the presence of IgM and IgG antibodies against specific of the outer membrane of *Salmonella typhi*. The test uses nitrocellulose strips

dotted with the 50 KDa specific outer membrane protein antigen. The test is carried out as per manufacturer's instruction. Briefly the reaction tray was divided into two columns and marked M and G. Required number of strips coded and labelled were placed separately in the M and G wells. 250  $\mu$ l of the sample diluent were dispensed into each of the wells and 2.5  $\mu$ l of sample were added and incubated at room temperature for 20 minutes on a rocker platform. The strips were washed thrice for a total of five minutes, and 250  $\mu$ l of anti human IgM and IgG were added to the respective wells and incubated for 15 minutes. The strips were washed as before, and 250  $\mu$ l of colour development solution were added and incubated for 15 minutes. The reaction was stopped, by washing the strips in distilled water and the results were read. When both the dots on the test strip were as dark or darker than their corresponding dots on the positive control strip, they were reported as positive.

### Typhidot M

Typhidot M is a dot enzyme immunoassay for the detection of specific IgM to *Salmonella typhi*. In this test IgG is inactivated before carrying out the assay as for the Typhidot. The test uses a nitrocellulose membrane strip dotted with the 50 KDa specific protein and a control antigen. 10  $\mu$ l of patient serum and controls are pre-absorbed for at least one minute with 90  $\mu$ l of IgG inactivation reagent. 900  $\mu$ l of sample diluent is then added into the reaction wells and the mixture incubated at room temperature on a rocker platform for one hour. The strips were washed thrice for a total of five minutes, and 1 ml of anti-human IgM conjugate was added and incubated for one hour. The strips were washed as before, and 1 ml of colour development solution was added and incubated for 15 minutes. The reaction was stopped, by washing the strips in distilled water and the results were read. When both the dots on the test strip were as dark or darker than their corresponding dots on the positive control strip, they were reported as positive.

### PanBio *Salmonella typhi* IgM and IgG ELISA Test

The PanBio utilises a direct ELISA format. Briefly, to the *Salmonella typhi* antigen coated microwell strips, diluted absorbed sample sera, control sera and cutoff calibrators were added and incubated at 37°C for 20 minutes. Residual serum was washed, and HRP conjugates anti human IgM or anti human IgG was added and incubated for another 20 minutes at 37°C. The microwells were washed and the colourless substrate system, tetra-methyl benzidine (TMB) and hydrogen peroxide, was added and incubated at room temperature for 10 minutes. The reaction was stopped

**Table I. Number positive by diagnostic assays evaluated.**

Groups	No	Typhidot	PanBio	Widal
<i>S. Typhi</i> culture positive	50	41	39	50
Clinical diagnosis, culture negative	50	18	12	50
Non-typhoid enteric fevers	44	12	6	24

**Table II. Sensitivity, specificity, PPV, NPV and efficiency of test of the kits evaluated.**

	No	Typhidot	PanBio	Widal
Sensitivity	100	82.0	78.0	98.0
Specificity	21.2	68.1	80.0	76.6
PPV	40.3	57.7	68.4	69.0
NPV	100	90.1	87.4	98.6
Efficiency of Test	48.6	72.9	79.9	84.0

and the wells were read at a wavelength of 450 nm. The test was taken as valid, when the absorbance readings of the above met the specifications of the manufacturers instruction. Results were interpreted based on PanBio units derived from the equation:

PanBio units = 10 x absorbance of sample/mean of cut off calibrator.

PanBio units <10 are as negative (No evidence of recent infection)  
>10 are read as positive (Suggestive of recent infection)

#### Data Analysis

Sensitivity, specificity, positive predictive values (PPV), negative predictive value (NPV), and efficiency of Test were calculated using standard equations.

% Sensitivity = true positives/(true positives + false negative) x 100,

% Specificity = true negatives/(false positives + true negative) x 100,

PPV = true positive/all positive tests

NPV = true negative/all negative tests

Efficiency of test = (true positive + true negative)/total samples

Note: *Salmonella typhi* culture positive (n=50) samples are taken as the gold standard.

