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Comparative evaluation of diagnostic efficacy of widal slide agglutination test & widal tube agglutination test in enteric fever

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Abstract

The serology is used as most common diagnostic modality for enteric fever. Slide Widal test is a rapid test and thus used as screening tool. Due to rapidity in availability of results of slide Widal test diagnosis being made solely on its basis in most of the laboratories. Hence based on above findings the present study was conducted to know the efficacy and diagnostic reliability of the slide Widal test in comparison to tube Widal test.

The present study was conducted in Department of Microbiology, Anugrah Narayan Magadh Medical College, Gaya for the duration of 4 months from March 2018 to July 2018. The patients suspected as having enteric fever during the study period were included in the study. The serum was separated from each blood sample following all standard precautions. The sera were then subjected to the Widal test by the slide agglutination method.

From the present study it can be concluded that Widal test is the common test used for diagnosis of the enteric fever in developing countries. The slide agglutination test can be used as screening tool and the positive results should be confirmed by the tube agglutination test.

Keywords: slide widal test, titre, tube widal test, enteric fever

Introduction

Widal test is a tube agglutination test employed in the serological diagnosis of enteric fever. The test is named after Georges Fernand Isidore Widal, a French physician and bacteriologist, born March 9, 1862, Algeria; died January 14, 1929, Paris.

In 1896 and named after its inventor, Georges-Fernand Widal, is a presumptive serological test for enteric fever or undulant fever whereby bacteria causing typhoid and malaria fever (caused by protozoa) are mixed with a serum containing specific antibodies obtained from an infected individual. In cases of Salmonella infection, it is a demonstration of the presence of O-soma false-positive result. Test results need to be interpreted carefully to account for any history of enteric fever, typhoid vaccination, and the general level of antibodies in the populations in endemic areas of the world. Typhidot is the other test used to ascertain the diagnosis of typhoid fever. As with all serological tests, the rise in antibody levels needed to perform the diagnosis takes 7–14 days, which limits its applicability in early diagnosis. Other means of diagnosing Salmonella typhi (and paratyphi) include cultures of blood, urine and faeces. These organisms produce H2S from thiosulfate and can be identified easily on differential media such as bismuth sulphite agar.

2-mercaptoethanol is often added to the Widal test. This agent more easily denatures the IgM class of antibodies, so if a decrease in the titre is seen after using this agent, it means that the contribution of IgM has been removed leaving the IgG component. This differentiation of antibody classes is important as it allows for the distinction of a recent (IgM) from an old infection (IgG).

The Widal test is positive if TO antigen titre is more than 1:160 in an active infection, or if TH antigen titre is more than 1:160 in past infection or in immunized persons. A single Widal test is of little clinical relevance due to the high number of cross-reacting infections, including malaria. If no other tests (either bacteriologic culture or more specific serology) are available, a fourfold increase in the titre (e.g., from 1:40 to 1:640) in the course of the infection, or a conversion from an IgM reaction to an IgG reaction of at least the same titre, would be consistent with a typhoid infection.

A new serological test called the Tubex test is neither superior nor better performing than the Widal test. Therefore, Tubex test is not recommended for diagnosis of typhoid fever [1].

Salmonella typhi and Salmonella paratyphi A, B and C cause enteric fever (typhoid and paratyphoid) in human. Laboratory diagnosis of enteric fever includes Blood culture, Stool Culture and Serological test. Widal test is a common agglutination test employed in the serological diagnosis of enteric fever. This test was developed by Georges Ferdinand Widal in 1896 and helps to detect presence of salmonella antibodies in a patient's serum.

Patients infected with Salmonella produce antibodies against the antigens of the organism. Antibodies in serum, produced

in response to exposure to Salmonella organisms will agglutinate bacterial suspension which carries homologous antigens. This forms the basis of Widal test.

