

Original Research Article

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## A Comparative Study of Blood Culture, Widal Test and Immunochromatographic Assay for Rapid Diagnosis of Typhoid Fever in a Tertiary Care Centre

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### ABSTRACT

Typhoid fever continues to be a major public health problem and the emergence of antimicrobial resistance by *Salmonellae typhi* adds to the complexity in treating the patients. *Salmonella enterica* subspecies *enterica* serovar *typhi*, the human specific, causative agent of typhoid fever, is one of the most common infectious diseases in developing countries like India 1. Widal test is associated with numerous limitations, but is still considered and extensively used as the diagnostic tool in our area. The bacteriological identification by blood culture is the best confirmative test of Typhoid fever. The aim of the study was to determine the reliability of Immunochromatographic test for the early diagnosis of typhoid fever when compared to the Widal test. The present study was carried out in Tirunelveli Medical College and Hospital, Tirunelveli for a period of one year from June 2017 to July 2018. A total number of 100 clinically suspected Typhoid fever patient's blood samples were taken and blood culture, widal test and Immunochromatographic tests were done. A total of 14 *Salmonella typhi* were isolated from 100 clinically suspected typhoid cases. A total of 47 samples were tested positive in Widal test. Out of this only one sample was positive for blood culture (True positivity rate – 2.1% and True negativity rate – 75.4%). Out of 26 samples positive for IgM in ICT, 10 samples were positive for blood culture (True positivity rate – 38.5%). Out of 74 samples that were negative for IgM, only 4 samples were found to be positive for blood culture (True negativity rate – 94.6%). In conclusion, the study implies that rapid ICT tests offers increased sensitivity, rapidity and simplicity over blood culture and Widal test, and can be used as a reliable, alternate early diagnostic tool to the most commonly used serological tests.

#### Keywords

Typhoid fever,  
Widal test,  
Immunochromatographic Test, Blood culture, *Salmonella typhi*

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### Introduction

Typhoid fever is a systemic infection caused by *Salmonella typhi*, and usually through

ingestion of contaminated food or water. It is a life threatening infection occurring in developing countries of the world and continues to be a major public health

problem. The acute illness is characterized by prolonged fever, headache, nausea, loss of appetite, and constipation or sometimes diarrhea. Symptoms are often non-specific and clinically indistinguishable from other febrile illnesses. However, clinical severity varies and severe cases may lead to serious complications or even death. It occurs predominantly in association with poor sanitation and lack of clean drinking water.

In 2015, there were 12.5 million new cases worldwide.<sup>[1]</sup> The disease is most common in India.<sup>3</sup> In 2015, it resulted in about 149,000 deaths worldwide – down from 181,000 in 1990 (about 0.3% of the global total). Typhoid fever emerged as an important infectious disease in the early 19th century. With an incubation period 3 to 21 days, it begins with mounting fever, headache, vague abdominal pain and constipation, which may be followed by appearance of rashes (Lesser and Miller, 2005; Gopalakrishnan *et al.*, 2002). These symptoms are for acute typhoid fever, specific antibody IgM is induced and it lasts for several weeks. It is later replaced by IgG<sup>9</sup> (Angraini *et al.*, 2004).

In the third week, the patient reaches a state of prolonged apathy, toxemia, delirium, disorientation and /or finally coma followed by diarrhea<sup>10</sup> (Gopalakrishnan *et al.*, 2002). Patients with typhoid fever carry the bacteria in their bloodstream and intestinal tracts for a long period of time. *Salmonella typhi* lives only in human beings. A delay in diagnosis and administration of appropriate therapy may significantly increase the risk of adverse outcome and mortality<sup>12</sup> (Bhutta, 1996). An accurate diagnosis of typhoid fever at an early stage is important for both etiological diagnosis, and also to identify patients that may become a potential carrier, becoming responsible for future acute typhoid fever outbreaks<sup>13</sup> (Parker, 1990). In Typhoid fever, the definitive diagnosis depends on the

isolation of *S. typhi* from blood, bone marrow, rectal swab, urine or duodenal aspirate culture (Gasem *et al.*, 1995 and Wain *et al.*, 2001). Even though blood culture is gold standard, the yield of it is quite variable. This test is highly specific but its sensitivity is affected by prior antibiotic intake and stage of illness<sup>17</sup> (House *et al.*, 2001). In most of the developing countries, irrational and widespread use of antibiotics is the main reason for the low sensitivity of blood cultures.

Bone marrow culture has a higher sensitivity despite 5 days of antibiotic therapy than blood culture but is more invasive procedure (Farooqui *et al.*, 1991; Gasem *et al.*, 1995): Bone marrow cultures though more sensitive is not feasible in mass public health screening. The sensitivity of stool and urine cultures is much lower and they become positive after the first week of infection.