#### RESULTS

Diagnosis of enteric fever was made in 144 cases, of which 50 were blood and/or stool culture positive for *S. typhi*, 50 were culture negative but Widal positive ( $\geq 640$ ) with a clinical diagnosis of typhoid fever made and 44 were non-typhoidal cases where a clinical diagnosis of enteric fever was made. In the last group 21 were culture positive for other *Salmonella*, 10 were diagnostically positive for viral hepatitis, four for dengue fever, four for other viral infections and two for scrub typhus. Three of them

were also culture positive for other organisms belonging to enterobacteriaceae family.

#### DIAGNOSTIC ASSAYS

Results of the diagnostic assays evaluated are shown in Table I, and their sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency of test are shown in Table II. The positive and negative controls in all the assays were appropriate, indicating that all the sera were tested correctly. Of the 144 samples, 121 were Widal positive. The sensitivity and specificity of Widal were calculated to be 100% and 21.2% respectively, with a PPV of 40.3%, NPV of 100% and efficiency of test of 48.6%. For the Typhidot kit, the sensitivity and specificity were found to be 82.0% and 78.0%. It had a PPV and NPV to be 57.7% and 90.1% with an efficiency of test to be 72.9%. The PanBio ELISA kits reflected a 78.0% sensitivity, 80.0% specificity, a PPV of 68.4%, a NPV of 87.4% and an efficiency of test of 79.9%. Nine samples from the *S. typhi* culture positive group which had tested negative by the Typhidot kit were repeated after removal of IgG and eight were found to be positive in the Typhidot-M kit. The combination of Typhidot and Typhidot-M increased the sensitivity to 98.0%, specificity to 76.6%, NPV to 98.6%, PPV to 69.0% and efficiency to 84.0%. Clearly the presence of IgG masked the detection of IgM.

An analysis was also carried out in the group where a clinical diagnosis of typhoid fever was made but was culture negative group (n=50). In this group all samples had a Widal titre of  $\geq 640$  for either H antigen (n=25) only or both H and O antigen (n=21). Among the group with titres  $\geq 640$  for the H antigen only, Typhidot picked up three samples, while eight tested positive by Pan Bio ELISA. Among the group which had Widal titres  $> 640$  for both H and O antigen, 16 tested positive by Typhidot and Typhidot-M and four tested positive by Pan Bio ELISA kit (data not shown).

#### DISCUSSION

The diagnosis of typhoid fever has relied on the isolation of *S. typhi* from the blood, faeces and bone marrow. The isolation rate varies from 30% to 70% with the rate in bone marrow aspirate culture being the highest. The Widal test, which is also relied on is time consuming and requires paired sera for interpretation. It is only moderately positive and cross-reactions with other *Salmonella* strains have been reported<sup>(1,2)</sup>. In low endemicity areas the frequency of weak and delayed H and O antibody responses severely limit the usefulness of the Widal

test<sup>(7)</sup>. The result of Widal test is also effected by variables such as frequency distribution of agglutinins in the population, effect of antibiotic treatment and antibody response to enteric fever<sup>(3)</sup>. Interpretation of Widal test should be based on base line titres in a healthy population. Also false positive results are seen with patients who had previous vaccination or infection or reported in association with a few autoimmune diseases. False negative results may be associated with early treatment, with hidden organisms in bone and joints and with relapse of typhoid fever<sup>(8)</sup>. The presentation of enteric fever is usually non-specific and the results of conventional diagnostic tests are often unavailable in the initial management of the patient. Hence, a rapid test for the early diagnosis would be useful and it should be sensitive, specific, rapid and inexpensive.

