



## Comparative Evaluation of diagnostic performance of Blood culture, Lateral flow immunoassay, Tube Widal and Slide Widal Test in diagnosis of Enteric fever using a Composite Reference Standard

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### ABSTRACT

**Background:** Enteric fever a potentially fatal multi systemic infectious disease is well recognised as a serious public health problem throughout the world. Undiagnosed and untreated cases may result in serious complications. Prompt and accurate diagnosis of the cases is the most important step for the efficient timely management of the disease.

**Aim:** to compare and evaluate the diagnostic performance of various diagnostic modalities for Enteric fever like Blood culture, Tube widal, Slide widal and Typhoid IgG/IgM Rapid Card test using a Composite Reference standard.

**Material and Methods:** This cross sectional analytical study was conducted in the Dept. of Microbiology of a rural tertiary care teaching hospital of western Uttarpradesh, India from July 2016 to June 2017 over a period of one year after obtaining permission from the institutional ethics committee.

Blood culture was done using standard microbiological techniques. Slide widal, Tube widal & Typhoid IgG/IgM Rapid Card tests were done as per instructions in the kit insert. Various diagnostic parameters like Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Accuracy, Positive Likelihood ratio and Negative Likelihood ratio for all the tests were calculated and compared using a Composite Reference standard.

**Results:** In this study the overall positivity rate for Blood culture was 12%, Tube Widal 32%, Slide Widal 46% and IgG/IgM RCT 48%. Amongst all four diagnostic tests under study highest sensitivity was exhibited by IgG/IgM RCT (77.42%) and highest specificity was observed for Blood culture (100%)

**Conclusion:** Amongst all the tests evaluated in this study IgG/IgM Rapid card Test which is a fast, reliable, simple and easy to perform test had emerged as the best diagnostic modality for appropriate and timely management of enteric fever.

**Keywords:** Enteric Fever, Blood culture, Widal test, Typhoid IgG/IgM Rapid Card test, Lateral flow immunoassay, Composite Reference Standard.

## Introduction

Enteric fever is well recognised as a serious public health problem throughout the world. As per the WHO records the global annual incidence of Enteric Fever is about 20 million cases with greater than 6 Lac deaths reported annually.<sup>1,2</sup>

Enteric Fever is mainly encountered in developing countries of South East Asia including India which accounts for almost 80% of the total worldwide cases.<sup>3,4</sup>

Enteric fever is a potentially fatal multi systemic infectious disease caused primarily by *Salmonella enterica* subsp. *enterica* serovar *typhi* and to a lesser extent by *Salmonella paratyphi* A,B & C.<sup>5-7</sup>

The main factors contributing towards this public health problem are rapid population growth, increasing urbanisation, contaminated water supply, poor hygiene and lack of sanitation.<sup>1,5,8</sup> In such community settings, the other causes of febrile illness exist like malaria, dengue, rickettsiosis, leptospirosis and melioidosis. These illnesses are not easily distinguishable esp. in the initial stage and hence creates confusion in clinical diagnosis. Undiagnosed and untreated cases may result in serious complications.<sup>5,9</sup>

Prompt and accurate diagnosis of the cases is the most important step for the efficient management and control of the disease.

Bone marrow culture and Blood culture have been recognised as the Gold standard diagnostic modalities in Enteric fever. Although cultures are more reliable but not that simple and easy to perform and are expensive and time consuming requiring infrastructure, equipments & trained Laboratory staff that is seldom available in resource constrained remote settings of developing countries.<sup>5,6,10</sup>

So, in most of these countries, widal test is the most preferred diagnostic modality for enteric fever as it is cost effective, simple, easy and doesn't require much training and resources<sup>[11]</sup>. But there is always a lack of uniformity in the interpretation of results as significant titre varies in different regions and in different community settings and also influenced by other co-existing

factors. However it is useful only in second week of illness and paired sera required for confirmation of result.<sup>1,11,12</sup>

Widespread, inappropriate, indiscriminate and irrational use of antibiotics prior to sample collection is the biggest obstacle in isolation of causative organism in bacterial cultures and also contribute in the emergence of multi drug resistant strains.<sup>6,10,13</sup>

