Research & Reviews

ISSN: 2320-3528

Research and Reviews: Journal of Microbiology and Biotechnology

Comparison of Widal test with Immunochromatography and Enzyme Immuno Assay for *Salmonella typhi* IgM and IgG Antibodies

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Research Article

Received: 02/06/2013
Revised: 14/06/2013
Accepted: 02/07/2013

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Keywords: Widal test, Typhifast test and Typhipoint test, Typhoid fever, *Salmonella typhi*

ABSTRACT

Typhoid fever is endemic in Asia Pacific Region, the Indian subcontinent, Africa, and South America. The protean manifestations of typhoid fever make this disease a true diagnostic challenge.Isolates from many parts of the world are now multidrug-resistant (MDR).There is a need for a quick and reliable diagnostic test for typhoid fever as an alternative to the Widal test. Collection and preservation of samples were done as per the manufacturer's instructions. Widal test was done by two quantitative methods i.e. slide and tube agglutination. About 80 and more than 80 titre was considered as significant titre. Typhifast test is immunochromatographic qualitative test assay performed in test device, which shows colour band when there is IgM in serum against coated antigen.Pink purplish coloured lines which confirm a positive test result. It was compared with positive control, which was also coated in respective test devices. Typhipoint is enzyme immunoassays (EIA) for the detection of IgM and IgG antibodies to the extracted protein of salmonella typhi in serum. Absence of this colored dot in the test region indicates negative test result. The results were compared with positive control, which was also coated in respective test devices. Total of 100 blood samples of suspected cases of typhoid fever was tested by three methods i.e. widal test, Typhifast &Typhipoit. Widal test in which, 48 samples (48%) were showed positive reaction with slide and tube agglutination method. Widal reaction was positive for TO & TH both in 62.5% of widal positive sample and 30% in suspected cases of typhoid fever&30% for TH in samples. In Widal positive reaction, maximum sample were from age group 21-40 years (58.33%).Out of 48 positive widal sample, 30 (62%) were males and 18 (38%) were females. Typhoid fever patients were outnumbered in MICU (22.2%).48 (48%) samples were positive and 52 (52%) were negative by widal. In typhifast 42 (42%) were positive and 58 (58%) were negative. In typhipoint 44 (44%) positive and 56 (56%) were negative. Out of100samples, 8 were used as positive and negative controls for typhipoint. 92 samples were used for testing. The result of the two methods i.e. Typhifast and Typhipoint were compared with the gold standard Widal test results. Typhipoint was more sensitive than both tests. It showed 7% sensitivity than typhifast. There was little difference among these three tests in positivity reaction point of view. Typhipoint was more sensitive than two tests.

INTRODUCTION

Typhoid fever occurs worldwide, primarily in developing nations whose sanitary conditions are poor. Typhoid fever is endemic in Asia, Africa, Latin America, the Caribbean, and Oceania, but 80% of cases come from Bangladesh, China, India, Indonesia, Laos, Nepal, Pakistan, or Vietnam [1]. Within those countries, typhoid fever is most common in underdeveloped areas. Typhoid fever infects roughly 21.6 million people (incidence of 3.6 per 1,000 populations) and kills an estimated 200,000 people every year.[9, 10-11]. Around 2200 different serotypes of Salmonella exist in animals and most are capable of causing Salmonellosis in humans.[1] these are divided into mainly 2 groups. a) Enteric fever group – It consist typhi & paratyphi causing typhoid fever & paratyphoid fever b) Food poisoning group – Causing gastroenteritis, septicemia & localized infection [2,3,4].

ISSN: 2320-3528

The classic presentation includes fever, malaise, diffuse abdominal pain, and diarrhea. Untreated, typhoid fever is a grueling illness that may progress to delirium, obtundation, intestinal hemorrhage, bowel perforation, and death within one month of onset. Survivors may be left with long-term or permanent neuropsychiatric complications [5]. The name *S typhi* is derived from the ancient Greek *typhos,* an ethereal smoke or cloud that was believed to cause disease and madness. In the advanced stages of typhoid fever, the patient's level of consciousness is truly clouded. Although antibiotics have markedly reduced the frequency of typhoid fever in the developed world, it remains endemic in developing countries [6]. *S typhi* has no nonhuman vectors. Oral transmission via sewage-contaminated water or shellfish (especially in the developing world) [7]. An inoculum as small as 100,000 organisms causes infection in more than 50% of healthy volunteers [8].

