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Critical evaluation of quality assurance in laboratory diagnosis of tuberculosis in selected nearby microscopic centers under RNTCP

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ABSTRACT

Objective: RNTCP relies on sputum smear microscopy for diagnosis, categorization of patients for treatment and assessment of their program. Therefore, it is crucial that the smear microscopy services provided are of highest quality possible. The current study is undertaken to do on site evaluation and Random blinded rechecking (RBRC) of slides at selected microscopic centers.

Material & Methods: Five microscopic centers were selected for onsite evaluation and Random Blinded rechecking. Slides were collected monthly from the respective DMCs. A questionnaire was developed to assess the overall operational conditions at the DMCs and a checklist was prepared to record the observation during the visit. RBRC slides were read by two microbiologists independently and results were compared with RNTCP results. Slides were read before and after restaining the slides.

Results: After the evaluation of checklist and questionnaire, it was found that 100% centers were following the charts for smear preparation, staining and grading with adequate stock supply. One out of 5 centers had maximum number of slides with poor quality of smear (16.7%), 8% uneven smear and 14% slides with improper thickness. There was 100% concordance when reading five positive and five negative smears. The mean time spent on microscopic examination was 4.4 minutes, compared with recommended time of 10 minutes. Out of 828 slides rechecked under RBRC one low false negative error was found.

Conclusion: The evaluation of quality control practices was found satisfactory. The laboratory staff was able to incorporate simple quality control procedures for AFB microscopy into their routine practice, resulting in reliable service. Onsite evaluation and RBRC are viable measures of laboratory performance and both should be continued.

Keywords: Sputum smear, Quality control, Tuberculosis, RNTCP, RBRC

INTRODUCTION

Quality is the degree of congruence between expectation and realization. Quality means meeting the pre-determined requirements of the users for a particular substance or service. Quality assurance (QA) is a wide-ranging concept covering all matters that individually or collectively influence the quality of a product. It denotes a system for continuously improving reliability, efficiency, and utilization of products and services. A quality assurance program is concerned with sampling, specifications and testing as well as with organization, documentation and release procedures that ensure satisfactory quality [1]. RNTCP relies on sputum smear microscopy for diagnosis, categorization of patients for treatment and assessment of their program. Therefore, it is crucial that the smear microscopy services provided are of highest quality possible. Reliable laboratory results are essential not just for diagnosis of patients, but also for proper categorization to follow their progress during treatment, including keeping them informed of the decision to start the continuation phase and to declare them cured at the end of treatment. If laboratory diagnosis is unreliable, microscopy errors can result in unnecessary treatment for non-TB cases, or in failure to detect persons with infectious TB, who will then continue to spread infection in the community. Errors in reading the follow-up smears can result in patients being placed on prolonged treatment or re-treatment, or in treatment being discontinued prematurely [2].QA is a total system consisting of internal quality control (QC), assessment of performance using external quality assessment (EQA) methods, and continuous quality improvement (QI) of laboratory services [3]. Total quality management means that every variable that could possibly affect the quality of the test results has been controlled. This ideal situation may not be possible, but a level of quality assurance that can control most of the factors that are likely to affect the test results can be attained. The current study is undertaken to check the Pre-analytic, analytic and post-analytic factors which influence the quality of the laboratory results.

MATERIALS AND METHODS

The study was conducted at Department of Microbiology, Lady Hardinge Medical College (LHMC) & associated Hospitals, New Delhi for a period of 15 months from October 2006 to December 2007 at the 5 nearby microscopic centers. The parameters evaluated in the study at the above centers were On Site Evaluation and RBRC of slide.

On Site Evaluation

A total of 30 visits were carried out during the study period, bimonthly. A questionnaire was developed to assess the overall operational conditions at the DMCs. A checklist was prepared to record the observation during the visit and to analyze the data to propose the corrective action to monitor improvements and it was filled at each visit. These study tools were pretested by visiting two microscopic centers and were modified accordingly. The study was carried out through personal interviews and interaction with the staff present at the center. Things assessed with the help of study tools were Infrastructure, Adequacy of supplies of reagents and other equipments, the condition of equipments, procedure of sputum collection and making smears, staining of smears and reading of smears, laboratory safety, and infection control measures, method of treatment and disposal of waste, handling of specimen, sharps, spillages, Stock registers and Workload. Five positive and five negative slides selected by systematic random sampling were examined in an unblinded manner. These slides were checked for its quality, size of smear, thickness, evenness of smear. Any error found on crosschecking was noted and LTs, STLS were also informed. Availability of control slides, both positive and negative, was also checked. Checklist was filled at the end of each visit.

