



## Study of the Baseline Widal Titres among Healthy Rural Population in and Around Jabalpur

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### ABSTRACT

**Background:** *In developing countries like India, Enteric fever is endemic. Widal agglutination test is used for its diagnosis. In a single serum sample the only test frequently relied upon for laboratory diagnosis is Widal test. A rising titre in paired sera or in single sera test if the antibody titres are higher than the cut-off value the test is considered positive. But in our area normal baseline titres in various individuals and cut-off values have not been established. So the aim of our study was to determine the base line titres of antibodies in apparently healthy individuals and thereby define the significant titres of the Widal test.*

**Materials & Methods:** *170 samples after being screened by Widal slide agglutination test and were further confirmed by the Quantitative tube agglutination test, to find out the baseline Widal titre among various individuals.*

**Result:** *The most frequently recorded titre for O agglutinins were 1:40 and for the H agglutinins were 1:40. The baseline anti-H agglutinin titre of the paratyphoid A (H) were 1:40 and Paratyphoid B (H) were 1:20.*

**Conclusion:** *In our locality for the diagnosis of enteric fever if a single Widal titre is used, probably the most appropriate cut-off titres against S. typhi being  $\geq 1$  in 80 for anti-O and  $\geq 1$  in 80 for anti-'H' agglutinins.*

**Keywords:** *Widal test, Slide agglutination test, Tube agglutination test, Cut-off titre, Baseline titre.*

### Introduction

In developing countries like India the major endemic health problem is enteric fever and continues to be a universal health problem, especially in tropics and subtropics <sup>[1]</sup>. In 2000, about 21.6 million people had typhoid fever. More than 90% cases in Asia only. In developing countries, these fevers are considered as a major cause of morbidity and mortality <sup>[2], [3]</sup>. Salmonella

enterica subspecies enterica serotype Typhi and Salmonella enterica subspecies enteric serotype Paratyphi A and Paratyphi B caused enteric fever. Mode of transmission is by close contact with patients or carrier. Other than humans these enteric fever serotypes have no known hosts in contrast to other Salmonella serotypes. For diagnosis laboratory tests are essential, since the clinical presentation of enteric fever is non-

descript. The definitive diagnosis and gold standard is by isolation of *Salmonella enterica* serotype Typhi, Paratyphi A and Paratyphi B from blood, stool or urine, bone marrow, etc. However, in countries like India, isolation of organism is often complicated by lack of facilities or inadequate and/or improper antibiotic use prior to culture and the rate of culture positivity is only 40-60% of cases usually in early course of disease [4]. In developing countries, for diagnosis of enteric fever Widal agglutination test has a greater scope. In diagnosis of enteric fever, Widal test though in vogue for more than hundred years is still plagued with controversy about its usefulness [5].

The Widal test is reliable only when there is evaluation of four-fold rise in antibody titre in subsequent samples [6]. In developing countries like India, most of the patients attending hospital are often late; in that case they require an immediate diagnosis and a specific treatment. Taking in account the above facts, diagnosis of enteric fever relies upon a single sample, instead of paired serum samples [7]. In that case, a single cut-off value of baseline titre among healthy individuals is required to interpret and diagnose enteric fever [8].

The significant titres of antibodies to 'O' and 'H' antigens varies and with time and place, since antibodies that react with *Salmonella* 'O' and 'H' antigens emerge in a variety of other conditions like malaria, dengue, other gram negative infections and in apparently healthy individuals in endemic areas [9], [10]. In endemic areas among the various individuals the use and interpretation of single Widal test depends on result of the baseline titres. There by, for appropriate interpretation of the results. it is necessary to establish the baseline titres periodically, so as to define the significant titres of 'O' and 'H' antibodies .

### Objectives

The objective of the present study is to determine the base line titres of antibodies for each of the 'O' and 'H' antigens of *Salmonella enterica*

serotype Typhi, Paratyphi 'A' & Paratyphi 'B' and thereby define the significant titres of Widal test in a single sera among patients attending the laboratory for investigations other than that for enteric fever.

### Materials & Methods

After the approval of the institutional ethical committee, Present community based, cross-sectional study was conducted in the Department of Microbiology under serology section at hospital in Jabalpur.

The study protocol and objectives was duly explained and after obtaining a written consent from apparently healthy volunteers of both the sexes and of the age groups which ranged from 18 to 50 years. A total of 170 non-repetitive blood samples from the Patients attending to the Microbiology laboratory for various investigations other than for enteric fever with no history of fever in the preceding six months and not vaccinated for typhoid in the preceding three years were included in the study. About 5 ml of venous blood sample was collected from each participant, left to clot for 15 minutes at room temperature. Sera were separated by using micropipette. The separated sera were properly labelled and stored in 2-8°C for no more than seven days. All serum samples were further subjected to standard tube agglutination method for the determination of antibodies against the antigens of *Salmonella typhi* 'O', 'H' and *Salmonella paratyphi* 'AH', 'BH' as per standard guidelines.

A commercially available antigens which contained Typhi- O & H, Paratyphi-A(H), B(H) were used, which were procured from Tulip diagnostic Ltd. Serial dilutions of serum were done from 1:20 to 1:640 and a drop of appropriate antigens was added to each tubes. Incubation condition for 'H' agglutinations was 37°C for 4 hours; results were noted after standing on the bench for 30 minutes. Similarly, for 'O' agglutinations, test tubes were incubated at 37°C for 4 hours and result were noted after overnight

refrigeration at 4°C. A known negative & Positive control sera was included in each batch of the tests for the reliability of test results [4]. All samples of blood donors (n=170) were screened by immunochromatography strip for Malaria parasite, HBsAg, HIV (Tridot), HCV (Tridot) & T. pallidum (RPR).

