*Annals of African Medicine Vol. 10, No. 1; 2011:41-4*

Original Article

Access this article online

Quick Response Code:

Website: www.annalsafrmed.org

DOI: 10.4103/1596-3519.76584



# Detection of *Salmonella typhi* agglutinins in sera of patients with other febrile illnesses and healthy individuals

**Ali M. Somily, Mustafa H. Adam, Mohamed O. Gad El Rab, Muhammad G. Morshed[1], Zahid Shakoor**

Department of Pathology, King Saud University & King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia, [1]Department of Pathology & Laboratory Medicine, University of British Columbia, Canada

**Correspondence to:** Dr. Ali Mohammed Somily, Department of Pathology/Microbiology, College of Medicine & King Saud University, King Khalid University Hospital, PO Box 2925, Riyadh 11461, Kingdom of Saudi Arabia.
E-mail: ali.somily@gmail.com

## Abstract

**Background and Purpose:** Widal test is frequently applied for the detection of *Salmonella* agglutinins to diagnose *Salmonella* enterica serotype *Typhi* infection. There are however a number of controversies challenging the diagnostic utility of this test. This study was performed to determine the prevalence of *Salmonella* agglutinins in patients with other febrile illnesses and healthy blood donors.

**Materials and Methods:** Sera from 50 healthy blood donors were compared for the presence of *Salmonella* agglutinins in various groups of patients with other febrile illnesses using Widal test in the division of Serology and Immunology at King Khalid University Hospital, Riyadh. The patient groups of other febrile illnesses included infections with Beta-hemolytic streptococcus (n = 50), Brucella (n = 46), *Helicobacter pylori* (n = 24), *Treponema pallidum* (n = 30), *Toxoplasma* (n = 44), and other parasites (n = 20).

**Results:** Majority of the patients and normal individuals were tested positive for Widal test at dilution of less than 1 : 40 both for the O (62.5%) and H (64.6%) antigen. A decreasing trend in Widal reactivity was observed with increasing dilutions of the serum samples. At 1 : 160 titer, which is generally considered as a cut off point for positive Widal test, 6.4 and 11% individuals had positive Widal test for O and H *Salmonella* antigens, respectively.

**Conclusion:** Detection of a significant number of positive Widal tests in conditions where it is expected to be nonreactive appears to be a serious problem in making a correct diagnosis of typhoid fever, thus challenging the diagnostic utility of the Widal test.

**Keywords:** False positive, other febrile illnesses, *Salmonella typhi*, Widal test

## Résumé

**Contexte et objet:** Widal test est souvent appliqué pour la détection de *Salmonella* agglutinines pour diagnostiquer la *Salmonella* enterica sérotype *Typhi* infection. Il y a cependant un certain nombre de controverses contestant l'utilitaire de diagnostic de ce test. Cette étude a été effectuée afin de déterminer la prévalence de *Salmonella* agglutinines chez les patients atteints d'autres maladies fébriles et donneurs de sang.

**Méthodes et matériaux:** La présence de Salmonella agglutinines dans divers groupes de patients, de donneurs de sang sain sérums de 50 ont été comparés autres maladies fébriles à l'aide de test Widal dans la division de la sérologie et d'immunologie à l'hôpital universitaire du Roi Khaled, Riyad. Les groupes de patients d'autres maladies fébriles inclus des infections à streptococcus bêta-hémolytique (n = 50), Brucella (n = 46), *Helicobacter pylori*(n = 24), *Treponema pallidum*(n = 30), Toxoplasma(n = 44) et autres parasites (n = 20).

**Résultats:** La majorité des patients et normale particuliers ont été testés positifs pour le test de Widal à dilution de

moins de 1 h 40, tant pour les O (62,5%) et l'antigène H (64,6%). On observe une tendance à la baisse de réactivité Widal avec l'augmentation des dilutions des échantillons de sérum. À titre de 1: 160, qui est généralement considéré comme une coupe au large de point pour test positif de Widal, 6.4 et 11% des individus avaient Widal positif pour les antigènes O et H *Salmonella*, respectivement.

