QUALITY ASSESSMENT OF WIDAL TEST IN MICROBIOLOGY LABORATORIES AT PRIMARY AND SECONDARY LEVEL BEFORE AND AFTER IMPLEMENTATION OF STANDARD OPERATING PROCEDURE (SOP): A COMPARATIVE STUDY

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Abstract

Context: *The use of standard operating procedures (SOP) in laboratory testing is one of the most crucial factors in achieving the quality. In primary and secondary level laboratory, the scope for microbiological test is limited. Enteric fever is one of the major public health problem in the developing countries, including Bangladesh. An undiagnosed and maltreated case of enteric fever may result in serious complication and even prove fatal. Widal test is the very extensively used serological test in laboratory at all level to aid in the diagnosis of enteric fever. It is the only available practical test for demonstrating antibodies to* Salmonella typhi *and* S. paratyphi. *In this study, an attempt has been made to evaluate the present status of microbiology laboratory by comparing the test results of investigator with that of laboratory staff for widal test at primary and secondary level before and after implementation of standard operating procedure (SOP) .*

Methods: *The present study was performed on clinically suspected cases of enteric fever attending at the primary and secondary level laboratories for widal test. A 120 blood samples were collected before implementing SOP and 50 blood samples were collected and tested after following SOP. A cross sectional, descriptive type of study was conducted in Narsingdi Sador Hospital as secondary level microbiology laboratory and Polash Upzilla Health Complex as primary level microbiology laboratory*

Results: *Before standard operating procedures (SOP), significant titre of widal test was found more by the investigator than the staff at both primary and secondary level. This difference in results was statistically significant ($p<0.05$). After SOP difference in the results of significant titre of widal test between investigator and staff was not statistically significant ($p>0.05$).*

Conclusion: *Implementing SOP for widal test and after practicing appropriate and standard techniques for dilution of serum at primary and secondary level, discrepancy in the results of widal test between investigator and staff was reduced and overall quality of tests were improved.*

Key words : *Standard operating procedures (SOP), widal test, enteric fever, quality assesment.*

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Introduction :

The microbiological test procedures should be incorporated in the standard operating procedure (SOP) to promote safe laboratory practice and to generate reliable, reproducible, and rapid laboratory result[1]. A standard operating procedure is a set of written instructions that document a routine and repetitive activity and describe both technical and administrative as well as operational elements of an organization[2]. The use of standard operating procedures (SOP) in laboratory testing is one of the most crucial factor in achieving the quality[3]. There are several components of a quality system. They should all be in place and operating before the end product of good quality laboratory report is likely to be achieved. Paying excessive

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attention to any one of the component while neglecting others will not achieve improvement in quality[4].

In primary and secondary level laboratory, the scope for microbiological test is limited but routine microscopic examination of urine, stool, malarial parasite in PBF, Gram staining, Ziehl-Neelsen staining of sputum and some serological tests (widal test, ASO titre etc.) are done almost in every laboratories at primary and secondary level[5]. The tertiary level laboratory must carry out the full range of tests required for the curative, preventive and promotive health needs of the community[3]. These tests depend largely on the skill and expertise of the staff and to control the quality of the output, every laboratory must follow the standard operating procedure manuals (SOPMS) in laboratory testing[1].

Enteric fever refers to either typhoid or paratyphoid fever[6]. It is one of the major public health problem in the developing countries, including Bangladesh[7]. Current estimate from the WHO suggest that there are 12 to 21 millions of enteric fever each year with 700,000 deaths[8]. An undiagnosed and maltreated case of enteric fever may result in serious complication and even prove fatal[6]. Widal test is the very extensively used serological test in laboratory at all level to aid in the diagnosis of enteric fever. It is the only available practical test for demonstrating antibodies to *Salmonella typhi* and *S. paratyphi*[9]. Demonstration of causative agents in the blood is considered to be the conclusive test in the diagnosis of the disease[8,10,11].

At present, in Bangladesh, microbiology laboratories at different level usually do not follow any SOP for tests which may be the reasons for variation in test results from laboratories to laboratories for the same test. In this study, an attempt has been made to assess the quality of blood for widal test in some microbiology laboratories at primary and secondary level with the aim to improve the quality of those tests after preparing and implementing SOP for those tests.

