

## Baseline widal titre among the healthy individuals: a study in and around Badnapur, Marathwada region at Tertiary Care Hospital

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### Abstract

**Objectives:** Present study was carried out to determine average baseline widal titer among the healthy individuals in and around Badnapur, Marathwada Region.

**Methods:** A total of 340 blood samples were collected from the apparently healthy individuals were further subjected to standard quantitative widal tube agglutination test to find out baseline widal titre among healthy individuals.

**Result:** The most frequently recorded titre for O agglutinins were 1:40 and For the H agglutinins were 1:80. The baseline anti-H agglutinin titre of the paratyphoid A(H) were 1:40 and Paratyphoid B(H) were 1:20.

**Conclusion:** Evaluation of baseline widal titre from single serum sample is mandatory for proper interpretation widal test to guide the clinicians. Therefore it was concluded that the cut-off values for TO, TH, A(H), B(H), were 1:40, 1:80, 1:40, and 1:20 respectively for the diagnosis purpose of enteric fever in this region.

**Keywords:** Enteric fever, Salmonella Typhi, Endemic titre

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2394-5478.2016.00038.8

### Introduction

Enteric fever continues to be a global health problem, especially in tropics and subtropics and is a major endemic health problem in developing countries like India, and it is considered as a major cause of morbidity and mortality in developing countries with more than 90% of cases found in Asia only<sup>1,2</sup>. Enteric fever is endemic in all parts of India and early Definitive diagnosis of enteric fever depends on isolation of typhoidal *Salmonella* from blood, stool, urine, bone marrow, bile or other body fluids<sup>3</sup>. 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<sup>1</sup>. Other demerit of blood culture is its cost and relatively long turnaround time. For these reasons, definitive diagnosis of enteric fever relies on widal test which was developed by Georges Fernand Isidore Widal in 1896, is an alternative to the microbial culture that are used for the diagnosis of enteric fever since its introduction 100 years back<sup>4</sup>.

The widal test is reliable only when there is evaluation of four-fold rise in antibody titre in

subsequent samples<sup>5</sup>. 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 mentioned facts, diagnosis of enteric fever relied upon a single sample, instead of paired serum samples<sup>6</sup>. In that case, a single cutoff value of baseline titre among healthy individuals is required to interpret and diagnose enteric fever<sup>7</sup>.

The significant titres of antibodies to 'O' and 'H' antigens varies from place to place and with time since antibodies that react with *Salmonella* 'O' and 'H' antigens appear in a variety of other conditions like malaria, dengue, other gram negative infections and in healthy persons in endemic areas<sup>7,8</sup>. The Widal titre among the healthy populations of different areas varies and it depends on the endemicity of typhoid in each area, which has been changing over time. Hence, it referred as the baseline antibody titre of that area<sup>1</sup>. Evaluation of baseline Widal titre among healthy individuals from place to place is mandatory for the proper interpretation of the Widal test in a single sample<sup>6,9,10,11</sup>.

Hence, the present study was an attempt to determine the baseline Widal titre (for *S.typhi* 'O', 'H' & *S.paratyphi* 'AH', 'BH') amongst the apparently healthy individuals of in and around Badnapur, Marathwada region in Maharashtra state, India to find out cutoff values for the interpretation of Widal test and to diagnose typhoid fever in endemic areas.

### Materials and 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 tertiary care hospital.

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 340 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 labeled 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<sup>1</sup>.

Highest dilutions of serum for anti-O, anti-H, anti-AH and anti-BH agglutinins showing visible agglutination were taken as end-point titre. All results data were analyzed by using EXCEL windows based program.

### Observations and Results

A total of 340 blood samples were collected from the apparently healthy individuals were further subjected to standard quantitative widal tube agglutination test to find out baseline widal titre among healthy individuals and test results were obtained as per standard criteria for the interpretation of widal tube agglutination test.