In India also, typhoid and paratyphoid fevers are endemic, occurring through all states. A typhoid fever outbreak in a slum of South Dumdum municipality, West Bengal, India in 2007 [12]. The outbreak also from the three villages of Kerala, Bhayla and Kalyangadh in Bavltaluka of Ahmedabad district as a result of contamination of the drinking water supply led to an epidemic, taking 137 people, mostly children, under its grip in 2009 [13]. In Vietnam, typhoid fever is highly endemic, with the southern provinces most heavily affected. In a study conducted in Dong Thap Province in 1995 and 1996, the incidence of confirmed serotype Typhi infection was 198 per 100,000 for all ages [14]. Emerging drug resistance among circulating serotype Typhi strains in Vietnam and elsewhere has complicated the treatment of typhoid fever and heightened the need for rapid accurate diagnosis [15]. Unfortunately, neither the Widal test, which remains in widespread use in the developing world, nor any of the serodiagnostic tests that have since been developed has proven sufficiently sensitive, specific, and practical to be of value in areas where this disease is endemic [16,17,18,19,20,21,22,23]. Hence, a study will be undertaken to compare the results of Widal test and Immunochromatographic test for Typhi IgG& IgM for the early diagnosis of disease and for early treatment of infection [32,33,34,35,36,37,38].

MATERIALS AND METHOD

Patient serum samples.

Criteria for sample collection

No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed and turbid samples. Clean and dry glassware free from detergents must be used for sample collection. Do not heats inactivate the serum. Though freshly collected serum is preferable, store samples at 2 to -80°C in case of delay in testing, for up to 72 hours.

WIDAL TEST KITS

The Manufactured by: Tulip Diagnostic (P) Ltd, Date of manufacturing: September 2012, Date of Expiry: February 2013, Storage Temperature: 4-8 °C. TYDAL® contains ready to use concentrated, smooth antigen suspensions of the bacilli; *S. typhi 'O', S. typhi 'H', S. paratyphi 'AH'*, S. *paratyphi 'BH',* polyspecific positive control , reactive with these antigens. The H agglutinable suspension of bacteria is prepared by adding 0.1 per cent formalin to a 24 hour broth culture or saline suspension of an agar culture. For preparation of O suspension of bacteria, the bacillus is cultured on phenol agar (1:800). The growth is scraped off in a small volume of saline and mixed with 20 times its volume of absolute alcohol. it is then heated in a water bath at 40°-50°C for 30 minutes, centrifuged and the deposit resuspended in saline to the appropriate density. Chloroform in then added as a preservative. S. typhi 901, O and H strains, are used for preparation of antigens. Each batch of prepared antigen should be compared with a standard. Widal kits of stained antigens are available commercially (Fig 1-3)

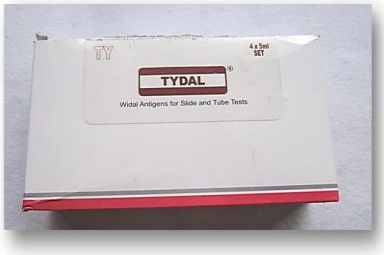

Figure1: Widal test kit for slide/ tube test method

Figure 2: Widal test kit

Figure3: Widal test reagents

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Additional material required

Slide test method

Stop watch, Variable Micropipettes.

Quantitative method

Timer, Kahn tubes / test tubes, Pipettes (0.1ml, 1ml), Physiological saline, Incubator (37°C), Test tube rack.

Separation of serum sample

The collected blood samples collected in plain tubes were centrifuged for 10 minutes at 2500 rpm. Sera were separated and used for serological tests either immediately or kept frozen at -20 °C until used.

Test Procedure

Keep the reagents and samples to room temperature before testing. Shake and mix antigens well before dispensing.

Slide Screen Method

Place one drop of positive control onto a reaction circle of the glass slide. Place 50 µl of physiological saline onto the next reaction circle of the glass slide. One drop of patient's serum to be tested onto each of the required number of reaction circles. Add one drop of appropriate TYDAL® antigen suspension to the reaction circles containing Positive control & physiological saline. Add one drop of appropriate TYDAL® antigen suspensions to the reaction circles containing the patient's serum. Mix contents of each circle uniformly over the entire circle with separate mixing sticks. Rock the slide gently back and forth, and observe for agglutination macroscopically at one minute.

Quantitative Method: Tube-test Procedure

Take appropriate number of sets (as required; one set for each antigen suspension) of 8 Kahn tubes / test tubes and label them 1 to 8.Pipette into tube No. 1 of all sets 1.9 ml of physiological saline. To each of the remaining tubes (2 to 8) add 1 ml of physiological saline. To tube No. 1 of all sets add 0.1 ml of serum sample to be tested and mix well. Transfer 1 ml of the diluted serum sample from tube No. 1 to tube No. 2 and mix well. Transfer 1 ml of the diluted serum sample from tube No. 2 to tube No. 3 and mix well. Continue this serial dilution till tube No. 7 in each set. Discard 1.0 ml of the diluted serum from tube No.7 of each set. Now the dilutions of the serum sample achieved from tube No. 1 to 7 respectively in each set is as follows: 1:20, 1:40, 1:80, 1:160, 1: 320, 1:640, 1: 1280. Tube No. 8 in all the sets, serves as a saline control. To all the tubes (1 to 8) of each set add one drop of the respective well-mixed TYDAL® antigen suspensions from the reagent vials and mix well. Cover and incubate at 37^0C overnight (approximately 18 hours). Dislodge the sediment button gently and observe for agglutination.