Methods:

Clinically suspected cases of enteric fever attending at the primary and secondary level laboratories were selected for widal test. A 120 blood samples were collected before implementing SOP and 50 blood samples were collected and tested after following SOP. A cross sectional, descriptive type of study was conducted in Narsingdi Sadar Hospital (district hospital), Narsingdi, as secondary level microbiology laboratory and Polash Upzilla Health Complex, Narsingdi, as primary level microbiology laboratory. Widal test was done by rapid slide agglutination test (SAT) by using Murex reagent (Murex Biotech Ltd, UK) and following the instruction of the kits. Patients serum was tested for 'O' and 'H' antibodies against *S. typhi* and *S. paratyphi A, B.* The presence of a visible agglutination was related with the presence of the corresponding antibody concentration in the samples. The test was carried out in slide agglutination card.

A) Sample collection techniques:

- Blood for widal test was collected from the study population by the investigator by exactly following SOP both at primary and secondary level.
- Then serum was separated and separated serum was used for widal test in the laboratory individually by the laboratory staff and the investigator.
- Reports were kept in record and these were compared to evaluate the test results at both level.
- Then after preparing and implementing a SOP for widal test for two weeks, blood was collected again by the investigator and was examined in the same way and test results were compared to see any improvement in quality of those tests in those laboratories.

B) Preparation and preservation of serum for Widal test[12,1]:

- Under aseptic precaution, using a 5cc sterile disposable syringe blood was collected in a sterile test tube.

- The test tube containing blood was allowed to clot. Then the tube was centrifuged at 1500 rpm for 15 minutes and serum was separated.
- Serum was transferred carefully to a clear, small vial.
- After labeling, the serum was stored in refrigerator at 2^0c to 8^0c for up to 72hours.
- Serum was preserved in micro tube at -20^0C until use for maximum six months
- Haemolysed or lipaemic samples were discarded.

C) Procedure of Rapid Slide Titration method[4,12]:

1) Using a suitable micropipette, 80, 40, 20, 10 or 5 ml of undiluted serum was taken on to a row of 3 cm diameter circles on a white tile.
2) The bottles containing O and H antigens were shakened well and one drop of the appropriate suspension *(S. typhi* O/H, *S.paratyphi* A, B O/H) was added to each serum aliquot.
3) The serum and antigen was mixed by stirrer and was spreaded over the entire area of the circle.
4) The test card was slowly rotated (at 100 rpm) for 1 minute and observed for any agglutination (clumping) by nacked eye.

D) Possible results:

Macroscopically the pattern of the agglutination were examined. The results were interpreted according to manufactures instructions as follows:

20 ml = 1/80; 10 ml = 1/160; 05 ml = 1/320 and for higher titres, serum was further diluted by equal volume of normal saline.

E) Interpretation[4,13]:

If agglutination was present , the results were interpreted as follows :

- High (e"1:320) or rising titre against O antigen suggested that active infection was present.
- High titre (≥1:320) against H antigen and low titre against O antigen suggested past infection or immunization.
- High titre against H and O antigen (e"1:320) was considered as active infection
- If the titre against TO was ≥1:160, it was considered as significant titre.
- If the titre against TO ≤1:80, it was considered as negative.

F) Quality control:

Each run of the tests were validated with a positive control and negative control.

The results of the study were recorded systematically. Data analysis was done by using computer SPSS programme and according to the objective of the study. Results were presented in the forms of tables. The tests of significance were calculated by using x^2. P value <0.05 was taken as minimum level of significance, P value <0.001 was taken as highly significant. This study was approved by the Ethical Review Committee of Dhaka Medical College, Dhaka.

Results

The results are presented in the following tables:

Table-I shows that before SOP at primary level, the titre of TO ≥1:160 was found in 15(25.00%) cases and TO ≥1:320 was found in 8 (13.33%) cases by the investigator. TO ≥1:160 was found in 13 (21.67%) and TO ≥1:320 was found in 6(10.00%) cases by the staff of primary level laboratory. Similarly, significant titre of TH was observed in 21(35.00%) cases, AH in 4(6.67%) and BH in 1(1.67%) cases by the investigator. At the secondary level, the titre of TO ≥1:160 was found in 16 (26.67%) and a titre of TO ≥1:320 was found in 9(15.00%) cases by the investigator. TO ≥1:160 was found in 14(23.33%) and TO ≥1:320 was found in 6(10.00%) cases by the staff of secondary level laboratory. Significant titre of TH was observed in 19(31.67%) cases, AH in 3(5.00%) and BH in 2(3.33%) cases by the investigator.