**Table 1: End titres against different serotypes of *Salmonella enterica* (N=340)**

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	22 (6.47)	328 (96.47)	99 (29.11)	214 (62.94)	04 (1.17)	01 (0.29)	Nil	Nil
<i>S. typhi</i>	Anti-TH	11 (3.23)	334 (98.23)	20 (6.17)	93 (27.35)	213 (62.64)	03 (0.88)	Nil	Nil
<i>S. paratyphi</i> A	Anti-AH	217 (63.82)	125 (36.76)	22 (6.47)	61 (17.94)	40 (11.76)	Nil	Nil	Nil
<i>S. paratyphi</i> B	Anti-BH	316 (92.94)	24 (7.05)	24 (7.05)	Nil	Nil	Nil	Nil	Nil

**Table 2: One sample T test**

Name of Antibodies	N	Mean	SD	SEM	Significance (two-tailed) P value
Anti-'TO' Ab	340	32.41	14.98	0.81	<0.01
Anti-'TH' Ab	340	63.65	25.44	1.38	<0.01
Anti-'AH' Ab	340	17.88	27.36	1.48	<0.01
Anti-'BH' Ab	340	1.41	5.13	0.28	<0.01

One sample T test results showed that, the two-tailed P value is less than 0.01. By conventional criteria, this difference is considered to be extremely statistically significant. (Table 2)

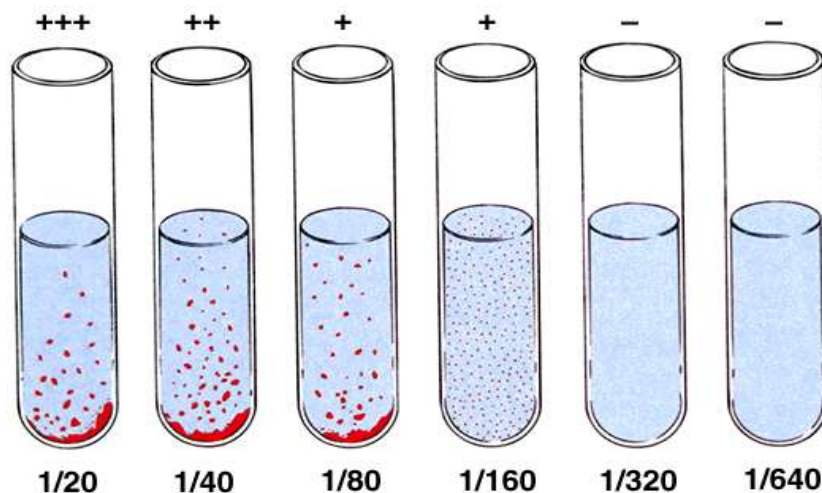


Fig. 1: Widal tube test showing visible agglutination reaction.(Image courtesy: www.microbiologyinfo.com)

### Discussion

Early diagnosis of Enteric fever is important for outpatient therapy. Laboratory diagnosis of enteric fever depends on gold standard bacterial culture and sensitivity for definitive diagnosis, but in resource constraint setting, it is not feasible to do on routine basis. 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. Widal test widely used in developing countries due to its cost, easy to perform and rapid result within a minute.

Studies conducted in different parts of our country, showed that baseline antibody titre for *Salmonella typhi* and *Salmonella paratyphi* varies from different geographical areas, even from same region. 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 1:80, 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 Badnapur region for *S. typhi*-O and *S. typhi*-H is 1:40(62.94%) & 1:80(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 >1:40 and >1:80 as being of diagnostic significance.

The baseline antibody titer 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 Shekhar Pal et.al.<sup>4</sup> found the baseline antibody titer for *S.typhi*-O was 1:40 & for *S.typhi*-H was 1:80 which is in accordance with present study.

Similarly, a study carried out by Madhusudhan NS et.al<sup>15</sup>. Observed that the baseline antibody titer for *S.typhi*-O was 1:40 & for *S.typhi*-H was 1:80 which is in accordance with titers observed in present study. The results of present study comparable with some other studies carried out by Kulkarni and Rego<sup>16</sup>, and Bharat e.al<sup>17</sup>.

In a study conducted by Shekar Pal et.al<sup>4</sup> 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) is 1:40 & for *S.paratyphi*-B(H) is 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

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 developing countries, such as 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.

Therefore present study concludes that the cut-off values for TO, TH, A(H), B(H), are 1:40, 1:80, 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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**How to cite this article:** Hatkar SS, Kotgire SA, Thakur AR. Baseline widal titre among the healthy individuals: A study in and around Badnapur, Marathwada region at Tertiary Care Hospital. *Indian J Microbiol Res* 2016;3(2):162-165.