A Prospective Comparative Study of Semi-Quantitative Slide Widal test& Quantitative Tube Widal test

Dr Nidhi K Sood *, Dr Manisha M Jain **, Dr Sachin Patel *, Dr Asha Mandalia ****, Dr H K Bhavsar *******

*Professor and Head, **,**** Tutor, ***Assistant Professor, Microbiology Department,

*****Superintendent and Assistant professor, Medicine Department GMERS Medical College and Hospital, Sola, Ahmedabad

Abstract: Objective: The diagnosis of enteric fever currently depends upon the isolation of Salmonella from a patient, most commonly by blood culture. This facility is not available in many areas where the disease is endemic. The Widal agglutination test which demonstrates the presence of somatic (O) and flagellar (H) agglutinins to Salmonella in the patient's serum using O and H antigen suspension is one of the most utilized diagnostic tests for typhoid fever in developing countries. In the recent past, a rapid slide test was developed which is now the most commonly used technique in local laboratories because of its convenience. The semi-quantitative slide test provides an approximation to the tube test titre. The present study was an attempt to compare the results of semi-quantitative slide agglutination with the quantitative tube method to ascertain any significant difference between two methods. **Material and Methods:** 300 precollected blood samples of OPD patients were collected over a period of 4 months and subjected to semi-quantitative rapid slide and quantitative tube Widal tests using commercially available Salmonella antigen test kits. **Results:** From the 300 samples studied, 17(5.7%) serum samples were positive by slide test method while 25(8.7%) seropositive by Tube test method. Present study shows, slight difference in the results obtained by the two methods of Widal test. **Conclusion:** The Tube Widal test is more sensitive and specific than slide Widal test, especially for rule out prozone phenomena, but slide Widal agglutination test is found to good alternative screening test because less time consuming, easy to perform, cost effective and can be applied in resource poor nations. There is an urgent need for the rational design and evaluation of effective and appropriate diagnostics for typhoid fever. [Jain M NJIRM 2014; 5(6):73-77]

Key Words: Salmonella, Slide Widal test, Tube Widal test

Author for correspondence: Dr Manisha M Jain; E 203, Nirmal Signature, Near Sahajanand Bunglow, Near Aditya green, new C G Road, Ahmedabad 382424, Gujarat, India **Mo:** +91 9925250032 **Email:** drjain.manisha@gmail.com

Introduction: Enteric fever which includes typhoid and paratypoid fever is a systemic febrile illness caused by the bacterium *Salmonnela enterica* serovar typhi and *Salmonella enterica* serovar paratyphi A,B or C respectivly. Salmonella enterica serotype typhi is found to be associated with more than 90% of cases of enteric fever. Many middle and low income countries are endemic regions of enteric fever due to an inappropriate level of sanitation. While in industrialised countries; it is mainly manifested after the travel to endemic countries.[2]

The standard method for the diagnosis of enteric fever is the isolation of organism from specimens especially blood, faeces, urine or other body fluids. In developing countries like India facilities for isolation & culture are often not available especilly in smaller hospitals.[1]However, the universally practiced diagnostic procedure is the Widal test because other methods are either invasive, expensive and have poor utility. Moreover, the isolation of causative agent from the culture requires a good microbiological laboratory facility which makes Widal test a better alternative method of diagnosis of typhoid and paratyphoid fever, mainly in many devloping countries like India.[2]

In this study, the Widal test was applied for the detection of *Salmonella typhi* and *Salmonella paratyphi* specific antibodies in serum and the results were compared between Tube Widal test and Slide Widal test.

Materials and Methods: The present study "A prospective comparative study of semi-quantitative Slide Widal test & Quantitative Tube Widal test'' was conducted in The Department of Microbiology, GMERS Medical Collage & Hospital, Sola, Ahmedabad from December 2013 to March 2014.

Source Population: Patients attended the Sola General Hospital medical OPDduring the study period.

Study Population: all febrile patients with symptoms clinically similar to typhoid fever attended the OPD at sola general hospital during the study period.

Sample Size: 300 patient's serum samples were studied for determination of antibody titre.

Inclusion Criteria:

- Fever for ≥ 7 days, with no obvious focus of infection,
- Abdominal discomfort- constipation or loose motions,
- Coated tongue, toxic look,
- Hepatomegally, splenomegally,
- Relative bradycardia, rose spot etc.

Exclusion Criteria:

- Persons who were immunized with typhoid vaccines.
- Patient suffering from fever other than typhoid.
- Patients on antibiotics

Laboratory Methods: Type of Specimen: Precollected patient's serum sample received at the receiving section of the Microbiology Department.

Collection of Specimens: Patients who presented with symptoms suspected of typhoid fever were subjected for the study. According to inclusion criteria like Fever for ≥ 7 days, abdominal discomfort, coated tongue, hepatomegally, splenomegally, relative bradycardia, rose spot etc. Details of clinical symptoms and signs were recorded in the proforma. Informed consent was taken from all patients. Precollected 2-3 ml blood samples suspected of typhoid were taken for serological semi-quantitative Slide Widal test & Quantitative Tube Widal test.

Serological Tests: Commercially available stained Salmonella antigen from RECKON DIAGNOSTICS PVT. LTD's kit containing S. typhi 'O', S.typhi 'H', S.paratyphi 'AH' and S.paratyphi 'BH' suspensions were used for the tests. Test procedures were performed according to manufacturer's instructions in the kit insert. Appropriate control tubes with normal saline were kept. Antibody titre was taken as the highest dilution of serum showing distinct agglutination. In semi quantitative slide Widal test antibody titres ≥ 1: 80 for 'O', 'H', 'AH'& 'BH' antigens and for quantitative tube Widal test antibody titres ≥1:120 for 'O', 'H', 'AH' & 'BH' antigens were consider as positive.

