

# EVALUATION OF A SINGLE WIDAL TEST IN THE DIAGNOSIS OF ENTERIC FEVER

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**Abstract.** The usefulness of a single Widal test was evaluated in an endemic area. This test was done on 62 bacteriologically proven enteric fever cases, 69 non-enteric fever cases and 50 healthy individuals. Using an 0 antibody cut-off of  $\geq 1:20$ , the Widal tube agglutination test yielded a sensitivity of 61% and a specificity of 88%. At a pre-test probability of around 50%, the positive predictive value was 83% and the negative predictive value was 72%. A 4% prevalence of 0 antibodies was noted in the healthy population.

## INTRODUCTION

The Widal test continues to be used in the diagnosis of enteric fever in countries where the disease is endemic and bacteriologic facilities are lacking. Several investigations in Asian and African countries showed that the sensitivity and specificity of a single Widal test ranged from 53 to 94% and 92 to 98%, respectively (Senewiratne *et al* 1977; Shehabi 1981; Abraham *et al*, 1981; Pang and Puthuchearu 1983; Hoffman *et al*, 1986). Such wide variations in test results have led to the impression that the assay is unreliable, particularly in countries where enteric fever is endemic.

Using *Salmonella* antigens commonly used in the Philippines, we sought to determine the diagnostic utility of a single Widal test in enteric fever.

## METHODS

### Study population

The subjects of the study were in-patients and personnel of three medical centers in Metro Manila: group 1, patients with a clinical picture

compatible with enteric fever and with at least one blood, urine or stool culture positive for *Salmonella typhi* or *paratyphi* A; group 2, febrile control patients without *Salmonella* infection; and group 3, healthy individuals who were normal on physical examination and who had been afebrile for the past year. Recruitment of all three groups was done during the same study period.

Excluded from the study were the following: 1) those who had been immunized with typhoid or paratyphoid vaccine; 2) those with diseases characterized by immune complex formation like rheumatic fever, systemic lupus erythematosus, and glomerulonephritis; and 3) those on immunosuppressive drugs.

### Widal test

Serum samples were obtained on admission for the first two groups. The Widal test was performed at the microbiology laboratory of the Research Institute for Tropical Medicine. Agglutinable suspensions of *Salmonella* groups A,B,C,D, prepared by Gamma Biologicals were used because these were the most commonly used antigens noted in a telephone survey of hospital laboratories in Metro Manila.

The slide agglutination method using serum dilutions of 1:20, 1:40, and 1:80 was used to screen all sera. Samples which displayed a "2+" macro-

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scopic agglutination (with clumping of 50% of the organisms) were tested by the tube agglutination method. Serial doubling dilutions of the subject's serum from 1:20 to 1:1280 were then tested. Both slide and tube agglutination methods were done according to standard microbiologic techniques with the appropriate controls (Farmer, 1985). The titer of the serum was the highest dilution showing a positive reaction (ie, a 2+ macroscopic agglutination). Only the responses to *Salmonella* groups A and/or D flagellar and somatic antigens were used in the analysis.

#### Analysis of the results

The following probability rates were determined: sensitivity, specificity, positive and negative predictive values (Sackett *et al.* 1985). Sensitivity or true positive rate was defined as the probability that the result would be positive when enteric fever was present. Specificity or true negative rate was the probability that the test result would be negative when enteric fever was not present. The positive predictive value was the likelihood that enteric fever was present when the test was positive, and the negative predictive value was the likelihood that enteric fever was not present when the test was negative. The latter two values were dependent on the sensitivity and specificity of the test and the probability of the patient having enteric fever before the test was

performed (pre-test probability). In group 1 we had estimated the pretest probability of enteric fever being present to be 50%.

Group differences in age and duration of illness prior to the Widal test were determined by the *t*-test.

## RESULTS

Table 1 shows the general characteristics of subjects included in the study. Group 2 patients were significantly older than those with enteric fever, while the latter were ill for a significantly longer period than the febrile controls.

Of the 62 patients with enteric fever, 38 were culture positive for *S. typhi* and 24 for *S. paratyphi* A. The organism was isolated from the blood in 54 patients, blood and stool in 4, stool in 3 and bone marrow aspirate in 1.