**Conclusion:** Des tests de détection d'un nombre important de Widal positif dans des conditions où il est censé être non réactives semble être un problème sérieux à faire un diagnostic correct de la fièvre typhoïde, défiant ainsi l'utilitaire de diagnostic de la Çiva Widal

**Mots clés:** Fausses positifs, d'autres fébriles maladies, *Salmonella typhi*, Widal test



## Introduction

Despite the introduction of several serological tests,[1-7] Widal test is commonly applied for diagnosis of typhoid fever. As the *typhi* antigen is shared by a large number of organisms from the *Salmonella* genus and other related organisms,[1,2,8] a positive Widal test is therefore likely to occur in several conditions other than the actual *Salmonella enteric* infection. False positive Widal test has already been reported in other febrile illnesses such as malaria, tuberculosis, and schistosomiasis.[4,9-12] This study was performed to assess the reactivity of Widal test by detecting *Salmonella* agglutinins in other febrile conditions or in otherwise healthy individuals.

## Materials and Methods

### Study population

Sera from 264 individuals comprising of 214 patients with other febrile illnesses and 50 healthy blood donors were tested for the presence of *Salmonella* agglutinins using Widal test. The patient groups included 50 patients with confirmed diagnosis of infection with *Beta-hemolytic streptococci*, with positive titers for antistreptolysin O antibodies; 46 patients infected with *Brucella abortus* and *melitensis* spp.; 44 patients tested positive for *Toxoplasma;* 30 had a positive Venereal Disease Research Laboratory test, *Treponema pallidum* Haemagglutination assay, and Enzyme Link Immunosorbent Assay IgG (index>1.1); 24 were positive for *Helicobacter pylori* IgG and IgA; and 20 patients had evidence of various parasitic infections. Findings of each group of patients were compared with a group of 50 normal healthy individuals to match the number of individuals in the largest group of patients with hemolytic streptococcus infection. These individuals were also matched for age and gender to serve as normal controls. The inclusion criteria for the healthy blood donors were no history of typhoid infection or vaccination in the past and no other febrile illness for at least six months prior to testing.

### Collection of blood sample

5 ml of blood was collected from each patient by venipuncture. Samples were left to clot and serum was collected, aliquoted, and kept at -20°C until used.

### Widal tube agglutination test

The standard Widal tube agglutination test was performed using a commercial kit (Murex Biotech Limited, UK) in accordance with the manufacturer instructions. Saline serum dilutions were made for each antigen to be tested. One drop of bacterial suspension was added to each tube, contents were mixed and incubated at 37°C overnight for both O and H suspensions, and agglutination was observed at the end of the incubation. Based on the previous study in the Kingdom of Saudi Arabia,[13] Widal test was interpreted as positive if the titer of agglutinins was either equal to or more than 1 : 160.

## Results

Among the patients, there were 126 males and 88 females with the mean age of 27 $\pm$ 9 years. The normal healthy individuals comprised of 34 males and 16 females with the mean age of 25 $\pm$ 7 years. Table 1 describes the Widal reactivity in the patients with other febrile illnesses and the healthy normal donors. Generally, Widal reactivity for both O and H antigens was present in all the groups including normal healthy individuals. Majority of the individuals reacted at less than 1 : 40 dilution both for the O (62.5%) and H (64.4%) *Salmonella* antigens. A decreasing trend in Widal reactivity was observed with increasing dilutions. At 1 : 40 dilution, the O and H reactivity was 7.9 and 7.2%, respectively and at 1 : 160 titer, which is considered as clinically significant, Widal reactivity for O antigen was 6.4% and H antigen was 11% in all the groups including normal healthy individuals. Comparatively smaller percentage of individuals, 2.7 and 3.4% had a positive Widal test for O and H antigens respectively at a titer of 1: 320. Among the groups at 1 : 160 dilution, the highest number of patients (eight patients) tested positive for H antigen were in the group infected with *Toxoplasma,* followed by patients infected with *Brucella* (four patients) and *H. pylori* (four patients). Among the healthy individuals, four tested positive for *Salmonella* H antigen. Similarly for O antigens, the highest number of positive Widal tests were detected

