abstract:
abstract objective: to study the diagnostic reliability of slide widal test with tube widal test in the diagnosis of enteric fever. methods: serum samples were obtained from 150 patients, clinically suspected to have typhoid fever. moreover, serum samples were also obtained from 25 patients with febrile diseases other than typhoid fever. all samples were tested using slide widal test, qualitatively and semi-quantitatively and tube widal test in the microbiology diagnostic laboratory, cmch over a period of 3 months (july 2011-september 2011). results: slide widal test showed many false positive reactions which were negative by tube widal test and clinically proved to be non-enteric cases. conclusion: slide widal test should not be used in the serological diagnosis of enteric fever and laboratories should perform only tube widal test, which is conventional, best for detecting antibodies and can be done in any set up. keyword: "enteric fever", "slide widal"

introduction:
typhoid fever is a life threatening systemic infection and an important cause of morbidity in developing countries. it is widely prevalent in india and several tropical countries with rapid population growth, increased urbanization, limited safe water, infrastructure, and health systems. it is also recognized that delay in diagnosis and institution of appropriate therapy may significantly increase the risk of adverse outcome and mortality. the diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other febrile illnesses. although the isolation of salmonella typhi on bone marrow culture or blood culture remains the gold standard for diagnosing typhoid fever, this may be problematic in endemic areas where adequate microbiologic facilities are limited. the widespread availability and use of antibiotics in the community makes it frequently difficult to isolate the organism on blood cultures and alternative methods such as bone marrow cultures are invasive and difficult to obtain routinely in pediatric patients. despite improved methods of bacteriologic isolation, there is a real need for rapid serologic diagnostic tests for typhoid fever.
During the American Civil War, 81,360 Union soldiers died of typhoid or dysentery. In the late 19th century, typhoid fever mortality rate in Chicago averaged 65 per 100,000 people a year. The worst year was 1891, when the typhoid death rate was 174 per 100,000. The most notorious carrier of typhoid fever was Mary Mallon, also known as Typhoid Mary. In 1907, she became the first American carrier to be identified and traced. She was a cook in New York. She was closely associated with fifty-three cases and three deaths. The Widal test has been used for almost 100 years old, is widely available in developing countries, and is still regarded as a useful test in endemic areas. The somatic O antigen is a phospholipid – protein-polysaccharide complex which forms an integral part of the cell wall. The H antigen present on the flagella is a heat labile protein. During infection, antibodies are produced in patient’s sera against these ‘O’ and ‘H’ antigens. Widal test detects the amount of antibodies formed in the patient’s serum. Persons who have had prior infection or immunisation may develop an anamnestic response during an unrelated fever. The Widal test is easy, inexpensive, and relatively non-invasive. It can be of diagnostic value when blood cultures are not available or practical. In later years, a rapid slide test was developed which is now the most commonly used technique in local laboratories because of its convenience. Slide widal test is an easy and rapid screening test. Hence the present study was done to find the diagnostic reliability of slide widal test in the diagnosis of enteric fever.

MATERIALS AND METHODS:
Microbiology at Coimbatore Medical College This cross sectional comparative study was conducted in the Department of Hospital over a period of 3 months from July 2011- September 2011 on 150 febrile patients, clinically suspected to have enteric fever, and 25 patients with febrile diseases other than enteric fever that have been diagnosed after both clinical examination and laboratory investigation (as 10 patients with urinary tract infection, 6 patients with respiratory tract infection, 5 patients with dengue infection and 4 patients with malaria) were included in this study as control group. A total of one hundred and seventy five blood samples were collected from both groups, were centrifuged and sera were separated. All serum samples were screened by slide agglutination test, qualitatively as per the (Span Diagnostics) manufacturer’s instructions. The results were interpreted and the samples showing clumping within a minute was considered as positive reaction, were further taken up for semi - quantitative slide agglutination test and Tube agglutination test in doubling dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, 1:640. All tubes were mixed well and incubated at 37°C for 24 hrs. The results were read as granular appearance for O positive reaction and as floccular appearance for H positive reaction.

RESULTS:
Of the 150 serum samples tested from clinically diagnosed enteric fever cases, O antigen was negative in 58 samples; positive 1:40 for 42 samples (28%); 1:80 for 28 samples (18%); 1:160 for 18 samples (12%); 1:320 for 4 samples (3%) in slide test, whereas in tube widal test, O antigen was negative in 118 samples; positive 1:40 for 12 samples (8%); 1:80 for 10 samples (7%); 1:160 for 4 samples (5%). In slide test, H antigen was negative in 118 samples; positive 1:40 for 12 samples (8%); 1:80 for 10 samples (7%); 1:160 for 4 samples (5%). In slide test, H antigen was negative in 16 samples; positive 1:40 for 70 (47%) samples;
1:80 for 32 samples(21%); 1:160 for 24 samples(16%); 1:320 for 8 samples(5%).