TYPHIFAST

Manufactured by: AB Diagnostic Manufacturing (P) Ltd, Date of manufacturing: September 2012, Date of Expiry: March 2013, Storage Temprature: 4-30 °C

Intende use

The Thyphifast Test Device is an Immunochromatographic assay design for the qualitative detection of specific IgM antibodies against Salmonella typhi in human serum, plasma or whole blood.

The typhifast Test Device is a qualitative immunochromatography test assay for the detection of human IgM antibodies against Typhoid Fever in human blood, serum and plasma. In this procedure, extracted Antigen from salmonella typhi is immobilized on to the nitro -cellulose membrane strips on the test region of the test device .when the sample has been added to the sample pad, followed by chase buffer, sample will migrate upwards. If specific IgM antibodies against S.typhi are present in the sample it will form an antibody-antigen complexes are subsequently visualized by a gold conjugated anti human IgM when the chase buffer is added and it migrates further the test region of the membrane, forming pink purplish colored lines which confirm a positive test result. Absence of this colored line in the test region indicates a negative test result. The control line contains rabbit anti goat IgM which bind with the goat anti-human IgM gold conjugate .the control band serves as an indication of proper migration plus reagent control (Fig 4-6).

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Kit content

Typhifast test devices in an individual seal aluminium pouch .Chase buffer in a dropper bottle (4ml* 1 bottle).Product insert (one)

Figure 4: Typhifast test kit

Figure 5: Typhifast test cassette

Figure 6: Typhifast test reagent

Test procedure

Allow the test device, reagent, and patient samples to reach room temperature Open the pouch and retrieve the device. Remove the test device from the sealed foil pouch. Once opened, the device must be used immediately. Place the device on a clean and level surface. Label the test device with the sample name. Proceed with the assay procedure as diagram below.

Positive

Positive for typhoid specific IgM antibodies if colored band appear at the test line (T) and control line (C). Any intensity of line should be considered positive.

Negative

Negative for typhoid specific IgM antibodies if only the control line (C) is visible through the viewing windows.

Invalid

Invalid if the control line (C) is absent. If this occurs, the assay should be repeated using a new test cassette.

TYPHIPOINT

Manufactured by: AB Diagnostic Manufacturing (P) Ltd, Date of manufacturing: September 2012, Date of Expiry: March 2013, Storage Temperature: 4-8 °C

Typhipoint is enzyme immunoassays (EIA) for the detection of IgM and IgG antibodies to the extracted protein of salmonella typhi in serum. Specific antiextracted protein antibodies (IgM and IgG) if present in human serum will bind to the protein dotted and blocked on nitrocellulose protein, forming an antigen antibody complex. This test only uses 1 type of protein only. Following a washing step ,enzyme-labeled rabbit anti human antibody (anti IgM or anti IgG)is added, which binds to the human S.typhi antibodies .the whole complex is then visualized by addition of 4-chloro-1-naphthol,which later visualize the reaction to a bluish purple dot.(Fig 7)

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Preparation of Reagent

A. Stock wash buffer (10X)

Allow the reagents to reach room temperature (18-25°C).Check the stock wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute stock wash buffer (10X) into a final concentration of 1X (please refer to the table below) the diluted wash buffer is sufficient for the entire kit Store it separately at 2-8°C and use when necessary.

Wash buffer 10X	Deionised water	Total volume (1X)
10ml	90ml	100ml
20ml	180ml	200ml

B. Colour Development Solution

Allow the reagents to reach room temperature (18-25°C) before mixing. Avoid exposing these reagents to strong lights. Calculate the volume to total reagent used the number of strips used. Total volume needed: 0.30ml x no of strips=X volume. Mix substrate A and substrate B with 1 part of substrate A+ 5 part of substrate B.

No of strips	6	10	14	24	40
Substrate A (ml)	0.3	0.5	0.7	1.2	2.0
Substrate B (ml)	1.5	2.5	3.5	6.0	10.0

Figure 7: Typhipoint test kit

Assay procedure

Note: All reagents and tests must be equilibrating to room temperature (20-25°C) before use.