The infections among febrile controls are shown in Table 2. None had any blood, urine and/or stool culture positive for *Salmonella*.

The Widal test results for all groups are shown in Table 3. Most patients had 0 antibody titers only, except for nine with H antibodies. Two of the nine patients had isolated H responses only,

Table 1  
Characteristics of patients with and without enteric fever.

Characteristic	Category of Subjects		
	Group 1 enteric fever patients	Group 2 febrile controls	Group 3 healthy controls
	(n = 62)	(n = 69)	(n = 50)
Male/female	33/29	42/27	22/28
Age (years), mean $\pm$ SD	24 <sup>+</sup> -11	39 <sup>+</sup> -24*	28 <sup>+</sup> -4
Duration of illness before Widal test (days), mean $\pm$ SD	13 <sup>+</sup> -10	9 <sup>+</sup> -9**	not appli- cable

\*  $p < .001$  for the comparison with group 1

\*\*  $p < .01$  for the comparison with group 1

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Table 2

Infections noted among febrile control patients.

Type of infection	Number of patients
I. Liver and gastrointestinal tract	
Viral hepatitis	5
Intestinal amebiasis	3
Cholecystitis	2
Amebic liver abscess	1
Peritonitis secondary to perforated sigmoid colon	1
Intra-abdominal abscess	1
II. Genitourinary tract	
Lower urinary tract infection (UTI)	8
Acute pyelonephritis	3
Chronic pyelonephritis	1
Postpartum endometritis	1
Fournier's disease	1
III. Central nervous system	
Tuberculous meningitis	3
Transverse myelitis	1
Viral encephalitis	1
Staphylococcal meningitis	1
IV. Skin and soft tissue	
Infected wound	2
Scalp infection	1
Gangrene, foot	1
Tropical myositis	1
V. Respiratory tract	
Pneumonia, bacterial	11
Pulmonary tuberculosis	3
VI. Miscellaneous	
Malaria	7
UTI and pneumonia	4
Viral meningoencephalitis and UTI	1
Measles	2
Mumps	1
Erysipelas	1
Pseudomonas arthritis	1

and belonged to Group 2. All but two healthy controls had no agglutinins.

Antibody titers of the enteric fever patients and the febrile control patients were used to calculate the sensitivity and specificity of the Widal tube agglutination test at various cut off points. Since there were few H responses, test characteristics were not greatly influenced by H antibody titers. The sensitivity and specificity of the O

antibody reactions are reported in Table 4. Specificity became 100% at a positivity criterion of 1:320, while sensitivity, which was highest at 1:20, decreased at higher titers.

The positive predictive values as well as the negative predictive values for the different O antibody titers, based on 50% pre-test probability of the disease, are summarized in Table 5.

DISCUSSION

This study was designed to determine the usefulness of the Widal test in diagnosing enteric fever in the Philippines. At an O antibody titer of 1:20, a single Widal tube agglutination test had a sensitivity of 61% and a specificity of 88%. With a prior test probability of 50%, the positive predictive value of an O antibody titer of 1:20 and above was 83% and the negative predictive value was 72%.

Investigations in other countries have shown the single Widal test to be useful, as it was positive in 58-97% of culture-proven cases and negative in 3-8% of non-typhoid patients (Shehabi 1981; Abraham *et al*, 1981; Pang and Puthuchery, 1983; Somerville *et al*, 1981; Levine *et al*, 1978). Differences in positivity criteria, antigens used and methods employed could explain the widely variable results. In their study of typhoid cases in Jakarta, Indonesia, Hoffman *et al* (1986) used an O antibody positivity criterion of 1:20, and found the test to be 53% sensitive and 98% specific. However, our results are not strictly comparable to this study since the latter employed the slide agglutination technique solely and used different *Salmonella* antigens.