RBRC

Slides were collected monthly from the respective DMCs. Slides for RBRC were selected by Lot Quality Assurance Sampling (LQAS) method[4]. After calculating the monthly sample size for each DMC, slides were selected by the STLS of the Chest Clinic and were first sent to District level laboratory for RBRC. After their evaluation the slides were collected from the respective DOTS centers. These slides were

read and then were cleaned with xylene and then were restained by Ziehl Neelsen staining method and were read again. The Senior Microbiologist rechecked slides. Reading of slides was done in a blinded fashion. Results of their RBRC were taken after reading the restained slides. Discrepancies were calculated for the positive and negative slides before and after restaining and the results were compared.

RESULTS

On Site Evaluation

A field visit is an ideal way to obtain a realistic assessment of the conditions and skills practiced in the DMC. At first visit Infrastructure of the Laboratory was assessed and evaluated in subsequent visits for any change or renovation. It was observed that 3 out of 5(60%)DMCs had infrastructure as per RNTCP guidelines. 100% centers had display of charts for smear preparation, staining and grading with adequate stock supply. It was observed that LTs do not accompany the patients while collection of sample. One out of 5 centers had maximum number of slides with poor quality of smear (16.7%), 8% uneven smear and 14% slides with improper thickness on reading slides during OSE at each center (**Table 1**).

Table 1 Result of reading 5 positive & 5 negative slides at the participatory centers during the bimonthly visits. Total number of slides observed (n) =300

Slide observed for	NDMC	LHMC	RML	CPH	JCC
Slide Quality					
Good (>10WBC/field):	277 (92.3%)	264 (88%)	258 (86%)	250 (83.3%	252 (84%)
Poor (<10WBC/field):	23 (7.7%)	36 (12%)	42 (14%)	50 (16.7%)	48 (16%)
Size of Smear					
2x3cm	298 (99.4%)	299 (99.7%)	298 (99.4%)	300(100%)	300(100%)
>/<2x3cm	2 (0.6%)	1 (0.3%)	2 (0.6%)	0	0
Staining					
Proper	300 (100%)	288 (96%)	300 (100%)	300(100%)	300(100%)
Not proper	0	12 (4%)	0	0	0
Thickness of Smear					
Proper thickness:	263 (87.6%)	269 (89.7%)	268 (89.3%)	258 (86%)	270 (90%)
Thick/thin smear:	37 (12.3%)	31 (10.3%)	32 (10.7%)	42 (14%)	30 (10%)
Evenness of smear					
Even:	282 (94%)	281 (93.7 %)	287 (95.7%)	276 (2%)	285 (95%)
Uneven:	18 (6%)	19 (6.3 %)	13 (4.3%)	24 (8%)	15 (5%)

There was 100% concordance when reading five positive and five negative smears. Sample collection, waste disposal and Internal Quality Control practices were not proper at any of the centers. Control smears were put only for fresh batch of reagents and not daily. The working hours for each DMC were calculated. The mean time spent on microscopic examination was 4.4 minutes, compared with recommended time of 10 minutes. A total of 828 slides were collected during the study period. The combined result of the RBRC of all 5 DMCs is shown in **Table 2**. All 828 slides were rechecked. 827 slides were correct. One slide that was read negative by LT was found to be scanty positive (2 bacilli). It was a Low false negative error which is a minor error. The results of RBRC were compared before and after restaining. No discrepancy was found after restaining. Two microbiologists read all slides before

and after restaining and the results were same. Both the Microbiologists read the slides in a blinded fashion. Results were compared at all the centers

Table 2 RBRC results of all 5 DMCs

Results of LTs	Result by Microbiologist				
	Negative (746)	Scanty (6)	1+ (34)	2+ (17)	3+ (25)
Negative (747)	Correct (746)	**LFN (1)**	-	-	-
Scanty (5)	-	Correct (5)	-	-	-
1+ (34)	-	-	Correct (34)	-	-
2+ (17)	-	-	-	Correct (17)	-
3+ (25)	-	-	-	-	Correct (25)

DISCUSSION

On Site Evaluation

Total quality management means that every variable that could possibly affect the quality of test results has been controlled. This ideal situation may not be possible, but a level of quality assurance that can control most of the factors that are likely to affect the test results can be attained. In present study, we evaluated the infrastructure of the laboratory. It was found that only 3 out of 5 centers had infrastructure according to RNTCP guidelines.