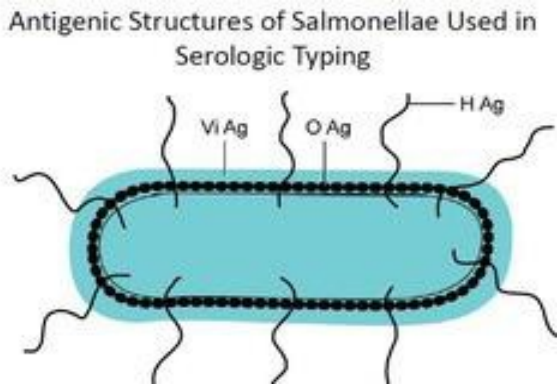

Fig 1

The organisms causing enteric fever possesses two major antigens namely somatic antigen (O) and a flagellar antigen (H) along with another surface antigen, Vi. During infection with typhoid or paratyphoid bacilli, antibodies against flagellar antigen of S. typhi (H), S. paratyphi A (AH), S. paratyphi B (BH) and Somatic Antigen of S.typhi (O) usually become detectable in blood, 6 days after the onset of infection. Those antigens specifically prepared from organism are mixed with patient's serum to detect the presence of antibodies. Positive result is indicated by the presence of agglutination. Absence of agglutination indicates a negative result. The paratyphoid O antigens are not employed as they cross react with the typhoid O antigen. If agglutination occurs with O antigen then it is considered positive for Salmonella typhi. If agglutination occurs in A or B antigen then it is confirmed as positive for Salmonella paratyphi. Agglutination will occur in H antigen for all the cases of antigens like O, A, and B.

The Widal test can be conducted in two ways:

Slide agglutination Widal test

1. Qualitative Slide Test
2. Quantitative Slide Test

Tube agglutination Widal test

Tube agglutination has more accuracy as compared to the slide agglutination technique. However, A slide widal test is more popular among diagnostic laboratories as it gives rapid results.

Limitations of Widal test

1. Tests done within 7 days of illness and after 4 weeks are usually negative.
2. The local titre of the place should be known for the results interpreted correctly.
3. This test (Quantitative) is highly time consumable.
4. Previous typhoid vaccination may contribute to elevated agglutinins in the non-infected population.
5. Other infections of non-enteric salmonella infection such as Typhus, Immunological disorders, chronic liver disease may cause false positive reaction.
6. Cross reaction between malaria parasites and salmonella antigens may cause false positive Widal agglutination test.

The serology is used as most common diagnostic modality for enteric fever. Slide Widal test is a rapid test and thus used as screening tool [3]. 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. However, due to rapidity in availability of results of slide Widal test diagnosis being made solely on its basis in most of the laboratories. Hence based on above findings the present study was conducted to know the efficacy and diagnostic reliability of the slide Widal test in comparison to tube Widal test.

Methodology

The present study was conducted in Department of Microbiology, Anugrah Narayan Magadh Medical College, Gaya, for the duration of 4 months from March 2018 to July 2018. The patients suspected as having enteric fever during the study period were included in the study.

The serum was separated from each blood sample following all standard precautions. The sera were then subjected to the Widal test by the slide agglutination method.

The approval of the institutional ethical committee was taken before conduct of this study. All patients were informed consents.

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 subjected to the tube agglutination test to find out exact titer of antibodies. The serum was diluted in doubling dilutions. 0.5 ml of each dilution was then added to a row of Felix tubes containing the same quantity of S. typhi O antigen and two rows of Dreyers' tubes containing the same quantity of S. typhi H and S. paratyphi A antigens. The rack containing all the tubes was then incubated at 37°C in a water bath overnight. Macroscopic agglutination was noted and recorded on the following day after keeping the rack at room temperature. The highest dilution of serum giving visible agglutination was calculated and matched against the currently used local cut off titre as mentioned above, to confirm positivity.

Results & Discussion

The total 550 samples were referred to the Department of Microbiology for the Widal Test. Out of that 550 samples 157 samples were observed to be positive. The 393 samples were found to be negative. From the 157 slide agglutination positive samples the 44 samples were positive by tube agglutination test.

Table 1: Comparison of Both tests

		Tube Widal Test				
		TO positive	**TO, TH positive**	**TO, AH positive**	**Negative**	**Total**
Slide Widal Test	TO positive	8	0	0	60	68
	TO, TH positive	4	28	0	50	82
	TO, AH positive	0	0	4	3	7
	Total	12	28	4	113	157

The slide agglutination test showed the 100% sensitivity and 85% specificity in all analysed samples. The positive predictive value was seen as 25% where as negative predictive values were seen as 100%.

According to Hoffman *et al.*, [4] the results of a single Widal test, tube dilution, micro-agglutination or slide agglutination are virtually un-interpretable unless the sensitivity and specificity as well as the predictive values of the test for the specific laboratory and patient population are known.

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%. [5] In contrast, some studies reported slide test to be sensitive and specific both [5-6]. However, in this study the specificity for slide Widal was relatively low (85%). Positive predictive value (25%) 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 [7]. Similarly in other studies too, many false positives were observed with slide Widal test [8-9].

However, it has been proved by many studies that the Widal agglutination test is still of significant diagnostic value, particularly in an area where there is a reasonably high suspicion (prior probability) of enteric fever, provided judicious interpretation of the test is made [10-11].

Conclusion

From the present study it can be concluded that Widal test is the common test used for diagnosis of the enteric fever in developing countries. 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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