Table - I

Results of the widal agglutination titre by the investigator and the staff of primary and secondary level laboratory before SOP

Widal agglutination titre		Primary level (n=60)		Secondary level (n=60)	
		Investigator	Staff	Investigator	Staff
TO	≤1:80	37(61.67)	41(68.33)	35(58.33)	40(66.67)
	≥1:160	15(25.00)	13(21.67)	16(26.67)	14(23.33)
	≥1:320	8 (13.33)	6(10.00)	9(15.00)	6(10.00)
TH	≤1:80	39(65.00)	43(71.67)	41(68.33)	46(76.67)
	≥1:160	12(20.00)	11(18.33)	11(18.33)	10(16.67)
	≥1:320	9(15.00)	6(10.00)	8(13.34)	4(6.66)
AH	≤1:80	56(93.33)	57(95.00)	57(95.00)	58(96.67)
	≥1:160	3(5.00)	3(5.00)	2(3.33)	2(3.33)
	≥1:320	1(1.67)	0(0.00)	1(1.67)	0(0.00)
BH	≤ 1:80	59(93.33)	60(100.00)	58(96.67)	59(98.33)
	≥1:160	1(1.67)	0(0.00)	2(3.33)	1(1.67)
	≥1:320	0(0.00)	0(0.00)	0(0.00)	0(0.00)

Figures in parentheses represent percentage

Table-II shows that before SOP at primary level , significant titre of TO was found in 23(38.33%) cases, significant titre of TH was observed in 21(35.00%) cases, AH in 4(6.67%) and BH in 1(1.67%) cases by the investigator. At secondary level, significant titre of TO was found in 25(41.67%) cases, significant titre of TH was observed in 19(31.67%) cases, AH in 3(5.00%) and BH in 1(3.33%) cases by the investigator.

Table - II

Difference in significant titre of widal test with the investigator and the staff of primary and secondary level laboratory before SOP

Significant titre	Primary level		Secondary level	
	Investigator	Staff	Investigator	Staff
TO	23(38.33)	19(31.67)	25(41.67)	20(33.33)
TH	21(35.00)	17(28.33)	19(31.67)	14(23.33)
AH	4(6.67)	3(5.00)	3(5.00)	2(3.34)
BH	1(1.67)	0(0.00)	2(3.33)	1(1.67)

Figures in parentheses represent percentage

For primary level		**For Secondary level**	
X^2 value	0.819	X^2 value	0.176
df	1	df	1
P value	$<0.05^*$	P value	$<0.05^*$

Table - III shows that after SOP at primary level, the titre of TO e"1:160 was found in 11 (44.00%) and a titre of TO ≤1:320 was found in 2 (6.00%) cases by the investigator. While TO ≥1:160 was found in 10 (40.00%) and TO ≥1:320 was found in 1 (4.00%) cases by the staff of primary level laboratory. Similarly, significant titre of TH was observed in 9 (36.00%) cases, AH in 1 (4.00%) and BH in no cases by the investigator. At secondary level, the titre of TO ≥1:160 was found in 10 (40.00%) and a titre of TO ≥1:320 was found in 2 (8.00%) cases by the investigator. TO ≥1:160 was found in 10 (40.00%) and TO ≥1:320 was found in no case by the staff of secondary level laboratory. Significant titre of TH was observed in 7 (28.00%) cases, AH in no and BH in 1 (4.00%) cases by the investigator.

Table-III

Results of the widal agglutination titre by the investigator and the staff of primary and secondary level laboratory after implementing SOP

Widal agglutination titre		Primary level (n=25)		Secondary level(n=25)	
		Investigator	Staff	Investigator	Staff
TO	≤ 1:80	12(48.00)	14(56.00)	13(52.00)	15(60.000
	≥1:160	11(44.00)	10(40.00)	10(40.00)	10(40.00)
	≥1:320	2 (6.00)	1(4.00)	2(8.00)	0(0.00)
TH	≤ 1:80	16(64.00)	16(64.00)	18 (72.00)	19(76.00)
	≥1:160	7(28.00)	7(28.00)	6(24.00)	6(24.00)
	≥1:320	2(8.00)	2(8.00)	1(4.00)	0(0.00)
AH	≤ 1:80	24(96.00)	24(96.00)	25(100.00)	25(100.00)
	≥1:160	1(4.00)	1(4.00)	0(0.00)	0(0.00)
	≥1:320	0(0.00)	0(0.00)	0(0.00)	0(0.00)
BH	≤ 1:80	25(100.00)	25(100.00)	24(96.00)	24(96.00)
	≥1:160	0(0.00)	0(0.00)	1(4.00)	1(4.00)
	≥1:320	0(0.00)	0(0.00)	0(0.00)	0(0.00)

Figures in parentheses represent percentage

Table- IV shows that after SOP at primary level , significant titre of TO was found in 13 (52.00%) cases, significant titre of TH was observed in 9 (36.00%) cases, AH in 1 (4.00%) and BH in no cases by the investigator. At secondary level, significant titre of TO was found in 12 (48.00%) cases, significant titre of TH was observed in 7 (28.00%) cases, AH in no case and BH in 1 (4.00%) cases by the investigator. Total number of significant titre was found in 23(92.00%) by the investigator and in 21(84.00%) by the staff at primary level. At secondary level, total number of significant titre was found in 20 (80.00%) by the investigator and in 17 (68.00%) by the staff.

Table - IV

Difference in significant titre of widal test with the investigator and the staff of primary and secondary level laboratory after SOP.

	Primary level		Secondary level	
Significant titre	Investigator	Staff	Investigator	Staff
TO	13(52.00)	11(44.00)	12(48.00)	10(40.00)
TH	9(36.00)	9(36.00)	7(28.00)	6(24.00)
AH	1(4.00)	1(4.00)	0(0.00)	0(0.00)
BH	0(0.00)	0(0.00)	1(4.00)	1(4.00)

Figures in parentheses represent percentage

For primary level		**For Secondary level**	
X^2 value	0.076	X^2 value	0.016
df	2	df	2
P value	0.963^{ns}	P value	0.992^{ns}

Table -V

Discrepancy in the results of significant widal agglutination titre before and after implementing SOP by the staff of primary and secondary level laboratory with the investigator.

Significant titre of widal test	Primary level		Secondary level	
	Before SOP (n=60)	After SOP (n=25)	Before SOP (n=60)	After SOP (n=25)
Discrepancy	10(16.67)	2(8.00)	12(20.00)	3(12.00)
No discrepancy	50(83.33)	23(92.00)	48(80.00)	22(88.00)
Total 60(100.00)	25(100.00)	60(100.00)	25(100.00)	

Figures in parentheses represent percentage.

For primary level		**For Secondary level**	
X^2 value	0.495	X^2 value	0.324
df	1	df	1
P value	0.482^{ns}	P value	0.569^{ns}

Table-V, shows the difference of widal test results between investigator and staff of primary and secondary level laboratory before and after following SOP. Before SOP, discrepancy of widal agglutination titre was found in 10 (16.67%) cases and no discrepancy was found in 50 (83.33%) cases with that of investigator at primary level. At secondary level, discrepancy of widal agglutination titre was found in 12 (20%) cases and no discrepancy was found in 48(80%) cases with that of investigator. Discrepancy was reduced to 8% from 16.67% at primary level and to 12% from 20% at secondary level after following SOP for widal test by the laboratory staffs.

Discussion:

Before SOP out of 60 samples for widal test, discrepancy was found in 10(16.67%) cases at primary level and in 12(20.00%) cases at secondary level. After implementing SOP at primary level, out of 30 samples, significant titre of TO was found in 13(52.00%) cases by the investigator and in 11(44.00%) cases by the staff. At secondary level out of 30 samples, discrepancy was found in significant titre of TO in 2(8.00%) cases and TH in one(4.00%) case. After following SOP by the staff, discrepancy was reduced to 8% from 16.67% at primary level and to 12% from 20% at secondary level laboratory. Before SOP, significant titre of widal test was found more by the investigator than the staff at both primary and secondary level. This difference in results was statistically significant ($p<0.05$). After SOP difference in the results of significant titre of widal test between investigator and staff was not statistically significant ($p>0.05$). In both primary and secondary level, discrepancy might be due to the fact that dilution of serum was not done in appropriate and standard method by the staffs, interpretation of widal test was not done in appropriate techniques and absence of direct supervising authority like a qualified microbiologist[3]. It was found that after implementing SOP for widal test and after practicing appropriate and standard techniques for dilution of serum at primary and secondary level, discrepancy in the results of widal test between investigator and staff was reduced and overall quality of tests were improved. Sufficient supply of good quality reagents would also help to reduce discrepancy further.

Conclusion

The use of standard operating procedure (SOP) as practical guideline in laboratory services, aimed at improving the reliability and efficiency in laboratory testing is the backbone of quality health care delivery at primary and secondary levels. Widal test should be done in proper dilution following SOP and should be correctly interpreted. It was found that at all levels, quality of the tests depend not only on the skill and expertise of the laboratory staff but also on the use of SOP for collection, preservation and processing of the sample

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