Original Article

Quality assessment of sputum for Acid Fast Bacilli(AFB) test in microbiology laboratories before and after implementation of Standard Operating Procedure (SOP): a comparative study.

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Abstract

Aims: To evaluate the present status of microbiology laboratory by comparing the test results of investigator with that of laboratory staff for sputum for Acid Fast Bacilli (AFB) withZiehl-NeelsenStaining (Z-N Staining) at primary and secondary level andto assess the present status of a microbiology laboratory for sputum for Acid Fast Bacilli (AFB) withZiehl-NeelsenStaining (Z-N Staining) at tertiary level. ***Material and Methods: Type of study :*** Cross sectional, descriptive type of study. ***Place of study:*** Department of Microbiology of Dhaka Medical College,Narsingdi Sadar Hospital,Narsingdi,PolashUpzilla Health Complex ,Polash, Narsingdi andDOTS centers. ***Duration of study:*** From July, 2007 to June,2008 ***Method of sampling:*** Non probability, purposive and convenient sampling ***Sample Size:*** Sputum for AFB: 300 sputum samples were collected for detection of AFB by direct Z-N staining, Z-N staining after bleach centrifugation and Auramine phenol staining before implementing SOP. After following SOP 150 sputum samples were collected and tested in direct Z-N method. ***Results:*** In the present study, before SOP out of 100 sputum smear at each level, discrepancy was found in 3% cases at primary level, 2% cases at secondary level and 1% case at tertiary level.After following SOP out of 50 sputum smear at each level, discrepancy was reduced to 2% cases at primary level and no discrepancy was found at secondary and tertiary level. ***Conclusion:*** Each laboratory must have SOP for laboratory testing to set the minimum acceptable standard for every test in order to improve and maintain the quality of laboratory services.

Key words: Quality assessment, Sputum for AFB test,Microbiology laboratories, Standard Operating Procedure (SOP).

Introduction

According to International Organization for Standards (ISO), quality is defined as totality of the characteristics of a product or service that make it suitable for the purpose for which it is intended1. In health laboratory services, the product is the report of analysis of the material received by the laboratory for processing i.e. laboratory testing and reporting. The use of standard operating procedures (SOP) in laboratory testing is one of the most crucial factor in achieving the quality[2].

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 results1. 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[3]. SOP must be written and implemented by a qualified laboratory officer and followed exactly by all members of staff. Each SOP must be given a title and identification number and be dated and signed by an authorized person. Essential components of a SOP are as follows[4]:

- Bio-safety precaution,
- disposal of infectious waste,

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- collection, transport and storage of specimens,
- criteria of rejection of samples,
- sample processing,
- sample testing,
- recording of results,
- reporting of results,
- maintenance of equipments,
- sterilization procedure and their control,
- disposal of infectious waste,
- procedure of quality control and
- referral.

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 attention to any one of the component while neglecting others will not achieve improvement in quality[5].

Tuberculosis (TB) has been a grave health problem in Bangladesh and is still one of the biggest killers among infectious diseases. Nearly one- third of the global population i.e. two billion people are infected with Mycobacterium tuberculosis4.In 2006, WHO ranked Bangladesh sixth among the world's 22 high burden TB countries[6]. More than 319,000 new cases, including 143000 sputum smear positive (SS+) pulmonary TB cases and 70,000 TB related deaths occur annually in Bangladesh[7,8]. National TB control programme of Bangladesh (NTP) has began implementing directly observed treatments(DOTS), short-course in 1993. By the end of 2004, the NTP estimated DOTS coverage was 99 percent[6].

The microbiological diagnosis of pulmonary TB by sputum smear microscopy plays a key role in routine and tuberculosis control programme in developing countries[9,10]. Fluorescent method produces brightly fluorescing tubercle bacilli and also increases the positivity of finding of Acid Fast Bacilli (AFB) than Ziehl-Neelsen (Z-N) method specially when they are few in number. But this method is expensive, requires special facilities for microscopy and due to its low specificity all positive smear should be checked by Z-N method[11]. Microscopy of sputum smear by bleach centrifugation methods (with 5% sodium hypochlorite solution) for concentration of the organisms is an important steps in the laboratory diagnosis of PTB using Ziehl-Neelsen staining and significantly increase the positivity of smear negative specimen. Centrifugation of bleach treated sputum increases the positivity of direct microscopy from 43.4% to 76.35% and also reduces the risk of laboratory acquired tuberculosis5. Over night sedimentation of bleach treated sputum also increases the positivity of smear microscopy[9,10].