However, we could not establish any relation of deficient infrastructure with quality assurance. The overall process of specimen collection, labeling, smear preparation and staining was satisfactory at all the centers. Mundy et al evaluated sputum smear microscopy in 2002 [5], they found that LTs were good in specimen processing and the performance that was found poor in the first month, improved considerably as a result of monitoring activities established by the authors. In present study, it was found that for sputum sample collection, patients are not accompanied by the LTs.

According to RNTCP guidelines, patients should be explained the procedure of sputum collection and should be accompanied by the LTs [3, 6]. It was noted that area for making smear on the slide was not marked by the LTs at any of the center. Marking the smear area on the slide is a good practice for making a slide as it helps to know where exactly the smear has been made and the correct size can be standardize and will be easier for the other reader to read the slide who has not made the smear. Proper selection, use and storage of reagents are a vital part of a good QA program.

In the present study supply of all the chemical reagents and equipments in relation to AFB microscopy from the State TB Office to all the centers was regular and stock of 3 months was available. All the study centers had one Binocular microscope in a good working condition and all were found to be covered by AMCs. The need for functioning microscopes and provision for their maintenance has previously been reported [7]. The present study found that waste disposal was not proper at any of the centers. However no relation was established with quality assurance due to poor management of waste. Overall workload of the laboratory was calculated for each DMC (**Table 4**).

Table 3 Method of waste management/disposal observed at the participatory centers during the bimonthly visits. (n=30)

S. No.	Method of waste management/disposal	NDMC Y (%)	LHMC Y (%)	RML Y (%)	CPH Y (%)	JCC Y (%)
1	Waste container with Lid	30(100)	30(100)	30(100)	30(100)	**30(100)**
2	Waste disposal					
	Pretreatment					
	• Disinfection	30(100)	30(100)	30(100)	30(100)	**30(100)**
	• Autoclave	30(100)	0(0)	0(0)	0(0)	**30(100)**
	Disposal					
	• Broom sticks and sputum cups					
	▪ Incinerator	0(0)	30(100)	30(100)	30(100)	**0 (0)**
	▪ Out-source agency	30(100)	0(0)	0(0)	0(0)	**30(100)**
	• Slides Handed over to outsource agency	30(100)	30(100)	30(100)	30(100)	**30(100)**
3	General cleanliness Satisfactory	30(100)	30(100)	30(100)	30(100)	**30(100)**
4	Safety Practices					
	Using gloves	6(20)	6(20)	6(20)	6(20)	**0(0)**
	Using aprons	30(100)	30(100)	30(100)	30(100)	**0(0)**

Y-No. of visits showing compliance

Table 4 Working hours calculated for each DMC

	NDMC	LHMC	RML	CPH	JCC
Total no. of slides stained in 15 months	5296	7881	5953	6947	8339
Total no. of working days	368	368	368	368	368
Number of slides stained /day	14	21	16	19	23
Time taken by LT for					
Entry in the register	42min	63min	48min	57min	69min
Sputum sample collection	84 min	126min	96min	114min	138min
Labeling slide	2.5min	3.5min	2.5min	3min	4min
Staining all the slides	25min	35min	30min	30min	40min
Reading all slides	60min	90min	60min	95min	115min
Reporting in register	14min	25min	16min	20min	25min
Tea time	15min	15min	10min	15min	20min
Lunch time	30min	40min	60min	60min	60min
Waste disposal	15min	15min	15min	15min	15min
Total time	287.5min (4.7hours)	412.5min (6.8hours)	337.5min (5.6hours)	409min (6.8hours)	486min (8.1hours)