Count the number of sample to be tested. For positive control, negative control and each sample, 2 strips are required. Align the strips with the mark side at the bottom. For each sample and controls, using a ball point, label 1 strip as M and the other one as G. Label code number for positive, negative control and each patients near to M or G . Place each strip in the well of the reaction tray with the mark side up. Add 300µ of sample diluents into each well. Allow the strips to be thoroughly wet. Add 3 µ of sample or controls into an appropriate well. Gently aspirate the solution to make sure it properly mix. Place the reaction tray onto the rocker platform. (If rocker platform not available, place the reaction well on the bench, and shake it manually every 5-10 minutes). Incubate for 25 minutes. Dilute washing buffer to 1x (see preparation of Reagent). Aspirate the first antibody from each well into the disinfectant. Add 300µl of 1x washing buffer and wash 3x for 5minutes each time. Add 300µl prediluted anti human IgM into the strips with M mark, and 300µl anti human IgG into the strip with G mark. Cover the strips from light and incubate for 45 minutes on the rocker platform (Meanwhile prepare colour development solution). Follow procedure 9 for washing steps. Add 300µl of colour development solution into each well. Place the tray back to the rocker platform. Cover the tray from light. Let the reaction happens for 10 minutes. Stop the reaction by aspirate the colour development solution into the disinfectant and rinse thoroughly with 3x with deionised water. Rinse the reaction tray thoroughly with distilled water. Store dry for re-use. Place IgM and IgG strips according to code and interpret the result.

Interpretation

The test is considered positive if the intensity of the is equal or greater than the intensity produce by the positive control. Comparison of IgM strips need to be made with IgM positive controls strips only and comparison of IgG strips need to be made with IgG positive control strips only. Interpretation must be done directly after the procedure is done.

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RESULTS

Total of 100 blood samples of suspected cases of typhoid fever were tested by **A.** Widal test (slide and Tube agglutination for detection of Typhoid antibodies, salmonella typhi "O" and "H" and Salmonella paratyphi "AH" and "BH".) **B.** Typhifast (Immunochromatographic test for detection of IgM antityphi antibody) **C.Typhipoint** (An enzyme immunoassay for the detection of IgM and IgG antibody). 100 samples were tested by conventional Widal test, using TO, TH, AH& BH antigens by slide and tube agglutination method 48 samples (48%) showed positive reaction in Widal test (Table 1-4)

Interpretation of Widal test result

A. 'O' Somatic antibody is specific for Typhoid fever **B.** 'H' Flagellar antibody – Group specific antibody it can show anamnestic reaction with other conditions **C.** O or H antibody titer ≥ 1:80 is suggestive of typhoid fever.

Table 1. Distribution of Positive Widal Test In 100 Samples

Antigens	Sample Tested	Positive Reaction	%
TO & TH Positive	100	30	30%
TO Only	100	10	10%
TH Only	100	8	8%
AH Only	100	0	0%
BH Only	100	0	0%
Total		48	48%

Out of 100 suspected samples for typhoid fever, Widal reaction was positive for both TO & TH in 30% samples. Only to 10%, Only TH 8%. Test for AH &BH were negative for all sample (Fig 5-7)

Table 2. Distribution of Positive Widal test Result

Antigens	Total No Of Positive sample	No of Positive	Out of 48 positive test (%)	Positive out of 100 samples
Both TO & TH	48	30	62.5%	30%
TO Only	48	10	20.9%	10%
TH Only	48	8	16.7%	8%
AH	48	0	00%	0%
BH	48	0	00%	0%

It was found that Widal test was test was positive for TO & TH both in 62.5% of widal positive sample and 30% in suspected cases of typhoid fever.

Table 3. Distribution of O and H antibody titer

Antigens	1:20	1:40	1:80	1:160	1:320	TOTAL
Both O & H Positive	0	0	04/30 (13.3%)	14/30 (46.6%)	12/30 (40.0%)	30
	0	0	04/30 (13.3%)	10/30 (33.3%)	16/30 (53.3%)	
Only O Positive	0	0	06/10 (60.0%)	02/10 (20.0%)	02/10 (20.0%)	10
Only H Positive	0	0	0	04/8 (50.0%)	04/8 (50.0%)	8

Widal test result

A. Accordingly 30% of samples from suspected cases showed TO & TH antibody titer ≥ 1:80 hence positive for typhoid fever.
B.10/100 samples showed only O antibody titer ≥ 1:80 considering O antibody is specific for Typhoid fever , these samples showed widal test result suggestive of Typhoid fever
C. 8/100 samples showed only H antibody ≥ 1:80. Presence of only H antibody of O antibody suggests
a) Anamnestic reaction, and this H antibody response could be due to other febrile condition
b) Persistence of H antibody after episode of typhoid fever. In these cases it is necessary to consider clinical details, recent history of typhoid fever, and result of other laboratory finding (Fig 8-10).

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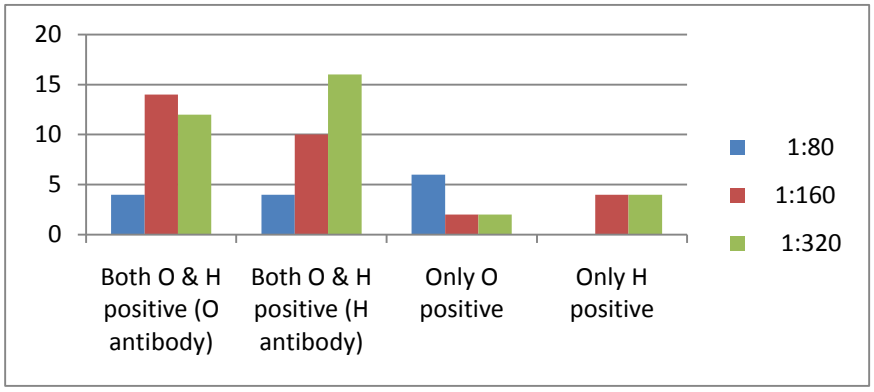

Figure 8: Widal test showing both TO & TH positive reaction, 14/30(46.6%) samples showed titer of 1:160.