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 some tests such assputum for AFB in Ziehl-Neelsen staining in some microbiology laboratories at primary, secondary and tertiary level with the aim to improve the quality of those tests after preparing and implementing SOP for those tests. An attempt has also been made to see the efficacy of Z-N method after bleach centrifugation by comparing with the traditional Z-N and Auramine- phenol method with the aim to include it in SOP for better detection of PTB cases at all level.

Materials and Methods

Table-I: Results of Ziehl-Neelsen stained sputum smear examination by the investigator and the staff of primary level dots centre before and after SOP.

	Before SOP (n=100)		After SOP (n=50)	
Microscopy outcome	**Investigator**	**Staff**	**Investigator**	**Staff**
Smear positive	14 (14.00)	11(11.00)	8(16.00)	7(14.00)
Smear negative	86 (86.00)	89 (89.00)	42(84.00)	43(86.00)
Total	100(100.00)	100(100.00)	50(100.00)	50(100.00)
Figures in parentheses represent percentage				
Before SOPAfter SOP				
	X^2 value	0.183	X^2 value	0.078
	Df	1	df	1
	P value	0.669^{ns}	P value	0.779^{ns}

Table-II: Results of Ziehl-Neelsen stained sputum smear examination by the investigator and the staff of secondary level dots centre before and after SOP.

	Before SOP (n=100)		After SOP (n=50)	
Microscopy outcome	**Investigator**	**Staff**	**Investigator**	**Staff**
Smear positive	13(13.00)	11(11.00)	7(14.00)	7(14.00)
Smear negative	87 (87.00)	89(89.00)	43(86.00)	43(86.00)
Total	100(100.00)	100(100.00)	50(100.00)	50(100.00)
Figures in parentheses represent percentage				
Before SOPAfter SOP				
	X^2 value	0.047	X^2 value	0.000
	Df	1	df	1
	P value	0.828^{ns}	P value	1.000^{ns}

Table-III: Results of Ziehl-Neelsen stained sputum smear examination by the investigator and the staff of tertiary level dots centre before and after SOP .

	Before SOP (n=100)		After SOP (n=50)	
Microscopy outcome	**Investigator**	**Staff**	**Investigator**	**Staff**
Smear positive	15 (15.00)	14(14.00)	7(14.00)	7(14.00)
Smear negative	85 (85.00)	86(86.00)	43(86.00)	43(86.00)
Total	100(100.00)	100(100.00)	50(100.00)	50(100.00)
Figures in parentheses represent percentage				
Before SOPAfter SOP				
	X^2 value	0.040	X^2 value	0.000
	Df	1	df	1
	P value	0.841^{ns}	P value	1.000^{ns}

Table-IV: Discrepancy in result of sputum smear examination in direct Z-N method by the investigator and staff of dots centre at primary, secondary and tertiary level before and after SOP.

Microscopy outcome	Primary level		Secondary level		Tertiary level	
	Before SOP	**After SOP**	**Before SOP**	**After SOP**	**Before SOP**	**After SOP**
Discrepancy	3(3.00)	1(2.00)	2(2.00)	0(0.00)	1(1.00)	0(0.00)
No discrepancy	97(97.00)	49(98.00)	98(98.00)	50(100.00)	99(99.00)	50(100.00)
Total	100(100)	50(100)	100(100)	50(100)	100(100)	50(100)
Figures in parentheses represent percentage						
Primary levelSecondary levelTertiary level						
	X^2 value	0.128	X^2 value	0.063	X^2 value	0.503
	Df	1	df	1	df	1
	P value	0.720^{ns}	P value	0.801^{ns}	P value	0.478^{ns}

Type of study : Cross sectional, descriptive type of study.

