

**To Study of diagnostic efficacy of widal slide agglutination test against widal tube****agglutination test in enteric fever**Praveen Singh Shekhawat¹, Ved Prakash Agrawal²¹ Senior Demonstrator Microbiology, ² Senior Demonstrator Anatomy,
Government Medical College, Bhilwara.**ABSTRACT:**

Background: Enteric fever is an important cause of morbidity in many regions of the world, with an estimated 13 million cases occurring annually in Asia alone.

Methods: Pre-collected blood samples on OPD and indoor bases with widal test request from enteric fever suspected cases were received in the microbiology lab for the processing and study was done over a period of 3 months.

Results: In our study, slide widal agglutination test showed very high false positivity for S.typhi H antigen and very low false positivity for S.typhi O antigen, when compared to tube widal agglutination test as a standard. The slide widal agglutination test also showed high positive predictive value for S.typhi O antigen and low positive predictive value for S.typhi H antigen, when compared to tube widal agglutination test as a standard.

Conclusion: The high false positivity and low positive predictive value shown by the slide agglutination test is to caution that the results of slide agglutination should not be solely relied upon for diagnosis and treatment of enteric fever.

Keywords: Agglutination, Slide, Tube, Widal test.

Introduction

Enteric fever is an important cause of morbidity in many regions of the world, with an estimated 13 million cases occurring annually in Asia alone. Estimates suggest an incidence rate of more than 21.5 million cases globally in the year 2000.¹

Salmonella is a leading cause of community-acquired bloodstream infections in many low- and middle-income countries. Accurate diagnosis is crucial to the management of the disease, but typhoid fever can be clinically confused with other febrile diseases, such as dengue fever and malaria.²⁻³

In India, enteric fever is caused by Salmonella enterica serotype Typhi and Paratyphi A. Serotypes B and C are very rare. Laboratory diagnosis mainly depends upon isolation of causative agents from specimens like blood and bone marrow. A blood culture gives positive results in 73-97% cases before the use of antibiotics.⁴ However, the availability of

microbiological culturing facilities is often limited in many typhoid endemic regions and blood cultures can be negative when patients have received prior antibiotic therapy. Hence, serological diagnosis using Widal test is relied upon in many cases.

Material and methods

Pre-collected blood samples on OPD and indoor bases with widal test request from enteric fever suspected cases were received in the microbiology lab for the processing and study was done over a period of 3 months. The serum was separated using all standard precautions. All the sera first tested with widal test by slide agglutination method. The antibody titre was taken as highest dilution of serum showing distinct visible agglutination. The widal test result with the antibody titres more than or equal to 1:80 for O antigen and the titre more than or equal to 1:160 for H antigen were considered as positive. When they result positive, they were checked by tube widal test for confirmation of result.

Table 1: Positive widal antibody titre result against different antigen

Antigen type	S.typhi O antigen	S.typhi H antigen	S.paratyphi A 'H' antigen	S.paratyphi B 'H' antigen
No. of positive by slide test	38	26	3	1
No. of positive by tube test	36	15	1	0
No. of slide widal test with negative tube widal	2	11	2	1

Table 2: Positive Predictive value of slide widal agglutination test when compared to tube widal agglutination test as a standard

Type of antigen	Positive predictive value (%)
S.typhi O antigen	92%
S.typhi H antigen	70.75%

In our study, slide widal agglutination test showed very high false positivity for S.typhi H antigen and very low false positivity for S.typhi O antigen, when compared to tube widal agglutination test as a standard. The slide widal agglutination test also showed high positive predictive value for S.typhi O antigen and low positive predictive value for S.typhi H antigen, when compared to tube widal agglutination test as a standard.

Discussion

The gold standard test for the diagnosis of typhoid fever is the isolation of bacteria from blood; however, the widespread and uncontrolled use of antibiotics leads to negative results. Moreover considering the poor facilities for the isolation of bacteria by culture methods in peripheral health centers and rural clinics, no other diagnostic tools is introduced thus far, other than widal test for the appropriate diagnosis of typhoid fever⁴.