So, there is always a need for a fast, reliable, simple and easy to perform sero-diagnostic test with a higher sensitivity and specificity than the conventional modalities for appropriate and timely management of enteric fever. Prompt and accurate diagnosis of enteric fever cases at early stage helps in identifying persons who may turn as potential carriers and can lead to outbreaks.<sup>1,11,14</sup>

Typhoid IgG/IgM Rapid card test is a Lateral flow Immunochromatographic test. It detects presence of IgM and IgG antibodies to *S.typhi* and *S.paratyphi* lipopolysaccharides. It could be used as a rapid screening test for Enteric fever as early as in first week of the febrile illness. The test is easy to perform and results can be interpreted visually within 15minutes. So, nowadays such immunoassays are emerging as preferred alternative for rapid and easy diagnosis of Enteric fever.<sup>11</sup>

So, we had undertaken this cross sectional study to compare and evaluate the diagnostic performance of various diagnostic modalities for Enteric fever like Blood culture, Tube widal, Slide widal test and Typhoid IgG/IgM Rapid Card test using a Composite Reference standard.

## Materials and Method

This cross sectional analytical study was conducted in the Dept.of Microbiology of a rural tertiary care teaching hospital of western Uttarpradesh from July 2016 to June 2017 over a period of one year after obtaining permission from the institutional ethics committee. The samples collected from the clinically suspected cases were processed as a part of standard diagnostic protocol. All the diagnostic tests evaluated in the

study were part of the routine diagnostic procedures and not meant exclusively for the research purpose. Only the diagnostic performance of these tests were compared. Personnel data regarding patients was not used for the research and was kept confidential.

**Inclusion criteria**<sup>2,6,11</sup> - A total of 100 clinically suspected cases of Enteric fever irrespective of age & sex presenting with any signs & symptoms suggestive of Enteric fever like:

- History of fever > 3 days
- Abdominal discomfort, constipation or loose motion
- Coated tongue
- Toxic look, hepatomegaly, Splenomegaly, rose-spots
- Relative bradycardia, etc.

were included in the study by simple random sampling.

**Exclusion criteria**<sup>2,5-7,11</sup>

- The patients with any other known cause of febrile illness other than Enteric fever.
- Repeat samples
- Patients with a history of prior antibiotic therapy and/or
- H/O Typhoid vaccination in recent past; were excluded from the study.

**Sample collection**<sup>5,11,15,16</sup>

Venous Blood samples 10ml from adults and 5ml from children were collected aseptically and inoculated into Adult & Paediatric blood culture bottles containing BHI Broth with SPS (Microexpress, India) respectively.

Another 3ml blood was collected in sterile plain vial and allowed to clot. Serum separated was used for Widal test and Rapid Card test(Lateral Immunoflow assay).

**Blood Culture/Sensitivity**

The inoculated blood culture bottles with blood to broth ratio 1:10 were incubated aerobically at 37°C for 24 hrs.

Subcultures were made on blood agar and MacConkey agar plates every alternate day till 7<sup>th</sup> day. Bacterial colonies grown over the culture plates were further processed using standard microbiological techniques. Isolation, Identification and characterisation of Salmonella sp. was done as per standard protocol through biochemical tests followed by Slide agglutination using antisera.<sup>5,12,15-20</sup>

Antimicrobial susceptibility testing was performed for all isolates using Kirby Bauer Standard Disc diffusion method as per CLSI 2016 guidelines.<sup>21</sup>

**Widal test:** Widal test was performed by tube method and Slide method both.<sup>2,6,8,11,13,19</sup>

Widal Tube Agglutination test was performed using Febrile Antigen Set (Arkay Healthcare, Surat, India).

Widal Slide test (Latex agglutination test) was performed using Widal antigen set (Tydal antigen suspension, Tulip diagnostics, Goa,India).

Test procedures were performed as per manufacturer's instructions given in the kit insert.