Widal test has been used for over a century in developing countries for diagnosing typhoid fever but it has a low sensitivity, specificity and positive predictive value, which changes with the geographical areas. Poor specificity is because of pre-existing baseline antibodies in endemic areas, cross reaction with other Gram-negative infections and non-typhoidal *Salmonella* and prior TAB or oral typhoid vaccination. The Widal test lacks sensitivity and specificity and single titer reading lacks reliability. Thus requiring a paired sera showing fourfold rise in titer, and it also requires more than 1 week for a significant titre to buildup in the blood. This makes it, though widely used, not a satisfactory and reliable diagnostic tool.

These limitations have thus prompted the emergence for other newer test like Immunochromatographic assay, ELISA, latex agglutination, co agglutination and the PCR (Haque *et al.*, 1999; Jesudason *et al.*, 1994; Mukherjee *et al.*, 1993).

Inexpensive, rapid and reliable serodiagnostic test recently available commercially and studied in many endemic areas with reports of higher sensitivity and specificity. ICT detects both IgM and IgG.

Thus ICT test offers simplicity, speed, early diagnosis and high negative and positive predictive values. The test become positive as early as in the first week of the fever, the results can interpreted visually and available within one hour (Ismail *et al.*, 1991; Choo *et al.*, 1994):

This study was undertaken to evaluate the Immunochromatographic assay for its usefulness in patients of Typhoid fever presenting to a tertiary care hospital in terms of their reliability, economical and rapid diagnostic value.

Thus facilitating early diagnosis and timely effective management thereby reducing the morbidity, mortality and carrier state due to Typhoid fever.

### **Materials and Methods**

This prospective cross sectional study was undertaken at the Department of Microbiology, Tirunelveli Medical College for a period of one year from June 2017– July 2018. One hundred clinically suspected Typhoid fever cases were selected on the basis of following inclusion criteria -

#### **Study population**

#### **Inclusion criteria (Butler and Scheld, 2004)**

- i) Fever for  $\geq 3$  days, with no obvious focus of infection,
- ii) Abdominal discomfort- constipation or loose motions,
- iii) Coated tongue, toxic look,
- iv) Hepatomegally, Splenomegaly
- v) Relative bradycardia, rose spot etc.

#### **Exclusion criteria**

- i) Persons who are immunized with typhoid vaccines.
- ii) Persons suffering from fever other than typhoid

Informed consent was obtained from all patients included in the study. The proforma was filled with the details like name, age, sex, ward, clinical diagnosis, risk factors, undergone any surgery, duration of hospital stay and other parameters significant to the present study.

#### **Sample collection and processing**

Blood was taken for both culture and serological tests. At least 7 ml of blood from each adult patient were collected from single venepuncture. The top of the rubber stoppers of the blood culture bottle were disinfected with 70% alcohol and 5ml of collected blood were injected immediately into the culture bottle. Rest 2 ml of blood from each sample were taken in a clean dry test tube for separation of serum. Tubes containing 2 ml of blood was kept at room temperature for one hour to allow clotting of blood and then it was centrifuged at 1500 rpm for 15 minute. Serum was separated and kept in a sterile Eppendorf's tube at -20C until further use.

#### **Procedure of conventional Blood culture method**

Blood culture was done by conventional method using bile broth. 5 ml of collected blood was inoculated immediately into 50 ml of bile broth (which was brought to room temperature 30 minutes before inoculation) respectively. The inoculated bottle was inverted 3-5 times to mix blood with broth. Inoculated culture bottle was incubated at 37°C aerobically. Subculture from conventional bottle was done after the first 24

hours, 48 hours and 7 days of incubation onto MacConkey agar, Nutrient agar and Blood agar plates.

The organisms were identified by their colony morphology, Gram staining methods, motility test and following biochemical reactions with suitable controls.

### **Serological tests**

#### **Antibody detection by Widal agglutination test**

##### **Slide test**

Slide agglutination test was done and when agglutination was visualised within 1 minute, tube test was done for the quantitative estimation of the titre of the antibody.

##### **Quantitative tube test**

Tube agglutination test was done and result was interpreted.

##### **Result interpretation of Widal test**

Antibody titre greater than 1: 80 was considered significant and suggested positive for Salmonella infection.

##### **Immunochromatographic test**

Lateral flow immunoassay test was done on serum by using Rapid typhoid IgG /IgM test device kit. This test is a qualitative antibody detection test with total assay time of 15 minutes. The test cassette consists of 1) a burgundy colored conjugate pad containing recombinant H antigen and O antigen conjugated with colloidal gold (HO conjugates) and rabbit IgG-gold conjugates. 2) a nitrocellulose membrane strip containing two bands G and M bands and a control band (C band).The M band is precoated with

monoclonal anti-human IgM for the detection of IgM anti-*S.typhi*. G band is precoated with reagents for the detection of IgG antibodies. C band is precoated with goat anti rabbit IgG. IgM antibodies if present in patient serum, will bind to HO conjugates.