Highest dilutions of serum for anti-O, anti-H, anti-AH and anti-BH agglutinins showing visible agglutination were taken as end-point titre.

### Observations & Results

A total of 170 blood samples were collected from the apparently healthy individuals and were further subjected to standard quantitative Widal tube agglutination test to find out baseline Widal titre among healthy individuals. The test results were obtained as per standard criteria for the interpretation of Widal tube agglutination test. [Table:1]. Correlation of results with age and sex was also made. [Table2].

**Table 1:** End titres against different serotypes of *Salmonella enterica* (N=170)

Serotype	Antibody Type	No Agglutination	No and % of positive samples	Dilutions					
				1:20	1:40	1:80	1:160	1:320	1:640
<i>S. typhi</i>	Anti-TO	11 (6.47)	164 (96.47)	50 (29.11)	107 (62.94)	2 (1.17)	1 (0.29)	Nil	Nil
<i>S. typhi</i>	Anti-TH	5 (3.23)	167 (98.23)	9 (6.17)	46 (27.35)	1 (0.88)	1 (0.88)	Nil	Nil
<i>S. paratyphi</i> A	Anti-AH	106 (63.82)	63 (36.76)	11 (6.47)	31 (17.94)	Nil	Nil	Nil	Nil
<i>S. paratyphi</i> B	Anti-BH	158 (92.94)	12 (7.05)	12 (7.05)	Nil	Nil	Nil	Nil	Nil

**Table 2:** Demographic distribution of individuals according to age group and sex

Total Participants		Frequency	%
		170	100
Sex	Male	129	76
	Female	41	24
Age groups	16-20 years	19	11
	21-30 years	91	54
	31-40 years	51	30

### Discussion

Early diagnosis of Enteric fever is important for outpatient therapy. Bacterial culture remains the gold standard for definitive diagnosis, but in resource constraint setting, it is not feasible to do on routine basis. Widal test is widely used in developing countries due to its cost, is easy to perform and rapid result within a minute. Widal agglutination test serve as one of the best alternative to detect anti-salmonella antibody directed against *Salmonella typhi* O, H and *Salmonella paratyphi* AH, BH antigens.

Studies conducted in different parts of our country, showed that baseline antibody titre for *Salmonella typhi* and *Salmonella paratyphi* varies

in different geographical areas. Evaluation of baseline antibody titre among healthy population at regular interval is necessary for the interpretation of Widal test.

In present study, most frequently recorded antibody titre for *S.typhi*-O were 1:40 and for *S.typhi*-H were 1:40, which is the cut-off baseline titre in this region. Hence, present study concludes that the current baseline antibody titre among the healthy individuals in and around Jabalpur region for *S. typhi*-O and *S. typhi*-H is 1:40(62.94%) & 1:40(62.64%) respectively. Based on this finding, we have set our own laboratory guidelines for the interpretation of O and H agglutinin Widal titres

and is >1:80 and >1:80 as being of diagnostic significance.

The baseline antibody titre for paratyphoid A(H) were 1:40 (17.94%) and Paratyphoid B(H) were 1:20 (7.05%), Based on these findings, significant diagnostic titre for Paratyphi A(H) is >1:40 and for Paratyphi B(H) is >1:20 in our region.

In a study carried out by Madhusudhan NS et.al<sup>[11]</sup>. They observed that the baseline antibody titre for *S.typhi*-O was 1:40 & for *S.typhi*-H was 1:80 which is almost in accordance with titre observed in present study.

Similarly, in a study carried out by Shekhar Pal et.al<sup>[12]</sup>. They found the baseline antibody titre for *S.typhi*-O was 1:40 & for *S.typhi*-H was 1:80 which is almost in accordance with present study.

The results of present study comparable with some other studies carried out by Kulkarni and Bharat<sup>[8]</sup> and Rego et al<sup>[13]</sup>.

In a study conducted by Shekar Pal et al.<sup>[12]</sup> he observed that baseline titre for *S.paratyphi*-A(H) & B(H) was 1:20 respectively, which is in concordance with present study; we found titre for *S.paratyphi*-A(H) was 1:40 and for *S.paratyphi*-B(H) was 1:20.

In present study, it was observed that the frequency of Antibodies for *Salmonella typhi* A & H is more than *Salmonella paratyphi* AH & BH, which indicates that the exposure to *Salmonella paratyphi* AH & BH is less in our study population which is similar to some other studies carried out in our country.

### Conclusion

More than fifty percent of healthy participants (52%) were positive to agglutinins for serotypes of *Salmonella*. The isolation of the various strains of *Salmonella enterica* subspecies *enterica* from blood remains the gold standard for the diagnosis of enteric fever. In the Indian subcontinent, many clinics and hospitals do not have a ready access to the blood culture method, thus making the Widal tube agglutination test the most common alternative laboratory procedure for the diagnosis of enteric fever. Hence evaluation of baseline

Widal titre from single serum sample is mandatory for proper interpretation Widal test to guide the clinicians. So the present study concludes that the cut-off values for TO, TH, A(H), B(H), are 1:40, 1:40, 1:40, and 1:20 respectively for the interpretation of Widal test and the diagnosis of enteric fever in this region.

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