Table No. 4 .Age wise distribution of widal positive patients

Age (In years)	No of positive patients	Percentage
0-10	4	8.33%
11-20	10	20.83%
21-40	28	58.33%
41-60	4	8.33%
Above 60	2	4.16%

Total no of widal positive sample- 48

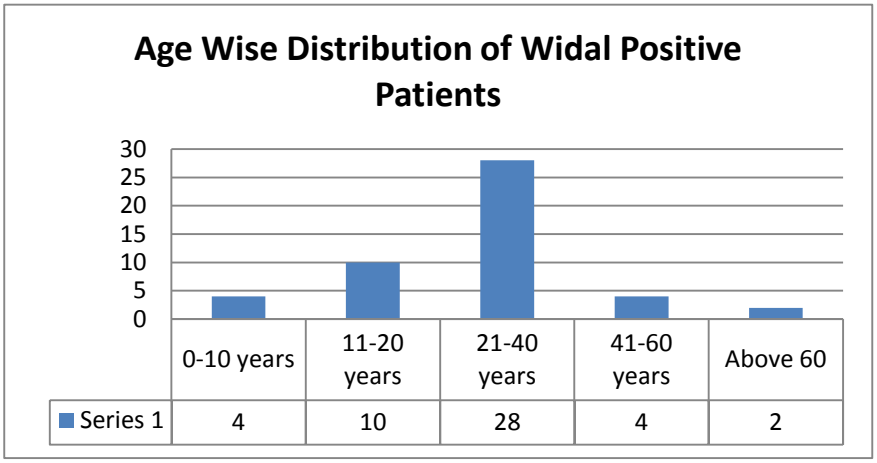

Figure 9 : In Widal positive reaction , maximum sample were from age group 21-40 years (58.33%), followed by 11-20 year (20.83%), 0-10 years (8.33%) and 41-60 years (8.33%)

Table 5. Male and Female distribution of widal positive reaction

Gender	Samples tested	Positive widal test	%
Male	52	30/52	57.6%
Female	48	18/48	37.5%
Total	100	48	

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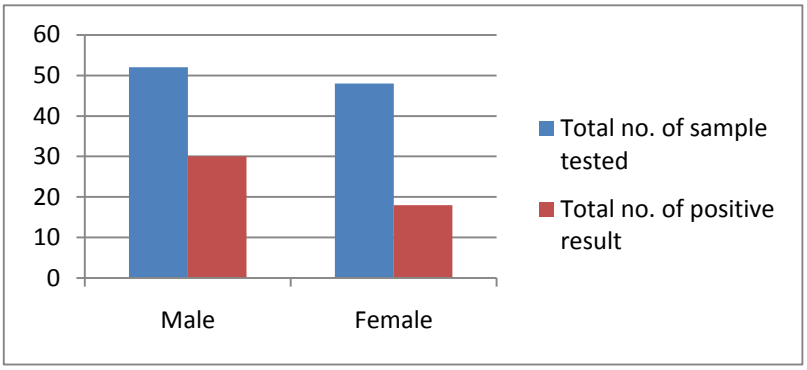

Figure 10: Gender wise distribution of widal positive patients.

Out of 48 positive widal sample, 30 (62%) were males and 18 (38%) were females. Thus, Males showed more positive Widal test than females.

Table 6. Distribution of widal positive patients attending OPD or IPD

Department	Sample tested	Positive reaction	(Percentage)%
IPD	36	20/36	55%
OPD	64	28/64	43%
Total	100	48	48%

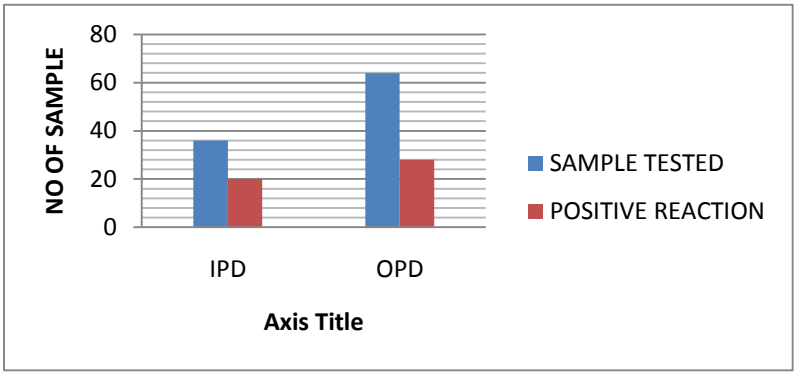

Figure 11: The patients show positive Widal test mainly from IPD.