In Tube test, H antigen was negative in 108 samples; positive 1:40 for 18 samples (12%); 1:80 for 12 samples(8%); 1:160 for 10 samples(7%), 1:320 for 2 samples(1%).

In slide test, AH antigen was positive 1:40 for 2 sample; 1:320 for 1 sample. In Tube test, AH antigen was negative for all samples in all the titres. BH antigen was negative in both slide and tube widal test in all the titres. Of the 25 hospital controls diagnosed with febrile illnesses other than typhoid, only one serum sample at a titer of 1/80 and 4 samples at a titre of 1/40 gave positive test result when using slide widal test, while none of the samples were positive at any titer in tube widal test. Higher rate of positivity was found in slide widal test than tube widal test. O and H antigens showed high positive reactions than AH and BH antigens. The H antigen showed very high positive reactions than O antigen in all the titres. The titre level was higher in slide widal than Tube widal.

**DISCUSSION:**

Widal test has been used in the diagnosis of typhoid illness for long time in our country. The Widal test is easy, inexpensive, and relatively non-invasive. It can be of diagnostic value when blood cultures are not available or practical. Sanaa S. Kabeil et al (2010) have reported widal test as a useful test. BL Sherwal et al (2004) and Zullfiqar Ahmed Bhutta et al (1999) in their study have reported that widal test had sensitivity of 57% and specificity of 83%. Jim Pruckler (2004) have reported that sensitivity of widal test is 64% and specificity is 76%.

In the present study, slide widal test gave many false positive reactions. Similarly, Karen H Keddy et al (2011) have reported that the semiquantitative slide agglutination test performed the worst and had very poor specificity and low PPV and NPV and hence an unreliable test. Many false positives were observed by Ayse Willke et al (2002) and Dr. Jagadish C Das (2007) in slide widal test. In contrast, slide test have been reported to be sensitive and specific by Henry Welch et al (1939) and Indro Handjo et al (2004).
In this study, high level of positivity was seen with O and H antigens in the slide - widal test similar to a study by Karen H Keddy et al (2011). Also, H antigens showed higher level of positive results than O antigen in slide widal similar to a study by Basaca - sevilla et al (1979) and Roohi Aftab et al (2009).

In the present study, slide widal test was positive in many non - enteric fever cases. There is also the possibility of cross-reactivity with non-bacterial infections such as malaria, dengue, hepatitis A, and infectious mononucleosis. Alfred Young Itah et al (2004), Ali M Somily et al (2011) and M.O.Ibadin et al(2004) have also observed similar cross reactions.

CONCLUSION:
It is concluded, that even today, the widal test remains one of the best, easily accessible, cheap and simple method for the diagnosis of typhoid fever. The slide widal test, performed by 90% of laboratories is convenient, simple to set up, a fast process, and involves less glassware. But this test has several limitations. It leads to many false positive reactions and has very poor specificity and low reliability. Patients without typhoid fever may receive unnecessary and inappropriate antimicrobial treatment which may lead to drug resistance. Also, the antibiotics nowadays are costlier. Why waste money and invite complications? Moreover, slide agglutination test is best for antigen detection and tube agglutination test is best for antibody detection. Hence, the slide widal test, though provides a rapid diagnosis should not be used as a diagnostic tool due to the above limitations. Slide widal test may be adopted as a screening procedure and the positive samples should be confirmed by tube widal test. Laboratories should perform only the tube widal test for serological diagnosis of enteric fever.

<table>
<thead>
<tr>
<th>Widal</th>
<th>Total no.of Positives &gt; 1:80</th>
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<tbody>
<tr>
<td></td>
<td>40</td>
</tr>
<tr>
<td>O</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>50</td>
</tr>
<tr>
<td>Slide</td>
<td>42</td>
</tr>
<tr>
<td>H</td>
<td>64</td>
</tr>
<tr>
<td>O</td>
<td>50</td>
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</table>

Table-1: Antibody titre of 150 clinically diagnosed enteric fever case using slide & Tube widal test

<table>
<thead>
<tr>
<th>Widal test</th>
<th>No of samples reactive at different titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Slide</td>
<td>4</td>
</tr>
<tr>
<td>Tube</td>
<td>0</td>
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</tbody>
</table>

Table - 2: Antibody titre of 25 cases of Non - Enteric fever cases

An Initiative of The Tamil Nadu Dr. M.G.R. Medical University
University Journal of Pre and Para Clinical Sciences
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