**Table 1: Widal test reactivity for O and H agglutinins in patients with other febrile illnesses and normal healthy individuals**

| | No. of samples | <1 : 40 | | 1 : 40 | | 1 : 80 | | 1 : 160 | | 1 : 320 | | 1 : 640 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | O | H | O | H | O | H | O | H | O | H | O | H |
| ASO | 50 | 44 | 44 | 4 | 3 | _ | 2 | 2 | 1 | _ | _ | _ | _ |
| Brucella | 46 | 24 | 30 | 3 | 2 | 14 | 5 | 3 | 4 | 2 | 3 | _ | 2 |
| *H. pylori* | 24 | 10 | 10 | 3 | 3 | 9 | 6 | 1 | 4 | 1 | 1 | _ | _ |
| Syphilis | 30 | 17 | 14 | 1 | 3 | 8 | 9 | 4 | 3 | _ | 1 | _ | _ |
| Toxoplasma | 44 | 31 | 29 | 2 | 1 | 7 | 3 | 2 | 8 | 2 | 3 | _ | _ |
| Schistosoma | 10 | 4 | 3 | 1 | 1 | 4 | 2 | 1 | 3 | _ | _ | _ | 1 |
| Hydatid | 5 | 2 | 1 | 2 | 1 | 1 | 2 | _ | 1 | _ | _ | _ | _ |
| Amoeba | 5 | _ | _ | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | _ | _ |
| Blood bank | 50 | 33 | 39 | 4 | 4 | 9 | 3 | 3 | 4 | 1 | _ | _ | _ |
| Total | 264 | 165 | 170 | 21 | 19 | 54 | 34 | 17 | 29 | 7 | 9 | _ | 3 |
| Percentage | | 62.5 | 64.4 | 7.9 | 7.2 | 20.5 | 12.9 | 6.4 | 11 | 2.7 | 3.4 | 0 | 1.1 |

ASO = Antistreptolysin antibody (evidence for beta-hemolytic streptococcal infection)

in patients suffering from syphilis (four patients), followed by the group with *Brucella* infection (three patients). Among the normal healthy individuals, there were three positive Widal tests. Percentage of individuals testing positive for O antigen was higher than H antigen at 1 : 80 dilution, whereas at the titer of 1 : 160 a higher Widal test reactivity for H antigen compared with O antigen was observed.

## Discussion

This study shows a significant number of patients suffering from other febrile illnesses, and normal individuals with no history of prior exposure to *Salmonella* either by vaccination or actual *Salmonella* infection had positive Widal tests. A number of studies have been performed in normal populations of different regions of the world to establish the background level of *Salmonella* agglutinins, in order to define a regional cutoff value for interpreting Widal test. Based on the pervious study from the Kingdom of Saudi Arabia, a titer equal to or more than 1 : 160 was regarded as clinically significant.[13] Because of the variable prevalence rates of *Salmonella* agglutinins, cutoff value for positive Widal test has been shown to exhibit a marked regional variation.[14-17] A recent study on single acute phase sample from Kenya adopting a cutoff titer of 1 : 360 for both O and H antigens revealed that only 26% of patients had a diagnostic titer.[18] Similarly, the majority of healthy individuals and those with various infections in the present study had reactive Widal test in titers less than 1 : 40 in contrast to another study from Kenya, where the majority reacted to titers either equal to or less than 1 : 80.[14]

A significant number of patients suffering from other febrile illnesses in the present study were found to have a reactive Widal test and in some cases approaching clinical significance. However, a similar study performed on a smaller group of 46 patients with other febrile illnesses detected only one patient with a titer of 1 : 160 for O antigen.[19] *Salmonella* organism is known for its ability to elicit cross reacting antibodies not only against O and H antigens, but also against other microbes as well.[16] Generation of *Salmonella* agglutinins therefore may occur either by specific or by nonspecific stimuli. The detection of *Salmonella* in a statistically significant population of children suffering from malaria may indicate that nonspecific stimuli may trigger the formation of *Salmonella* agglutinins.[20] Similarly, patients receiving treatment for carcinoma bladder with intravesical Bacillus Calmette-Guérin have been shown to exhibit high levels of *Salmonella* agglutinins in their sera, which returned to normal after the cessation of therapy.[11] Reactive Widal test in a significant number of patients with other febrile disorders in this study could have been the result of microorganisms from unrelated species triggering the production of *Salmonella* agglutinins.