**Interpretation of Widal test:** Widal test irrespective of the method employed was interpreted as positive for a titre of > 80 for O agglutinins and a titre of > 160 for H agglutinins based on the prevailing basal titres in that region and in that community settings as being evaluated prior to the commencement of the study.

**Typhoid IgG/IgM Rapid Card Test**<sup>11,14</sup>

Typhoid IgG/IgM Rapid Card Test(CTK Biotech, San Diego. CA) is a Lateral flow immunochromatographic test for qualitative detection and differentiation of IgG and IgM anti- S.typhi and anti- S. paratyphi antibodies in the test serum.

One drop (30-40µl.) of test serum is dispensed into the sample well of test device followed by one drop of sample diluent/buffer. Results were read within 15 minutes.

**Interpretation:**

**1) Negative/Non-reactive result:** If only C-band is present(pink/purple line in control region) with no band in both the test regions M & G(regions

specifying IgM & IgG respectively) indicates Negative result.

**2) Positive/Reactive result:** C-Band must be present and in addition to it if

a) Only M band is present, the result is IgM positive.

b) Only G-band is present then the result is IgG positive.

c) Both M and G bands are present then the result is both IgM & IgG reactive/positive.

**3) Invalid:** If no C-band develops in the control region, the assay is considered invalid regardless of appearance of any band in the test region.

Test procedures and interpretation of results were done according to manufacturer's instructions in the kit insert.

A comparative evaluation of diagnostic performance of Blood Culture, Tube widal, Slide widal test and Typhoid IgG/IgM Rapid Card Test was done against a Composite Reference Standard using tests for statistical significance.<sup>5,12,22-24</sup>

The composite reference standard was based on the following criteria:

I) All the blood culture positive cases

II) Blood culture negative cases with the 2 tests positive out of the following 3 serodiagnostic tests:

1) Tube Widal Test

2) Slide Widal Test

3) IgG/IgM Rapid Card Lateral flow Immunoassay.

Were considered as Composite Reference standard positive confirmed cases.

**Statistical analysis:** Various diagnostic parameters like Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Accuracy, Positive Likelihood ratio and Negative Likelihood ratio for all the tests were calculated and compared. The statistical analysis of results was done to establish the test of significance (Mc Nemar's Chi square test) for clear and confirmatory statements. p-value < 0.05 was considered statistically significant.<sup>13,25</sup>

**Table-1:** Composite Reference Standard Positive Cases of Enteric Fever

S.No	Diagnostic Test	Composite-Reference Standard Positive Cases
A.	Blood Culture Positive cases	12
1.	RCT only	4
2.	RCT+SW	1
3.	RCT+TW	5
4.	RCT+SW+TW	2
B.	Blood Culture Negative cases	50
1.	TW + SW	8
2.	TW+ RCT	13
3.	SW + RCT	21
4.	TW+ SW +RCT	2
	Total (A+B)	62

**Table-2:** Laboratory test Results in correlation with the duration of illness

Duration of illness in weeks	Number of Diag. Test +ve cases	Diagnostic Test Positive cases			
		Blood culture	Tube Widal	Slide Widal	RCT
1 <sup>st</sup>	30	10	2	3	20
2 <sup>nd</sup>	54	2	23	30	25
3 <sup>rd</sup>	16	0	7	13	5
Total	100	12	32	46	50

**Table-3:** Diagnostic Test Characteristics of Laboratory Tests for Enteric Fever

Diagnostic Test	Sensitivity 95% CI	Specificity 95% CI	PPV 95% CI	NPV 95% CI	Accu racy	+LR 95% CI	-LR 95% CI
Blood Culture	19.355 (10.42%- 31.37%)	100 (90.75%- 100.00%)	100	43.2 (40.22% - 46.20%)	50	-	0.81 (0.71-0.91)
IgG/IgM RCT	77.42 (65.03%- 87.07%)	94.74 (82.25%- 99.36%)	96 (86.09% - 98.94%)	72 (61.72% - 80.40%)	84	14.7 (13.79- 57.06)	0.24 (0.15- 0.38)
Tube Widal	48.39 (35.50- 61.44%)	94.74 (82.25%- 99.36%)	93.75 (79.16%- 98.34%)	52.94 (46.6%- 59.15%)	66	9.1935 (2.33- 36.30)	0.545 (0.42-0.70)
Slide Widal	55 (41.68%- 67.52%)	68.42 (51.35%- 82.50%)	74 (62.76%- 82.65%)	48 (39.57%- 56.83%)	60	1.74 (1.03- 2.92)	0.66 (0.47- 0.94)