Table 7: Distribution of Widal Positive patients in different wards (IPD only)

Wards	Total no. of tested samples	No. of positive sample	%age
Medicine ward	8	4	11.1%
Casualty	6	2	5.5%
ICU	10	6	16.6%
MICU	12	8	22.2%
Total	36	20	55.5%

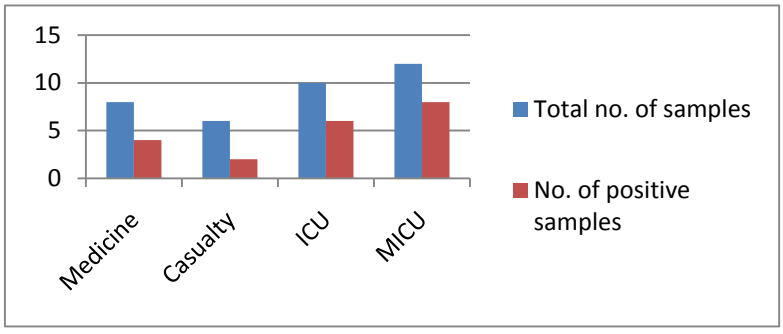

Figure 12: Typhoid fever patients were present in MICU (22.2%) followed by ICU (16.6%), Medicine ward (11.1%) & Casualty (5.5%).

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Comparison of Widal test with other method

A) Typhifast – Immunochromatographic test for IgM antibodies for Salmonella typhi.
B) Typhipoint – Enzyme Immunoassay test for IgG &IgM antibodies for Salmonella typhi (Fig 11-12). Total 100 samples were tested by Widal test, Typhifast (immunochromatographic test) and Typhipoint (EIA). Out of which 48 (48%) samples were positive and 52 (52%) were negative by WIDAL. In typhifast 42 (42%) were positive and 58 (58%) were negative. In typhipoint 44 (44%) positive and 56 (56%) were negative (Table 8).

Table 8

Methods	Total samples	No. of positive samples	%age
Widal	100	48	48%
Typhifast (ICT)	100	42	42%
Typhipoint (EIA)	100	49	49%

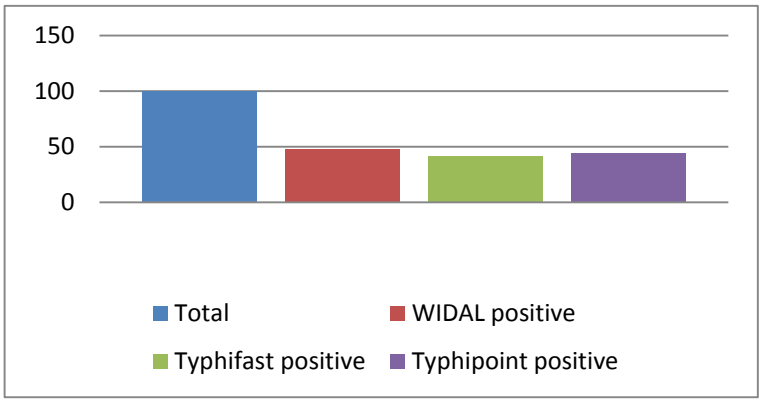

Figure 13: Comparison of Widal test with Typhifast and Typhipoint.

Table 9: Comparison of Typhifast with Widal test

Test	Widal	Typhifast
Positive	48	42
Negative	52	58
Total	100	100

Table 10. Comparison of Typhipoint with Widal test.

Test	Widal	Typhipoint	
		IgM	IgG
Positive	48/100	38/92	36/92
%	48	41.3%	39.13%

Total no of sample were 100 but in typhipoint 8 samples were used as positive and negative controls, only 92 samples were tested(Table 9-10 & Fig 14-21).

Table 11: Comparison of Widal test with typhifast and typhipoint

Test	Widal	Typhifast	Typhipoint	
		IgM	IgM	IgG
Positive	48/100	42/100	38/92	36/92
%	48%	42%	41.3%	39.13%

The result of the two methods i.e. Typhifast and Typhipoint were compared with the gold standard Widal test results.