##### **Procedure**

Serum samples were added to the sample well followed by adding the supplied diluents. The positive control forms a colored band in the test and control line. Any test sample showing similar or darker bands was defined as positive. The absence of any visible band was considered as a negative test result.

##### **Interpretation**

The immunocomplex is then captured on the membrane by the pre coated anti-human IgM antibody, forming a burgundy colored M band, indicates positive test result. IgG antibodies if present in patient serum, will bind to HO conjugates.

The immunocomplex is then captured by the precoated reagents on the membrane, forming a burgundy colored G band, indicating positive test result. Absence of M and G bands suggests negative test.

##### **Results and Discussion**

In the present study, among 100 clinically suspected typhoid cases 59% were males and 41% were females. A total of 100 clinically suspected fever cases with fever of  $\geq 3$  days has been included 70% of cases presented with fever of 3-7 days and 30% were having fever of more than a week duration.

Out of the 100 tested samples, 14 samples(14%) were positive for *S. typhi* and hence bacteriologically proven typhoid fever or "true positive cases". The remaining 86

patients were culture negative.

Widal test was carried out for all the clinically proven typhoid cases. The cut off value of Widal test was considered as 1:80 for both TO and TH. In our study about 57.44% cases with fever of more than a week showed an antibody titer of  $\geq 160$  (Table 1).

Among clinically suspected 100 typhoid fever cases 53 cases were both blood culture and Widal test negative. Out of 47 positive samples for Widal test, only one sample was positive for blood culture (True positivity rate – 2.1%). Out of 53 negative samples for Widal test, 13 samples were negative for blood culture (True negativity rate – 24.5%) (Table 2).

Immunochromatography assay showed 26% samples to be positive for IgM and 6% samples to be positive for IgG. None of the samples were positive for both IgG and IgM. Out of the 26 positive ICT IgM cases the number of positive cases appear to gradually decrease as the duration of fever at presentation increases (Table 3).

Out of 26 samples positive for ICT, 10 samples were positive for blood culture (True positivity rate – 38.5% (Table 4). Out of 74 samples negative for ICT only 4 samples were found to be positive for blood culture (True negativity rate – 94.6%). IgG antibody are not considered as a comparison, because long-term persistence of the IgG antibody after exposure to typhoid infection or vaccination.

In the present study, among 100 clinically suspected typhoid cases 59% were males and 41% were females. This finding was similar to that of Roxas and Mendoza (1989) with 56% males and 44% females. The age of the patients ranged from a minimum of 16 years to a maximum of 74 years. Most of the isolates (39%) were from patients aged between 31 and 45 years. This is similar with

the studies of Riyaz chungathu *et al.*, (2015), Varsha Gupta *et al.*, (2013). A study done by Butler *et al.*, (1991) also showed that infection rate is slightly higher in male population, because men are more in the habit of travelling more for work and more frequently exposed to outdoor food and water that may be contaminated and also males are more likely to report in hospitals. Health education and awareness regarding food and personal hygiene will bring this number down. This is comparable with the other studies of Shoora shetty Manohar Rudresh *et al.*, (2015) and Sarika Jain *et al.*, (2012).

In this study 100 clinically suspected fever cases with fever of  $\geq 3$  days has been included 70% of cases presented with fever of 3-7 days and 30% were having fever of more than a week duration. Isolation of Salmonella is possible in the earlier days of disease and antibiotic intake will be less during this period. To compare the antibody level it was better to test the samples of patients presenting later into the week. This was comparable with the studies of Raveesh *et al.*,

In this study from blood samples of 100 febrile patients clinically suggestive of Typhoid fever 14 samples (14%) were positive for *S. typhi* and hence bacteriologically proven Typhoid fever or "true positive cases". The remaining 86 patients were culture negative.

Similar culture findings were also reported by Hossain *et al.*, (2001) from Bangladesh of 16.67%. But, Saha *et al.*, (2001) from Bangladesh and Jesudasson and Sivakumar from India reported an isolation rate of 8.40% and 6.92% respectively, which was even lower.

The overt abuse of antibiotics and it being difficult to obtain large enough volume of blood for the culture is the main cause for low



isolation rate. As seen with the studies by Parande *et al.*, (2011) and Walia and Kalaivani *et al.*,

The Widal test is still the widely used serological test for Typhoid fever. Here the antibody against antigens O and H are detected. In this study, Widal test was carried out for all the clinically proven typhoid cases. The cut off value of Widal test was considered as 1:80 for both TO and TH. In our study about 57.44% cases with fever of more than a week showed an antibody titer of  $\geq 160$ .