**Table-4:** Comparative assessment of diagnostic tests with reference to CRS

Test	Result				Mc Nemar's Chi-square Test	p-Value
	Positive		Negative			
Reference Standard	RF +ve	RF -ve	RF +ve	RF -ve		
Blood culture	12	0	50	38	48.02	0
Tube Widal Test	30	2	32	36	24.735	0
Slide Widal Test	34	12	28	26	5.625	0.00885
IgG/IgM RCT	48	2	14	36	7.682	0.0028

## Results and Discussion

In this study we had found that out of the total 100 clinically suspected cases of Enteric fever, blood culture came out to be positive in only 12 cases all of which were *Salmonella enterica* serotype typhi. As evident by a number of studies conducted in the recent past, a very low blood culture positivity rate was seen in clinically suspected cases of Enteric fever. Due to its limited sensitivity and higher specificity it could not be practically considered as a Reference Gold standard. So, an alternative Composite reference standard was designed for this study. Such reference standards were previously been used in a number of studies involving the comparison of diagnostic tests (Baughman AL et al 2008<sup>23</sup>, Siba V et al 2012<sup>12</sup>, Naaktgeboren CA et al 2013<sup>22</sup>, Mitra S et al 2016<sup>24</sup>, Maheshwari V et al 2016<sup>5</sup>).

Out of 100 clinically suspected cases of Enteric fever, a total of 62 cases fulfilling the criteria of the Composite Reference standard and accordingly considered as confirmed cases of Enteric fever, included in Reference standard

positive category and remaining 38 cases as Reference Standard negative [Table-1].

Highest positivity rate for Blood culture was found during first week of illness (83.3%). In case of Tube Widal as well as Slide Widal most of the test positive cases were found to be reported in the 2<sup>nd</sup> and 3<sup>rd</sup> weeks of illness (93.75% and 93.48% respectively). In case of IgG/IgM Rapid Card Test diagnostic utility was found significant during 1<sup>st</sup> and 2<sup>nd</sup> weeks of illness with 89.6% positive outcome [Table-2]. Similar observations were reported by Baragundi M et al 2010<sup>26</sup> and Maheshwari V et al 2016<sup>5</sup>.

In this study the overall positivity rate for Blood culture was 12%, Tube Widal 32%, Slide Widal 46% and IgG/IgM RCT 48%. The low isolation rate in case of Blood culture may be due to a number of factors like prior antibiotic therapy, inadequate volume of blood inocula and late presentation (Maheshwari V et al 2016<sup>5</sup>, Sharanya K et al 2016<sup>11</sup>, Mohanty SK et al 2007<sup>27</sup>). A number of studies in the past have reported low isolation rates for blood culture (Sharanya K et al



2016<sup>11</sup>, Porwal A et al 2016<sup>28</sup>, Poudel S et al 2014<sup>29</sup>, Yadav S et al 2014<sup>30</sup>, Bargundi M et al 2010<sup>26</sup>). Mohanty & Ramana 2007<sup>27</sup> attributed the relative low sensitivity of the blood culture in diagnosing Enteric fever due to widespread and irrational use of antibiotics and insufficient volume of blood collection from paediatric subjects.

In this study out of 100 samples tested Tube Widal came out to be positive in 32 cases and Slide widal in 46 cases. Sensitivity of Tube Widal came out to be 6.67% in first week of illness, 42.6% in second week and 43.75% in third week. Sensitivity of Slide widal was 10% in first week, 55.56% in second week and 81.25% in third week. These findings are similar to study conducted by Sanjeev H et al 2013<sup>10</sup>. Usually O antibodies appear on 6<sup>th</sup> to 8<sup>th</sup> day and H antibodies on 10<sup>th</sup> to 12<sup>th</sup> day after disease onset which accounts for the same<sup>19</sup>.