Place of study: Department of Microbiology of Dhaka Medical Collegeas tertiary level laboratory,NarsingdiSador Hospital as secondary level microbiology laboratory,PolashUpzilla Health Complex as primary level microbiology laboratoryand DOTS center of primary, secondary and tertiary level.

Duration of study: From July, 2007 to June,2008

Method of sampling:Non probability, purposive and convenient sampling

Ethical Issue: This study was approved by the Ethical Review Committee of Dhaka Medical College, Dhaka.

Sample Size: Sputum for AFB: 300 sputum samples were collected for detection of AFB by direct Z-N staining, Z-N staining after bleach centrifugation and Auramine phenol staining before implementing SOP. After following SOP 150 sputum samples were collected and tested in direct Z-N method.

Study Population

Inclusion criteria:Sputum specimens were collected from new tuberculosis suspects attending the dots

center at primary, secondary and tertiary level with history of cough with or without blood for more than three weeks.
Exclusion criteria:Patients who had been taking anti tubercular treatment or treatment failure cases of tuberculosis

Table-V: Results of sputum smear examination by direct Ziehl-Neelsen method, Fluorescent method (FM) and Ziehl-Neelsen method after bleach centrifugation by the investigator (n=300).

Method of sputum smear examination	Smear positive	Smear negative
Direct Z-N method	42(14.00)	258(86.00)
Direct FM method	46(15.33)	254(84.67)
Z-N method after bleach centrifugation	58(19.33)	242(80.67)

Figures in parentheses represent percentage
X^2 value 1.700, df 1, P value <0.01**

Results

Table-I shows the results of Ziehl-Neelsen stained sputum smear examination by the investigator and the staff of primary level dots centre. Before SOP, out of 100 sputum smear prepared from new TB suspects at primary level laboratory, 14 were smear positive and 86 were smear negative by the investigator. When the same samples were examined by the staff, [11] were smear positive and 89 were smear negative. After following SOP,8 (n=50, 16.00%) were smear positive by the investigator and 7(n=50, 14.00%) were smear positive by the staff.

Table-II shows the results of Ziehl-Neelsenstained sputum smear examination by investigator and staff of dots centre at secondary level . before SOP, out of 100 sputum smear prepared from new TB suspects at secondary level laboratory, 13 were smear positive and 87 were smear negative by the investigator. But incase of staff, 11 were smear positive and 89 were smear negative. After following SOP, 7 (n=50, 14.00%) were smear positive by both by the investigator and the staff.

Table-III shows the results of Ziehl-Neelsenstained sputum smear examination by the investigator and the staff of tertiary level dots centre. Before SOP, out of 100 sputum smear prepared from new TB suspects at tertiary level laboratory, 15 were smear positive and 85 were smear negative by the investigator. When examined by the staff, 14 were smear positive and 86 were smear negative. After following SOP, 7 (n=50, 14.00%) were smear positive by both by the investigator and by the staff.

Table-IV shows that in the results of smear microscopy in direct Ziehl-Neelsen method, discrepancy was found in 3 cases at primary level, 2 cases at secondary level and 1 case at tertiary level before SOP. After SOP, discrepancy was reduced to only one cases at primary level and no discrepancy was found at secondary and tertiary level.

Table-V shows that out of 300 sputum smear at all level, 42(14%) were smear positive by Ziehl-Neelsen method, 46(15.33%) were smear positive by fluorescence microscopy and 58(19.33%) were smear positive by Ziehl-Neelsen method after bleach centrifugation by the investigator.

Discussion:

The role of diagnostic medical laboratories in saving lives is well established today. These laboratories with their timely and correct reporting aid the physicians in their treatment. A well equipped and properly managed diagnostic laboratory is the very soul of correct diagnosis, prognosis and monitoring of both communicable and non-communicable diseases1. Improvement in the quality of laboratories can be done by the application of good laboratory practices and to establish good laboratory practice a standard operating procedure manual(SOPM) is essentia[12].