This study shows that compared with H antigen, O antigen was frequently detected at lower dilution but at the higher dilutions the observation was reversed. Although it is difficult to explain the diagnostic importance of this phenomenon, the titers of agglutinins against H antigen have been suggested to be a better indicator of *Salmonella* infection.[21] Moreover, it has also been claimed that titers for any of the two antigens may reach diagnostic levels in the first two weeks of *Salmonella* infection.[14] Further investigations are needed to gain a better understanding of the dynamics and behavior of O and H antigen titers in typhoidal and other febrile illnesses.

Widal test continues to be a subject of differing opinions with regards to its performance as a reliable diagnostic test for enteric fever. Among several issues, a higher percentage of false positive results are a major problem.[22] Several febrile illnesses such as tuberculosis, malaria, and hepatitis B-associated

polyarteritis nodosa have been shown to be associated with high incidence of false positive Widal test.[23-25] Detection of reactive Widal test in different clinical conditions other than enteric fever in the present study could either be the result of high levels of false positive Widal test in these conditions or the presence of cross-reacting antibodies, thus adding to the existing controversies.



Nested polymerase chain reaction (nPCR) has recently been shown to be a very sensitive and a specific tool for accurate diagnosis of enteric fever. Comparative analysis of the data shows that nPCR performs better than culturing the organism in patients with febrile illnesses of undetermined origin.[26] Similarly, immunoblotting has also been suggested to be a useful method for providing serological evidence of infection with *Salmonella typhi,* particularly for rapid diagnosis of typhoid fever.[27] The availability of such facilities however would still remain limited to specialized centers, and reliance on Widal test for diagnosis of enteric fever will probably continue until the introduction of a relatively simple, cost effective, and reliable test for detection of *Salmonella* infection.

## Conclusion

Despite the associated problems, Widal test is commonly being applied in the laboratories for the diagnosis of enteric fever. A better understanding of the dynamics of *Salmonella* agglutinins with regards to the background levels in an endemic area, along with the significance of O and H agglutinin titers in association with the clinical findings, is vital for a reliable diagnosis of enteric fever.