A study done by Shukla *et al.*, (1997) also found that 44.2% had TO titre of  $\geq 160$  in single sample collected from patients suspected to have typhoid in an endemic area of South India. Second specimens are often not sent to the laboratory to verify the rising titre. It is possible that the Widal test would have performed better if paired sera were tested to demonstrate the rising titers. Patients rarely return for follow-up once treated so that obtaining paired sera in a routine clinical setting is unlikely. Clinicians cannot wait for results from two samples hence widely rely on “positive” Widal test done on a single serum sample.

In the present study 30% of samples were collected from patients with fever of  $>7$  days. In such patients antibody titre was found to be  $\geq 320$ . This is due to the increase in antibody titre as the duration of fever increases. The incidence of false negative Widal test among the bacteriologically proven cases of this study was 13(24.5%). These findings were similar to when compared with findings of Sudeepa Kumar *et al.*, 11.3% (Saha *et al.*,) and 6.9% in Malaysian populations (Malik, 2001).

In the present study, among clinically suspected 100 typhoid fever cases 53 cases

were both blood culture and Widal test negative. Out of 47 positive samples for Widal test, only one sample was positive for blood culture (True positivity rate – 2.1%). Out of 53 negative samples for Widal test, 13 samples were negative for blood culture (True negativity rate – 24.5%). This correlates with findings of Olopoenia and King, 2000; Parry *et al.*, 2002; Rodrigues, 2003). Suboptimal sensitivity is due to prior antibiotic therapy and failure to mount an immune response by certain individuals (Olopoenia and King, 2000). The IgM antibody starts appearing later into the first week.

The sensitivity, specificity, Positive predictive Value and Negative predictive Value of Widal test were 7%, 46.5%, 2.1% and 75.4%. These values are in concordance with studies published by Sherwal *et al.*, Widal test has a low sensitivity, specificity and low PPV, but it has good NPV which indicates that negative Widal test result have a good indication for the absence of the disease.

In the current study Immunochromatographic test was evaluated for its usefulness in patients of typhoid fever presenting to our hospital and observed that it has a sensitivity of 71.4% and specificity of 81.4%, which was higher than that of Widal test (sensitivity-7% and specificity-46.5%) and comparable to the studies done elsewhere in India and outside.

ICT had a comparable sensitivity of 94% and specificity of 77%, while Widal test had sensitivity and specificity of 63% and 83% only in a study conducted in Pakistan. The effectiveness of ICT in early diagnosis of typhoid fever patients was also studied in two different studies in Malaysia. Its sensitivity and specificity was reported as 90.3% and 91.9% respectively in the first study, and was significantly higher. The second study, also showed a sensitivity and specificity of 98% and 76.6% respectively.

**Table.1** Relationship with Widal positive results and duration of fever

Duration	< 7 days	7-10 days	> 10 days
Positive	1(2.12%)	27(57.44%)	19(40.42%)

**Table.2** Comparison of blood culture and Widal test results

Widal test	Blood culture		Total (N=100)
	Positive	Negative	
Positive	1 (2.1%)	46 (97.9%)	47
Negative	13 (24.5%)	40 (75.5%)	53
Total	14 (14%)	86 (86%)	100

**Table.3** Comparison of duration of fever with ICT positive results

Duration	< 7 days	7-10 days	> 10 days
Positive	10	12	4

**Table.4** Comparison of blood culture and ICT test results

Immunochromatography assay (IgM)	Blood culture		Total (N=100)
	Positive	Negative	
Positive	10 (38.5%)	16 (61.5%)	26
Negative	4 (5.4%)	70 (94.6%)	74
Total	14	86	100

Immunochromatography assay showed 26% samples to be positive for IgM and 6% samples to be positive for IgG. None of the samples were positive for both IgG and IgM. Out of the 26 positive ICT IgM cases the number of positive cases appear to gradually decrease as the duration of fever at presentation increases.

Out of 26 samples positive for ICT, 10 samples were positive for blood culture (True positivity rate – 38.5%). Out of 74 samples negative for ICT only 4 samples were found to be positive for blood culture (True negativity rate – 94.6%). IgG antibody are not considered as a comparison, because long-term persistence of the IgG antibody after exposure to typhoid infection or vaccination. Widal test has been used for over a century in

developing countries for diagnosing typhoid fever but it has a low sensitivity, specificity and positive predictive value, which changes with the geographical areas. In this study we have compared the relative diagnostic accuracy of Widal test with a rapid Immunochromatographic test (ICT) taking blood culture positive cases as relative standard.

Rapid Immunochromatographic test evaluated in this study offers increased sensitivity, rapidity, early diagnosis and simplicity over blood culture and Widal test and it can be used as a reliable alternate diagnostic tool to the most commonly used serological tests. Thus, making it an ideal alternate, economical and a reliable diagnostic tool in our setup to be considered.

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