This cross sectional study was done to assess the quality of some tests in microbiology laboratories at primary, secondary and tertiary level with the aims to improve the quality of those tests in those laboratories after preparing and implementing SOP for those tests. Specimens were collected and evaluation and re-evaluation test were done before and after implementing SOP at all level to see the performance of the laboratories regarding those tests. In the present study, it was tried to see the extent of difference in results between investigator and staff of primary, secondary and tertiary level laboratory before and after implementing SOP. If there was any difference in results between the investigator and the staff then it was considered as discrepancy.
In the present study, it was found that at primary level before SOP, out of 100 sputum smear prepared from new TB suspects and stained with Ziehl-Neelsen staining, 14(14%) were smear positive by the investigator and 11(11%) were smear positive by the staff of primary level dots centre. At secondary level out of 100 sputum smear, 13(13%) were smear

positive by the investigator and 11(11%) were smear positive by the staff of secondary level DOTScentre before SOP . Similarly out of 100 sputum smear stained with Ziehl-Neelsen staining, 15(15%) were smear positive by the investigator and 14(14%) were smear positive by the staff of tertiary level dots centre. In the present study, number of smear positivity was found more by the investigator than the staff of DOTScentre but this difference in results was not statistically significant.

In the present study, before SOP out of 100 sputum smear at each level, discrepancy was found in 3(3%) cases at primary level, 2(2%) cases at secondary level and one (1%) case at tertiary level.After following SOP out of 50 sputum smear at each level, discrepancy was reduced to one (2%) cases at primary level and no discrepancy was found at secondary and tertiary level .

Before SOP, discrepancy in smear positivity might be due to over burden of new TB suspects and follow up TB patients attending the DOTScentre of all level for sputum examination. Due to this higher rate of patients attendance, procedure of early morning sputum collection technique and its importance could not be properly explained to the patients by the staff. Also the processing the sample for smear preparation, staining of the smear by Ziehl-Neelsen method and smear microscopy was not done properly following SOP by the staff. After implementing SOP for sputum sample collection, processing, smear preparation, staining of the smear in Ziehl-Neelsen method and smear microscopy for AFB, there was significant improvement in the performance of sputum smear examination by the staff and discrepancy between the investigator and the staff was reduced.

In the present study, it was found that out of 300 sputum examined by the investigator, 42(14%) were positive by direct Ziehl-Neelsen method, 46(15.33%) were positive by fluorescence technique and 58(19.33%) were positive by Ziehl-Neelsen method after bleach centrifugation. The difference in results of sputum smear examination in direct Z-N method and Z-N method after bleach centrifugation is statistically significant ($p< 0.05$). In contrast to the findings of the present study, Matuet al.,(2008) from Kenya Medical Research Institute reported that culture positive cases of tuberculosis were 54%, smear positive by direct Z-N method was 37.2%, direct FM positive was 44.6% and smear positive by Z-N method after bleach centrifugation techniques was 46.20%. The higher rate of smear positivity in that study might be due to the fact that, prevalence of tuberculosis is higher in Kenya.

From the present study, it might be concluded that, 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, secondary and tertiary level. Every laboratory must follow standard operating procedure (SOP) manuals in laboratory testing. Proper training of the staffs of primary, secondary and tertiary level laboratory should be done periodically and they should be instructed to follow the SOP strictly under the super vision of a qualified microbiologist. If sputum smear microscopy for AFB can be done in Ziehl-neelsen staining after bleach centrifugation at all level laboratories, more smear positive PTB case detection can be possible as culture of M tuberculosis is only feasible at few reference laboratories at tertiary level. Lastly, one of the limitation of the study should be mentioned. Even after extensive and vigorous search, sufficient number of literature was not available on similar type of study. Hence some of the variables of the present study could not be compared with others adequately.

Conclusion

In the present study it was observed that each laboratory must have SOP for laboratory testing to set the minimum acceptable standard for every test in order to improve and maintain the quality of laboratory services. It was found that at all level, quality of the tests depend not only on the skill and expertise of the laboratory staff but also on the strict follow up of SOP for proper collection, preservation, processing of the sample. If the staffs could be given adequate training periodically and being continuously monitored by qualified supervising authority to strictly follow the SOP then the quality of the test would also improve. Sufficient supply of good quality reagents might help in further improvement in the quality of the tests. Sputum smear microscopy in Ziehl-Neelsen method could be done after bleach centrifugation at all level of microbiology laboratories for better isolation of PTB cases.

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