1. Widal Tube Test: Principle: Agglutination test In the kit of tube Widal test, killed coloured bacterial suspension of *Salmonella* contains specific O and H antigen for S. typhi and AH & BH antigens for S. paratyphi A & B respectively. These antigens react with antibodies present in patient's serum and give visible agglutination clumps.

Table 1: Widal tube test

Tube no.	1	2	3	4	5	6	7	8
Serum dilution	1:15	1:30	1:60	1:120	1:240	1:480		Saline control
Normal saline	7ml	3.5ml	3.5ml	3.5ml	3.5ml	3.5ml		3.5ml
Patient's serum	0.5ml	–	–	–	–	–		–
Transfer diluted serum	3.5ml to tube no. 2	⇨ 3.5ml	⇨ 3.5ml	⇨ 3.5ml	⇨ 3.5ml	⇨ 3.5ml	Preserve in refrigerato	3.5ml

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Transfer dilution to respective labelled O,H,AH,BH tubes	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml		0.5ml

2. Widal Slide Test: Principle: Agglutination test the bacterial suspension (antigen) is mixed with suspected patient's serum in various dilutions. Appearance of agglutination in highest dilutions determines the titre of the antibodies in patient serum.

Table 2: Widal Slide Test

Circle	Serum volume	Appropriate antigen drop	Equivalent titre
1	20 µl	1drop	1:80
2	10 µl	1 drop	1:160
3	5 µl	1 drop	1:320

Result: A total of 300 patients attending the Sola Civil Hospital, Ahmedabad from December 2013 to March 2014 and were screened for the antibodies against the *Salmonella enterica* subspecies *enterica* serotypes *S. typhi*, *S. paratyphi* A and *S. paratyphi*B by the Widal slide and tube test.

Table 3: Total serum samples positive for Typhoid, Paratyphoid A, Paratyphoid B [Slide test [(≥1:80) andTube test (≥1:120)]

Antibody type	Tube test Positive	Slide test Positive
Typhoid	23(7.66%)	15(5%)
Paratyphoid A	2(0.66%)	2(0.66%)
Paratyphoid B	0(0%)	0(0%)
Total	25(8.3%)	17(5.7%)
Seropositivity in percentage	8.3%	5.7%

Table 4: Sex Distribution of Patients

Sex	Total &percentage	Positive & Percentage
Male	137(45.7%)	10(3.3%)
Female	163(54.3%)	15(5.0%)
Total	300(100%)	25(8.3%)

Discussion: Typhoid fever is a major public health problem associated with significant morbidity and mortality in many countries. The "gold standard" test for the diagnosis of typhoid fever is the isolation of bacteria from blood, faeces, urine, bone marrow or other body fluids; however the widespread and uncontrolled use of antibiotics leads to negative results. Moreover, considering the poor facilities for the isolation of bacteria by the culture methods in the peripheral health centres and rural clinics, no other diagnostic tool is introduced thus far; Widal test can be use as a screening test for typhoid fever[1]. The Widal test is one of the most utilized tests for typhoid fever in India with tube Widal test being largely replaced by a semiquantitative slide test for its rapidity and convenience.

In this study, 300 patients were screened for the antibodies against the Salmonella enterica subspecies enterica serotypes, S. typhi, S. paratyphi A and S. paratyphiB by the Widal slide and tube test and the results of 300 patients compared and discussed with result of other studies.

Table 5: Showing the Difference in Result Obtained by Slide & Tube Widal test

Studies Done By	Difference in result obtained by slide & tube Widal test (%)

Lavanya V[1]	22%
Thelma E. Tupasi et al[19]	8-10%
Brusch[3]	9%
Present study	2.6%

Here the aim of the study to compare the results between tube & slide Widal tests like in present study the difference of results is only 2.6 % so slide Widal test can be used as good screening test for rapid diagnosis & can be replaced the tube Widal test which is cumbersome and more time consuming .

Table 6: Showing the Sex Ratio in Different Studies

Studies	Sex ratio (M:F)
Wain J et al (1998)11	1 : 1
Yaramis A et al (2001)12	1.4 : 1
Olsen SJ et al (2004)14	1.05 : 1
Chirag S et al (2005)15	2.8 : 1
Balakrishna (2010)10	1.47 : 1
Retnosari S et al.(2001)13	1 : 2.3
Present study	1 : 1.5

Table 7: Showing the age wise distribution in different studies

Studies	Positivity with percentage (0-15 year's age gr.)	Positivity with percentage (16-45 years age gr.)	Positivity with percentage (>45 years age gr.)
Balakrishna10	24 (12%)	18 (9%)	-
Present study	8 (2.66%)	15(5.0%)	2(0.66%)

Conclusion: Although blood culture is gold standard for diagnosis of typhoid fever, the rising titer of Widal test is also helpful for diagnosis of typhoid fever. The Widal test is an easy, inexpensive and relatively non-invasive test that can be of diagnostic value in situations where blood cultures cannot be obtained.

In present study out of 300 patient's samples, 8.3% & 5.7% samples were positive by tube Widal test, & slide Widal test respectively. The difference of positivity between two tests was 2.6%. Patients of 16 to 45 years age group showed more positivity and results shows that female patients are more prone to typhoid than male with positivity ratio of M: F was 1: 1.5.

The Tube Widal test is more sensitive and specific than slide Widal test, specially for rule out prozone phenomena, but slide Widal agglutination test is found to good alternative screening test because less time consuming, easy to perform, cost effective and can be applied in resource poor nations.

There is an urgent need for the rational design and evaluation of effective and appropriate diagnostics for typhoid fever.

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