It was observed in the study that all DMCs had sufficient staff and working hours to perform their routine laboratory work except one. Study by Mundy et al in 2002 [5] compared the actual times for processing specimens with recommended times. Although the microscopy results were good, staff spent approximately 4 minutes examining each smear instead of the recommended 10 minutes for negative smears. Because of the overall high laboratory workload, it is not feasible for district laboratory staff in Malawi[5] to spend 10 minutes examining 200 fields in every negative smear. They concluded that examination of 100 fields was adequate and formally reduced the recommended number of fields to 100 as a result of this study. The present study found that screening the slides for 5 minutes is sufficient for 100 fields but it take more time to read a negative or scanty grade slide because they have to be screened twice.

As a part of OSE, five positive and five negative slides selected systematically from the RNTCP TB laboratory register were checked for overall performance of LTs. In the present study, a total of 300 slides during 15-month study period were rechecked in an unblinded fashion (Table 1). There was no discrepancy found in reading the slides by the LTs. This was in concordance to other studies[8, 9, 10]. It can be argued that the high agreement could have been due to the availability of the smear results for the STLS before rereading[10].

RBRC

RBRC is a method of external quality assurance. The primary function of quality assurance should be to identify laboratory in need of additional training and supervision to improve the quality of initial reading rather than identification of slide errors[9, 10]. The aim of AFB microscopy quality assurance rechecking should be to identify center or individuals with unsatisfactory performance due to technical problem may be related to stains and staining technique and these may remain undetectable even to the best controller if rereading is not performed prior to rereading. For this reason, systematic restaining of all slides before rechecking has been recommended[11,12].

A single error should be considered as a warning of possible problems and requires further evaluation[3].The sample size is determined by the LQAS method[3]. The lot quality assurance sampling methodology, developed in industry for quality control, is increasingly adopted for use in public health service settings[4, 13, 14]. In the present study, a total of 828 slides selected by LQAS method were examined. The result of 827 slides was in concordance with the result of LTs. One slide that was read negative by LT was found scanty grade positive (Table 2). This was a low false negative error and is considered a minor error. The feedback of the results to the LTs is a major component of RBRC in improving their technical efficiency. In the present study, it was observed that LTs always try to improve their microscopy after getting the feedback of RBRC.

The implication of our study is that rechecking of slides is important for the assessment of laboratory technicians as it always motivates LTs to improve their performance. Recent reports reveal that poor storage conditions result in fading of the basic fuchsin color of AFB in sputum smears[15, 16, 17]. This indicates that the restaining of the slides before rereading would be useful to assess false-positive errors more precisely[18]. Results of present study are not in accordance with other studies. Practice of keeping slides in the slide boxes by the LTs in the present study may be a reason that slides were not faded as seen in other studies. Kantor et al[17] in 2000 concluded that recommendation of restaining sputum smears prior to rereading is questionable. This would need much additional work for implementation.

The present study observed that restaining and reading of slides under RBRC increases the workload of the LTs. Moreover, no discrepancies were found before and after restaining .We are of the opinion that restaining of slides is not required.

CONCLUSION

Laboratories should be renovated according to RNTCP guidelines. LTs should be motivated to accompany the patients during the sample collection and to give proper instructions to patients. Five

minutes time is enough to read the 100 fields in a smear. LTs should be trained in BMWM during their training period. Rechecking of 5 +ve and 5 -ve slides during OSE and RBRC are good parameters for QA and should be continued.

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REFERENCES

1. Kumari S, Bhatia R, Heuck CC. Quality Assurance in Bacteriology and Immunology. WHO regional Publication, South-East Asia Series No.28.WHO, Regional Office for South-East Asia, New Delhi. 1998.
2. Agarwal SP, Mundade Y, Chauhan LS. Quality Assurance of sputum microscopy under RNTCP.Chapter 17 Pg 155-163.
3. Revised National Tuberculosis Control Program Laboratory Network. Guidelines for Quality assurance of smear microscopy for diagnosing tuberculosis. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi. April 2005
4. Selvakumar N, Prabhakaran E, Murty B.N, Sivagamasundari S, Vasanthan S, Govindaraju R, Perumal M, Wares F, Chauhan T, Santha T, Narayanan P.R. