

Original Research Article

Diagnostic reliability of Widal slide agglutination test for enteric fever- still a query

Kiran Yadav^{1*}, Geeta Parihar¹, Suresh Kumar Yadav²

¹Department of Microbiology, Jawaharlal Nehru Medical College, Ajmer, Rajasthan, India

²Department of Pediatrics, Jawaharlal Nehru Medical College, Ajmer, Rajasthan, India

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*Correspondence:

Dr. Kiran Yadav,

E-mail: emailkiran123@gmail.com

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ABSTRACT

Background: Typhoid fever is a major public health problem associated with significant morbidity and mortality in many countries. The Widal slide agglutination test is a commonly used test for diagnosing enteric fever. Limited literature is available on its diagnostic ability in comparison to the tube agglutination test. Aim of this study was carried out to evaluate the efficacy of the Widal slide agglutination test for the diagnosis of enteric fever.

Methods: This cross-sectional study was done in department of microbiology in Jawahar Lal Nehru medical college and hospital, Ajmer over a period of six months from September 2013 to March 2014. A total of 640 serum samples received in the microbiology department were processed for detecting the presence of anti O and anti H agglutinins against *S. typhi*, *S. Paratyphi A* and *S. Paratyphi B* by slide and tube Widal agglutination tests as per standard protocols. The significant titre for O and H agglutinin, 1:160 was taken as positive.

Results: Of the 154 slide Widal positive samples, 115 (18%) samples tested negative by the tube agglutination test whereas only 39 (6%) samples were positive by both slide and tube agglutination.

Conclusions: Slide Widal test has high sensitivity and NPV (100%) can be used as a screening tool but due to low specificity (83.93%) any positive sample should be confirmed by tube Widal test.

Keywords: Slide Widal test, Titre, Tube Widal test, Typhoid fever

INTRODUCTION

Typhoid fever is a life threatening systemic infection and a major cause of morbidity and mortality worldwide. It is widely prevalent in India and other tropical countries with fast growing population, limited safe water, and health system.¹

Isolation of salmonella is the most reliable method for diagnosing enteric fever. Blood culture is considered as the gold standard diagnostic modality and 70-75% diagnostic yield seen if being done in first week of illness.² However, lack of well-trained technical staff and laboratory equipment in most of the primary health care

facility in the developing countries limited its use.³ Along with that frequent use of antibiotics in the community make it difficult to isolate the organism from blood culture and alternate methods like bone marrow culture are invasive and needed expertization.⁴

Thus, in the developing countries serology is used as most common diagnostic modality for enteric fever. Slide Widal test first introduced by Henry Welch, is a rapid test and thus used as screening tool.⁵ The tube agglutination test takes more time, more cumbersome and requires well trained technical staff. It is useful to clear doubts regarding the equivocal agglutination reactions obtained by the slide Widal test.⁶ However, due to rapidity in

availability of results of slide Widal test diagnosis being made solely on its basis in most of the laboratories. Therefore, the present study was conducted to know the efficacy and diagnostic reliability of the slide Widal test in comparison to tube Widal test.

METHODS

This cross-sectional study was done in department of microbiology in Jawahar Lal Nehru medical college and hospital, Ajmer over a period of six months from September 2013 to March 2014. A total of 640 clinically suspected enteric fever cases samples which came to microbiology laboratory for Widal test during the study period were included in the study. A total of 640 samples were centrifuged and serum was separated. The serum was subjected to Widal test by slide agglutination method according to manufacturer's instruction.

Briefly, 50µl of serum was placed upon the slide provided in the kit followed by addition of 50µl of antigen. The slide was rocked gently for one minute and observed for agglutination. The sample positive for agglutination was titrated by using the semi-quantitative Widal test as per manufacturer's recommendations.

All the samples were then tested by the tube agglutination test. Doubling dilution of the testing serum was made. Then 0.5 ml of *S. typhi* O antigen, *S. typhi* H antigen, *S. paratyphi* A and *S. paratyphi* B antigen were added to

same quantity of diluted serum samples. The rack containing all the tubes was incubated in a water bath at 37°C overnight. Macroscopic agglutination was noted and recorded on the following day. The highest dilution of the serum showing visible agglutination was taken as titre of the antibodies in the testing sera. The titre of 1:160 was taken as positive towards O and H antigens.

Statistical analysis

The results of both slide and tube agglutination test were compared and analyzed using Fisher's exact test. sensitivity, specificity, positive predictive value, and negative predictive value calculated.

RESULTS

A total of 640 samples were received during the study period. The samples were first subjected to slide agglutination test. Out of 640 samples, 486 (76%) samples were negative while 154 (24%) were found to be positive. All the samples were also tested simultaneously by tube agglutination test. Out of 154 (24%) slide agglutination positive samples, 39 (25.3%) were also positive by tube agglutination whereas all the sera which were negative by slide were also negative by tube agglutination method (Table 1). From these data, it can be inferred that only 6% samples were positive by both slide and tube agglutination while 18% samples were incorrectly tested positive by slide agglutination test.

Table 1: Distribution of positive samples of the slide agglutination test in comparison to tube agglutination test.

		Tube Widal test				Total
		TO positive	TO, TH positive	TO, AH positive	Negative	
Slide Widal test	TO positive	7	0	0	64	71
	TO, TH positive	3	27	0	49	79
	TO, AH positive	0	0	2	2	4
	Total	10	27	2	115	154

TO positive \geq 1:160, TH positive \geq 1:160, AH positive \geq 1:160.

Table 2. Sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV) of Widal slide agglutination test in comparison with tube Widal test (Fisher's exact test).

Parameter	Sensitivity	Specificity	PPV	NPV
Slide agglutination test	100%	83.93%	25.32%	100%

Sensitivity, specificity, positive predictive value and negative predictive value of slide agglutination test calculated (Table 2).

DISCUSSION

The isolation of bacteria from blood, bone marrow or body fluids remains the gold standard for diagnosing enteric fever. The relatively low rate of isolation from

blood culture and alternative methods such as bone marrow cultures which are of invasive nature become problematic for clinicians to use them as sole diagnostic tool. Although, the Widal test has moderate sensitivity and specificity but still it remains the most common alternative to other methods in diagnosing enteric fever. We had done similar study recently in which Widal test had the sensitivity of 45% and specificity of 86%.⁷ Now days, the tube Widal test being largely replaced by slide

agglutination test due to its rapidity, convenience and low cost.

In present study, the slide agglutination test performed well as a screening tool since it had good overall sensitivity (100%) and negative predictive value (100%). In most of the previous studies done worldwide, the slide Widal test showed high sensitivity (92%) and tube Widal showed high specificity (100%). Similarly, a study conducted in India has reported that tube Widal test had sensitivity of 57% and specificity of 83%.⁸ In contrast, some studies reported slide test to be sensitive and specific both.^{5,9}

However, in this study the specificity for slide Widal was relatively low (83.93%). Positive predictive value (25.32%) was significantly low, which is an important measure of diagnostic tools. These results agree with another study that show the semi-quantitative slide agglutination test performed the worst and had very poor specificity and low PPV and hence an unreliable test.¹⁰ Similarly in other studies too, many false positives were observed with slide Widal test.^{1,11}

High false positivity by slide agglutination test in this study may be due to endemicity of disease and the presence of cross reactivity with non-bacterial infections such as malaria, dengue, infectious mononucleosis.¹²⁻¹⁴ The data from this study shows that slide agglutination test can be used as a reliable test for screening out negative samples but the reliability of positive samples is still questionable. Slide Widal cannot be solely relied upon for diagnosing enteric fever as large number of samples got falsely labelled as positive and this could lead to starting of unwanted antibiotics in otherwise healthy persons. This could lead to serious consequences by further aggravating the current scenario of antibiotic resistance.

As truly said by Henry Welch “No Widal test is infallible and it is not likely that any will be developed that will lower the validity of the isolation of the etiological agent.”²⁵ A culture is still the gold standard for the diagnosis of enteric fever.

The slide Widal test, performed in most of the laboratories is simple, rapid, easy to do and may be used as a good screening test. But due to large number of false positive reactions it cannot be solely used for diagnosing enteric fever. Therefore, whenever using slide agglutination test instead of the standard tube Widal test, it should always be confirmed with the results of the tube agglutination test.¹⁵

CONCLUSION

It is concluded, that even today, the Widal test remains one of the most commonly used test for diagnosing enteric fever in developing world. The slide Widal test, performed by most of the laboratories though convenient,

simple and rapid test but high false positivity and low positive predictive value limits its use as a sole diagnostic modality. The slide agglutination test can be used as screening tool and the positive results should be confirmed by the tube agglutination test.

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