Among Filipino patients, Roxas and Mendoza showed that a single Widal slide agglutination test was 57.5% sensitive and 100% specific at an O or H antibody titer of 1:320. Although the same source of antigens was used as in this study, the report did not provide details of the procedures performed. Moreover, the data on Widal test results among enteric fever cases were obtained retrospectively from medical records (Roxas and Mendoza, 1989). In another study of 44 Filipinos with enteric fever, the Widal tube agglutination test was shown to be 64% sensitive and 100% specific (Buck *et al*, 1987). Compared to our study

Table 3

H and O antibody titers among enteric fever cases and controls.

Group	Antigen	No. of patients with antibody titer							
		< 1:20	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Group 1	H	55	0	0	1	2	1	1	2
	O	24	5	6	9	5	3	5	5
Group 2	H	67	1	1	0	0	0	0	0
	O	61	4	3	0	1	0	0	0
Group 3	H	50	0	0	0	0	0	0	0
	O	48	0	0	1	1	0	0	0

Table 4

Sensitivity and specificity of a single Widal test at different O antibody titers among 62 enteric fever patients and 69 febrile controls.

O antibody titer	Sensitivity (%)	Specificity (%)
1:20	61	88
1:40	53	94
1:80	44	99
1:160	29	99
1:320	21	100

Table 5

Positive and negative predictive values for a single Widal test at different O antibody titer levels.

O antibody titer level	Positive predictive value (%)	Negative predictive value (%)
1:20	83	72
1:40	89	69
1:80	96	66
1:160	95	61
1:320	100	59

however, different *Salmonella* antigens were used, and a higher O antibody positivity criterion of  $\geq 1:80$  was employed.

The positive predictive value answers the question for the clinician: "Given a positive test, what is the likelihood that enteric fever is present?" The negative predictive value answers the converse. We consider a test to be useful in clinical decision-making if it gives a predictive value of 80% and above. In this report, O antibody titer of  $\geq 1:20$  yielded a high positive predictive value. In addition,

the sensitivity was highest at this titer without unduly compromising the specificity.

In an endemic area, individuals may have a background antibody titer higher than those from a non-endemic area. Ara *et al* (1980) noted O and H antibody responses in 11.3% of 510 healthy residents of Pakistan. Using the Widal tube agglutination method, Levine *et al*, (1978) found 32% of 41 Peruvians in the same age group as our healthy subjects to have O antibody titers greater than 1:20. It has been reported that 55% of 3,307 healthy Filipinos have detectable O or H agglutinins. Forty-one percent of them have H antibody reactions, with 77% of such reactors having a titer of 1:20 and 1:40. The 4% prevalence of O antibodies in the healthy population of the present study is surprisingly low, and compares with that from a non-endemic area. This may be because these subjects were medical personnel belonging to the higher socio-economic bracket.

Certain workers have raised the question of which antibody (O or H agglutinins) is of greater diagnostic value. It is widely held that O agglutinins are of greater significance (Pang and Puthuchery, 1983). However, one study has shown H antibodies to be equally useful (Abraham *et al*, 1981). Our study confirms the former view.

We used the tube agglutination technique to determine the agglutinin titers and performed the slide technique only as a screening procedure. Tube agglutination is the preferred method, since slide agglutination results in wide discrepancies in titers for a given serum (Freter, 1976). However, two studies have demonstrated the diagnostic value of the slide agglutination method (Abraham

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*et al*, 1981; Hoffman *et al*, 1986). A study using the slide agglutination method alone needs to be done to assess its usefulness in the local setting.

The sensitivity and specificity of the test may vary at different stages of the illness. Our patient population had a mean period of 13 days of illness before testing. Unfortunately the number of samples tested is too small to permit the determination of sensitivity and specificity for each week of illness of the test.

In summary, this study showed that a Widal 0 agglutinin titer of 1:20 had a specificity of 88%, sensitivity of 61%, positive predictive value of 83%, and a negative predictive value of 72%. Thus, in an unimmunized Filipino adult patient with a febrile illness, a *Salmonella* O antibody titer to groups A and/or D of  $\geq 1:20$  obtained by the Widal tube agglutination method is suggestive of enteric fever. A negative titer does not rule out the disease.

Confirmation by culture remains the gold standard in the diagnosis of enteric fever and should be performed where facilities are available.

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