We evaluated the Typhidot Dot EID kits and PanBio ELISA kits. The Typhidot test procedure is simple and does not require any sophisticated laboratory equipment and requires lesser technical expertise. In previous studies carried out by Ong et al<sup>(9)</sup>, the Typhidot test showed 100% correlation with culture positivity. The results of the Typhidot test showed a higher specificity (68.1%), PPV (57.7%) and efficiency (72.9%) than Widal (21.2%, 40.3%, 48.7% respectively). In studies conducted by Choo et al<sup>(10,11)</sup> in children aged between one to 12 years of age, a sensitivity (95.2%), specificity (74.6%) and NPV (96.1%) was noted while the earlier paper gave Widal sensitivity and specificity as 89%. Studies conducted by Jackson et al<sup>(12)</sup> showed a higher specificity (91%), sensitivity (90%), PPV (76%) and NPV (97%), but the study group included only 21 samples in Typhoid group compared to 144 samples in our study. When compared with the studies by Karamat et al<sup>(13)</sup>, sensitivity (92%) and specificity (96%) were higher, but here the study group consisted of only 25 samples. The sensitivity of Typhidot improved, when sera were pre-absorbed to remove IgG, and an overall increase in analytical values was noted, indicating the importance and usefulness of Typhidot M in analysis of samples negative by Typhidot. Hence, while using the Typhidot test, it would be appropriate to test the negative samples using Typhidot M to further confirm, and also to increase the detection of *S. typhi* infection. Specificity (98%) and NPV (98.6%) of the combined Typhidot and Typhidot M results was higher than studies conducted et al<sup>(14)</sup> (95.2% and 95.5% respectively).

ELISAs standardised to detect antibodies using capsular polysaccharide<sup>(15)</sup>, lipopolysaccharide<sup>(16)</sup> and outer membranc protein<sup>(17)</sup> as antigen have been found to be useful in the diagnosis of typhoid fever. The antigen used for the PanBio ELISA kits

is not mentioned. In our present study, PanBio ELISA was found to be more specific (80%) with a higher PPV (68.4%) and efficiency (79.9%) than Widal (21.1, 40.3 and 48.6% repectively). While there was an increase in sensitivity, specificity and PPV when compared to the Typhidot test, the efficiency and NPV was found to be lower. However, comparison of PanBio ELISA with results of combined Typhidot and Typhidot M test showed PanBio ELISA to have a lower sensitivity, PPV, NPV and efficiency but a higher specificity thus reflecting on the antigen used in the assay which seemed to be more specific for *S. typhi*.

Our analysis of culture negative samples showed that the Typhidot was able to show positivity in more samples than the PanBio kits. The reason may be associated with the type of antigen used by both kits where as a result of greater sensitivity, specificity is compromised as can be seen by the differences obtained in sensitivity and specificity of both the kits.

When comparing the practical aspects of the tests evaluated, Typhidot is simple and easier to perform, and requires approximately one hour to complete. It does not require any special equipment and hence is convenient to conduct the test in the field and in small hospitals where facilities are lacking. The PanBio ELISA on the other hand requires specialised equipment and is better done in laboratories where facilities are available. Also the PanBio ELISAs require five wells, for the blank, controls and cutoff calibrator for each run and is therefore better to run PanBio with many samples to avoid wastage. On comparison of the analytical evaluation of the tests, the efficiency of test is enhanced when the Typhidot kit is used together with Typhidot M kit.

## CONCLUSION

Prompt diagnosis of typhoid fever is essential for the patient and the community management of typhoid fever. It is therefore important to have a satisfactory test to replace conventional tests used for the diagnosis of typhoid fever. In the present study, evaluation using a limited number of sera (n=144), the Typhidot appears to be a practical alternative. It is also observed that in areas of high endemicity a step to include removal of IgG in the detection of IgM would enhance the sensitivity of the assay as a diagnostic tool.