Widal agglutination was introduced as a serologic technique to aid in diagnosis of typhoid fever. The test was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of Salmonella typhi. While the definitive diagnosis of typhoid fever depends on

the isolation of S typhi from blood, stools, urine or other body fluids.⁵⁻⁷

Serological diagnosis relies classically on the demonstration of a rising titer of antibodies in paired samples 10 to 14 days apart. In typhoid fever, however, such a rise is not always demonstrable, even in blood culture-confirmed cases. This situation may occur because the acute-phase sample was obtained late in the natural history of the disease, because of high levels of background antibodies in a region of endemicity, or because in some individuals the antibody response is blunted by the early administration of an antibiotic. Furthermore, patient management cannot wait for results obtained with a convalescent-phase sample. For practical purposes, a treatment decision must be made on the basis of the results obtained with a single acute phase sample. False-positive Widal test results have been reported for patients with non-enteric fever salmonellae infections, malaria, typhus, C. neoformans meningitis, immunological disorders, and chronic liver disease.⁸

The study done by Gaikwad UN et al.⁹ also showed the high false positivity and low positive predictive value by slide agglutination test and suggested to caution that the results of slide agglutination test should not be solely relied upon for diagnosis and treatment of enteric fever.

The study done by Karen H Keddy¹⁰ showed the semiquantitative slide agglutination test performed the worst among four rapid serology tests evaluated and showed low PPV and NPV of the same.

Conclusion

The high false positivity and low positive predictive value shown by the slide agglutination test is to caution that the results of slide agglutination should not be solely relied upon for diagnosis and treatment of enteric fever.

References

1. House D, Wain J, Ho VA, Diep TT, Chinh NT, Bay PV, et al. Serology of typhoid fever in an area of endemicity and its relevance to diagnosis. *J Clin Microbiol* 2001;39:1002-7.
2. Reddy EA, Shaw AV, Crump JA. . Community acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis*: 2010;10:417–432. 2.
3. Deen J, Von Seidlein L, Andersen F, Elle N, White NJ, Lubell Y. Community-acquired bacterial bloodstream infections in developing countries in south and Southeast Asia: a systematic review. *Lancet Infect Dis*. 2012;12:480–487.
4. Wain, J., T. S. Diep, V. A. Ho, A. M. Walsh, T. T. Nguyen, C. M. Parry, and N. J. White. 1998. Quantitation of bacteria in blood of typhoid fever patients and relationship between counts and clinical features, transmissibility, and antibiotic resistance. *J. Clin. Microbiol.* 1998;36:1683-1687.
5. Lavanya, V.; Solabannavar, Shivakumar S.; Sonth, Suresh B. Comparison of Results Obtained by Semiquantitative Slide Agglutination and Tube Widal Tests in the Diagnosis of Suspected Typhoid Fever Cases. *International Journal of Biological & Medical Research*;2013;4(1):3001.
6. Manson-Bahr PEC, Bell DR. Manson's tropical diseases, 19th edn. Bailliere-Tindall, London 1987:194– 206.
7. Gilman RH, Terminel M, Levine MM, HernandezMendoza P, Hornick R. Comparison of relative efficacy of blood, stool, urine, bone marrow and rose spot cultures for recovery of *Salmonella typhi* in typhoid fever. 1975;1:1211–1215.
8. Geddes AM. Unexplained fever. *BMJ*.1974;4:397–398.
9. Gaikwad UN, Rajurkar M. Diagnostic efficacy of widal slide agglutination test against widal tube agglutination test in enteric fever. *Int J Med Public Health* 2014;4:227- 30.
10. Karen H Keddy, Arvinda Sooka, Maupi E Letsoalo, Greta Hoyland, Claire Lise Chaignat, Anne B Morrissey, et al. Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub-Saharan African sites. *Bulletin of the World Health Organization* 2011; 89:640-647.