## References


1. Chart H, Waghorn DJ, Rowe B. Serological detection of patients infected with *Salmonella* enteritidis. Serodiagn Immunother Infect Dis 1994;6:79-81.
2. Wang W, Perepelov AV, Feng L, Shevelev SD, Wang Q, Senchenkova SN, *et al.* A group of *Escherichia coli* and *Salmonella* enterica O antigens sharing a common backbone structure. Microbiology 2007;153:2159-67.
3. Duthie R, French GL. Comparison of methods for the diagnosis of typhoid fever. J Clin Pathol 1990;43:863-5.
4. Rai GP, Zachariah K, Shrivastava S. Comparative efficacy of indirect haemagglutination test, indirect fluorescent antibody test and enzyme linked immunosorbent assay in serodiagnosis of typhoid fever. J Trop Med Hyg 1989;92:431-4.
5. Barrett TJ, Blake PA, Brown SL, Hoffman K, Mateullort J, Feeley JC. Enzyme linked immunosorbent assay for detection of human antibodies to *salmonella* vi antigen. J Clin Microbiol 1983;17:625-7.
6. Nardiello S, Phizzella T, Russo M, Galanti B. Serodiagnosis of typhoid fever by enzyme linked immunosorbent assay of anti-*salmonella typhi* lipopolysaccharide antibodies. J Clin Microbiol 1984;20:718-21.
7. Verdugo RA, Lopez VY, Puente JL, Ruiz PG, Calva E. Early diagnosis of typhoid fever by enzyme immunoassay using *Salmonella typhi* outer membrane protein preparation. Eur J Clin Microbiol Infect Dis 1993;12:248-54.
8. Wei W, Perepelov AV, Lu F, Shevelev SD, Quan W, Senchenkova SN, *et al.* A group of *Escherichia coli* and *Salmonella* enterica O antigens sharing a common backbone structure. Microbiology 2007;153:2159-67.
9. Ghosh K, Javeri KN, Mohanty D, Parmar BD, Surati RR, Joshi SH. False positive serologiacl tests in acute malaria. Br J Biomed Sci 2001;58:20-3.
10. Senewiraine B, Senewiratne K. Reassessment of the widal test in the diagnosis of typhoid. Gastroenterology 1977;73:233-6.
11. Skoutelis A, Dimitrakopoulos G, Bassaris H. False positive widal reaction in high-titer disseminated BCG infection. Eur J Clin Microbiol Infect Dis 1994;13:261-3.
12. Hoffman SL, Flanigan TP, Klaucke D, Leksana B, Rockhill RC, Punjabi NH, *et al.* Widal slide agglutination test, a valuable rapid diagnostic test in typhoid fever patients at the infectious diseases Hospital of Jakarta. Am J Epidemiol 1986;123:869-75.
13. Pang T, Putucheary SD. Significance and value of the widal test in the diagnosis of typical fever in an endemic area. J Clin Pathol 1983;36:471-5.
14. Jumba MM, Mirza NB, Mwaura FB. *Salmonella typhi* and *Salmonella* para*typhi* antibodies in Kenya. East Afr Med J 1995;72:755-7.
15. Frimpong EH, Feglo P, Essel-Ahun M, Addy PA. Determination of diagnostic Widal titres in Kumasi, Ghana. West Afr J Med 2000;19:34-8.
16. Levré E, Valentini P, Armani G, Grassi R, Caroli G. Serological recognition of agglutinins against O and H antigens of various *Salmonella*e in "normal" adult subjects. Ann Sclavo 1979;21:646-57.
17. Aftab R, Khurshid R. Widal agglutination titer: A rapid serological diagnosis of typhoid fever in developing countries. Pak J Physiol 2009;5:65-76.
18. Omuse G, Kohli R, Revathi G. Diagnostic utility of a single Widal test in the diagnosis of typhoid fever at Aga Khan University Hospital (AKUH), Nairobi, Kenya. Trop Doct 2010;40:43-4.
19. Pu SJ, Huang HS. Diagnostic value of a single Widal test. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi 1985;18:256-63.
20. Ibadin MO, Ogbimi A. Antityphoid agglutinins in African school aged children with malaria. West Afr J Med 2004;23:276-9.
21. Abraham G, Teklu B, Gedebu M, Selassie GH, Azene G. Diagnostic value of the Widal test. Trop Geogr Med 1981;33:329-33.
22. Parry CM, Hoa NT, Diep TS, Wain J, Chinh NT, Vinh H, *et al.* Value of a single-tube widal test in diagnosis of typhoid fever in Vietnam. J Clin Microbiol 1999;37:2882-6.
23. Sharma JR, Parmar IB, Sharma SJ, Kesavan A. False positive Widal reaction in malaria. Indian Pediatr 1993;30:1343-7.
24. Capron JP, Chivrac D, Delcenserie R, Dupas JL, Orfila J, Lorriaux A. False-positive Widal test in HBs antigen-associated polyarteritis nodosa. Gastroenterology 1978;75:770.
25. Jhaveri KN, Nandwani SK, Mehta PK, Surati RR, Parmar BD. False positive modified Widal test in acute malaria. J Assoc Physicians India 1995;43:754-5.
26. Nandagopal B, Sankar S, Lingesan K, Appu KC, Padmini B, Sridharan G, *et al.* Prevalence of *Salmonella typhi* among Patients with febrile illness in rural and peri-urban populations of Vellore district, as determined by nested PCR targeting the flagellin gene. Mol Diagn Ther 2010;14:107-12.
27. Dera-Tomaszewska B, Głonicka R. An immunoblotting as a method for the diagnosis of typhoid fever. Med Dosw Mikrobiol 2007;59:241-50.


**Source of Support:** Nil, **Conflict of Interest:** None declared.