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## REFERENCES

1. Merican I. Typhoid fever: present and future. *Med J Malaysia* 1997; 52:299-308.
2. Ivanoff B and Levine MM. Typhoid fever: continuing challenges from a resilient bacterial foe. *Bull Inst Pasteur* 1997; 95:129-42.
3. Topley and Wilson's Principles of Bacteriology, virology and immunity. Eighth Edition, Volume 3, Chapter 3, 1990; 22:427-30.
4. Losonsky GA, Ferrecci C, Koltoff KL, Kaintuck S, Robbins JB and Levine MM. Development and evaluation of an enzyme linked immunosorbant assay for serum antibodies of chronic *Salmonella typhi* carriers. *J Clin Micro* 1987; 25(12):2266-9.
5. Ismail A, Kader ZSA, Ong KH. Dot enzyme immunosorbant assay for the serodiagnosis of typhoid fever. *Southeast Asian J Trop Med Public Health* 1991; 22:563-6.
6. Pang T and Puthuchery S. Significance and value of the Widal test in the diagnosis of typhoid fever in an endemic area. *J Clin Pathol* 1983; 36:471-5.
7. Brodic J. Antibodies and the Aberdeen typhoid outbreak of 1964. II. Coombs complement fixation and fimbrial agglutination tests. *J Hyg (Lond)*. 1977; 79(2):181-92.
8. Duthic R and French GL. Comparison of methods for the diagnosis of typhoid fever. *J Clin Pathol* 1990; 43:863-5.
9. Ong KH, Ismail A, Choo KE, Kader ZK. Clinical field trails on the Dot Enzyme Immunosorbent Assay (EIA) for the diagnosis of Typhoid fever in proceedings of First Asia-Pacific Symposium on Typhoid fever, October 91; 207-14.
10. Choo KE, Oppenheimer SJ, Ismail AB and Ong KH. Rapid serodiagnosis of typhoid fever by dot enzyme immunoassay in an endemic area. *Clin Infect Dis* 1994; 19:172-6.
11. Choo KE, Razif AR, Oppenheimer SJ, Ariffin WA, Lau J, Abraham T. Usefulness of the Widal test in diagnosing childhood typhoid fever in endemic area. *J Paediatr Child Health* 1993 Feb; 29(1):36-9.
12. Jackson AA, Ismail A, Ibrahim TAT, Kader ZS and Nawawi NM. Retrospective review of dot enzyme immunoassay test for typhoid fever in an endemic area. *Southeast Asian J Trop Med Public Health* 1995; 26(4): 625-30.
13. Karamat KA, Usman J, Ahmed TA, Abbas SH. Detection of serum IgM against *Salmonella typhi*: A rapid diagnostic technique. *J College Physicians Surgeons Pakistan* 1998; 8(4):170-3.
14. Choo KE, Davis TME, Ismail A, Tuan Ibrahim TA, Ghazali WNW. Rapid and reliable serological diagnosis of enteric fever: comparative sensitivity and specificity of Typhidot and Typhidot M tests in febrile Malaysian children. *Acta Tropica* 1998; 72(2): 175-83.
15. Losonsky GA, Ferreccio C, Koltoff KL, Kaintuck S, Robbins JB and Levine MM. Development and evaluation of an enzyme-linked immunosorbant assay for serum Vi antibodies for detection of chronic *Salmonella typhi* carriers. *J Clin Micro* 1987; 2266-9.
16. Sippel J, Bukhtiar N, Awan MB, Krieg R, Duncan JF, Karmat KA, et al. Indirect immunoglobulin G (IgG) and IgM enzyme linked immunosorbant assays (ELISAs) and IgM capture ELISA for detection of antibodies to lipopoly-sacchridein adult typhoid fever patients in Pakistan. *J Clin Micro* 1989; 27(6):1298-302.
17. Verdugo-Rodriguez A, Gam LH, Devi S, Koh CL, Puthuchery SD, Calva E, et al. Detection of antibodies against *Salmonella typhi* outer membrane protein (OMP) preparation in typhoid fever patients. *Asian Pac J Allergy Immunol* 1993; 11(1):45-52.

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