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Widal test result (SLIDE Agglutination)

Both O &H positive

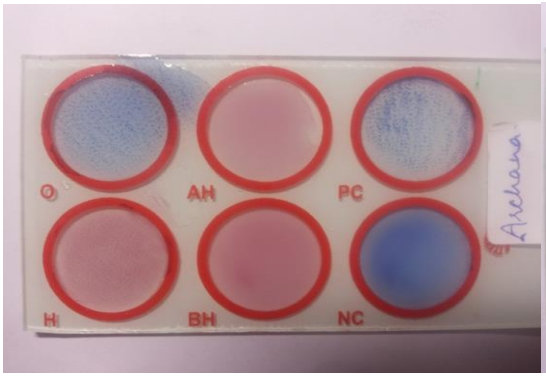

Figure 14: O positive agglutination

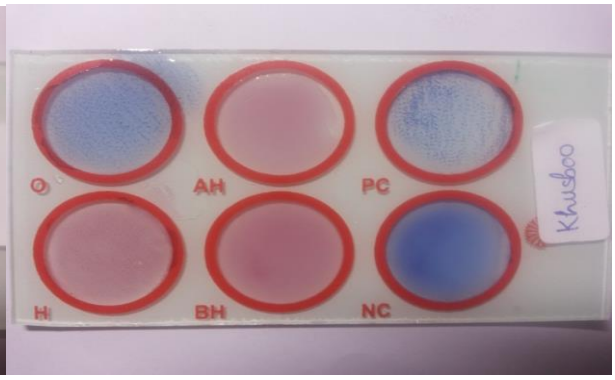

Figure 15: H positive agglutination

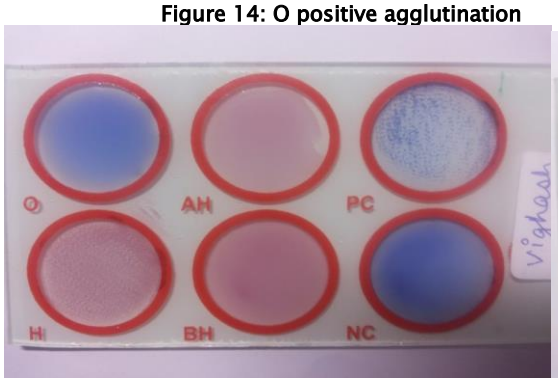

Figure 16: Negative for Widal test

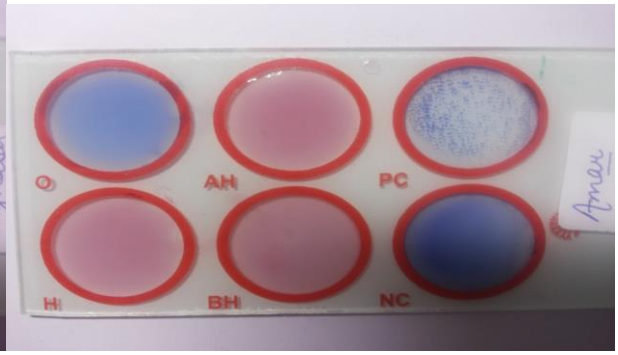

Figure 17: Widal test result (Tube agglutination):– 'O' Agglutination Test (T) and Control (C) 'H' Agglutination Test (T) and Control (C)

Typhifast Result

Figure 18: Typhifast Positive

Figure 19: Typhifast negative

Typhipoint result

Figure 20: Typhipoint positive for IgM

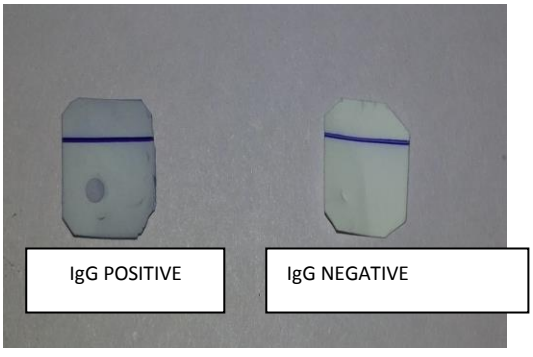

Figure 21: Typhipoint positive for IgG

ISSN: 2320-3528

DISCUSSION

Total of 100 blood samples of suspected cases of typhoid fever were tested in MGM Medical College & Hospital, Kamothe, Navi Mumbai, over a period of one year from February 2012 to January 2013, by: **A.** Widal test (slide and Tube agglutination for detection of Typhoid antibodies, salmonella typhi "O" and "H" and Salmonella paratyphi "AH" and "BH".) **B.** Typhifast (Immunochromatographic test for detection of IgM Salmonella typhi antibody) **C.Typhipoint** (An enzyme immunoassay for the detection of IgM and IgG salmonella typhi antibody)

Table 12 .Showing the sex ratio in different studies among the suspected cases

Study series	Sex ratio (male: female)
Wain J et al (1998) [42]	1:1
Yaramis A et al (2001) [31]	1.4:1
Retnosari S et al. (2001) [58]	1:2.3
Olsen SJ et al (2004) [39]	1.05:1
Chirag S et al (2005) [44]	2.8:1
Dr .Balakrishna T.P (2010) [58]	1.47:1
Present study	1.08:1