Out of 100 samples tested in this study IgG/IgM Rapid Card Test gave positive results in 50 cases out of which 48 were reference standard positive. Amongst all four diagnostic tests under study highest sensitivity was exhibited by IgG/IgM RCT (77.42%) followed by Slide widal (55%), Tube Widal (48.39%) and least sensitivity by Blood culture (19.35%). Amongst all the tests under study highest specificity was observed for Blood culture (100%) followed by both Tube widal and IgG/IgM Rapid card test exhibiting same specificity (94.74%) and least specificity for Slide widal (68.42%) [Table-3].

In this study Tube widal had scored over Slide widal in terms of Specificity (94.74% and 78.95% respectively). These findings are in accordance with number of studies conducted in the past. (Willke et al 2002, Oslén et al 2004<sup>34</sup>, Das JC et al 2007<sup>33</sup>, Keddy KH et al 2011<sup>32</sup>, Gaikwad UN et al 2014<sup>31</sup>, Sood NK et al 2014<sup>7</sup>, Ramaraj R 2016<sup>8</sup>, Radhika R et al 2017<sup>13</sup>).

But in this study Tube widal test has slightly lower Sensitivity as compared to Slide widal test (48.4% and 55% resp.). This finding is in accordance with the studies conducted by Olsen et

al 2004<sup>34</sup>, Indro Handojo et al 2004<sup>35</sup>, Gaikwad UN et al 2014<sup>31</sup>, Radhika R et al 2017.<sup>13</sup>

As compared to Slide widal, Tube widal test with a much better Specificity, positive predictive value (PPV), Negative Predictive value (NPV), Positive Likelihood ratio (LR<sup>+</sup>) and Negative Likelihood ratio (LR<sup>-</sup>) and a comparable sensitivity has been proved to be much superior to Slide widal in terms of diagnostic performance [Table-3].

Likelihood ratios (LR) being independent of disease prevalence are always better than predictive values while quantifying and comparing diagnostic performance of different tests.

Positive LR shows how much more likely someone is to get a positive test if he/she has the disease, compared with a person without disease and vice versa for Negative LR.

Positive and Negative Likelihood ratios were highest for the Blood culture. Blood culture also has 100% specificity. This could be attributed to the design of Composite reference standard of which blood culture is an integral part.

IgG/IgM Rapid card test having comparatively high values of Sensitivity, Specificity, PPV, NPV and significant Likelihood ratios (LR<sup>+</sup> & LR<sup>-</sup>) had came out to be the best diagnostic modality for Enteric fever amongst all the tests evaluated in this study [Table-3].

Test Accuracy was also highest for IgG/IgM RCT followed by Tube widal and Slide widal which had almost same accuracy.

The difference in the findings of the diagnostic tests under study was found to be highly statistically significant as evident in Table-4.

Conclusion: Amongst all the tests evaluated in this study IgG/IgM Rapid card Test which is having comparatively higher values of Sensitivity and Specificity and which is a fast, reliable, simple and easy to perform test had emerged as the best diagnostic modality for appropriate and timely management of enteric fever.

This test should be considered as one of the most potential alternative to the long established Widal

test. It could be used as a rapid screening test for Enteric fever as early as in first week of the febrile illness. However it could be used as a rapid screening test but can never replace the Blood culture as the antibiotic susceptibility testing is highly recommended in the current scenario with emerging threat of multi drug resistant strains.

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**Conflict of interest:** Authors declare that they have no Conflict of interest.

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**Abbreviations:**

BHI- Brain Heart Infusion Broth

CLSI-Clinical and Laboratory Standards Institute

Ig- Immunoglobulin

LR- Likelihood Ratio

NPV-Negative Predictive value

PPV-Positive Predictive value

RCT-Rapid Card Test

WHO-World Health Organisation