The present study showed a male preponderance with a ratio of 1.08:1 which is comparable with that of Olsen SJ et al. In the present study Widal positive reaction , maximum sample were from age group 21-40 years (58.33%), followed by 11-20 year (20.83%), 0-10 years (8.33%) and 41-60 years (8.33%) which is quit comparable with the study of Dr Balakrishna T.P. It showed 33% of patients belong to the age group 11-20 years. 24%were in the age group of 21-30 years. 15.5% were in the age group of 31-40 years the youngest patients were 3 years old and oldest was 68 years [58]. In the other study by Sharma N, (et al) found that 71% typhoid fever case were less than 30 year [59] In the present study widal positive reaction from the age group of 0-10 is 8.33% which is quit comparable with the study Razel L Kawano et al who showed 14% incidence of widal positive patients in the age group of 0-10 years [57,58,59,60]. Out of the 100 samples tested, 52% males & 48% were females. Widal test positivity was 30/52(57.6%) for male 18/48(37.5%) for female which is quite comparable with the studies of Dr. Safia Sultana, Department Of Microbiology, Mymen Singh Medical College, Bangladesh, who in her study reported 52% males & 48% females to be positive by widal test [24,25,26,27,28,29,30]. Another study done by Butler and others also showed similar result that infection ratewas slightly higher in male (Butler et al. 1991) [40,41,42,43,44,45,46,47,48,49]. Butler expressed his opinion that greaterexposure of male to contaminated food and water outside the home might be region of higherof infection among this population [63,64,65,70].

Table 13: Widal positive in various studies among the suspected typhoid cases

Study series	Percentage of positivity
Sherwal BL et al.(2004) [51]	57%
D Narayanappa et al. (2008) [50]	48%
Gopalakrishnan V et al. (2002) [61]	34%
Bhutta ZA et al. (1999) [66]	54%
Retnosari S et al (2001) [68]	11%
Yaramis A et al (2001) [62]	20%
Present study	48%

In the present study, Widal test was positive in 48 (48%) which is quite comparable with the study of D Narayanappa et al. (48%) ,Sherwal BL et al. (57%), Bhutta ZA et al (54%)

Table 14: Typhidot positive in various studies among the suspected typhoid cases

Study series	Percentage of positivity
Sherwal BL et al.(2004) [51]	79%
D Narayanappa et al. (2008) [50]	78%
Bhutta ZA et al. (1999) [66]	70%
Retnosari S et al (2001) [68]	72%
Membrebe FA et al .(1999) [67]	56%
Jesudason MV et al. (2006) [69]	9%
Present study	44%

In the present study, 44% of the cases were positive by Typhidot test. Other studies have shown higher percentage of

ISSN: 2320-3528

positivity expect Jesudason MV [62]. Results of Typhidot test was considered positive if IgM was positive regardless of whether IgG was positive or negative. Typhidot test might find masking effect in which IgM was masked by high IgG levels where IgG was likely to come from past infection or in the initial state of reinfection. Retnosari S [58]. (2001) conducted Typhidot-M on same samples and the percentage of positivity increased from 72% to 81%.

CONCLUSION

Total of 100 blood samples of suspected cases of typhoid fever were tested by A. Widal test (slide and Tube agglutination for detection of Typhoid antibodies, Salmonella typhi "O" and "H" and Salmonella paratyphi "AH" and "BH".) **B.** Typhifast (Immunochromatographic test for detection of IgM antityphi antibody) C.Typhipoint (An enzyme immunoassay for the detection of IgM and IgG antibody). About 100 blood samples of suspected cases of typhoid fever were tested in MGM Medical College& Hospital, Kamothe over a period of one year. In the Widal test 48/100 (48%) sample showed positive reaction. Widal test in 100 suspected cases the result where as follow. Both TO & TH positive in 30 (62.5%), Only TO Positive 10 (20.9%. Only TH positive 8 (16%), AH & BH were negative. In the dilution 46.6% sample showed the titre in 1:160 for 'O' antibody and 53.3% sample showed titre of 1:320. In Widal positive reaction, maximum sample were from age group 21-40 years (58.33%). The numbers of samples were more for male patient and Males again showed more positive Widal test than females. The numbers of samples received for Widal test were more for OPD but positivity was 28/64 (43%) Whereas number of samples from indoor patients for Widal test was less but positivity was more 20/36 (55%). Thus, more Typhoid fever patients Were Present in MICU (22.2%). In the typhifast (immuno chromatography test for detection of Igm antibody test was positive in 42/100 patients were as in same samples Widal test were positive in 48/100 samples .The different in two test could be because, in the typhifast method only Igm antibody are detected. In the typhipoint (EIA for IgG & IgM antibody) test IgM antibody positive in 41.3% samples, IgG 39.30 % .where as widal was positive in 48% samples .the different in positivity reaction could be because of the specific nature (monoclonal antibody IgG & IgM) detected in the EIA method. The overall positivity of the reaction was for Widal test 48%, typhifast 42% & typhipoint IgM. About 41.3% & typhipoint IgG 39.13% though there is little difference in the positivity reaction. It is not satisfactory significant. IgG antibody are not considered as comparison because long-term persistence of the IgG antibody after exposure to typhoid infection or vaccination.

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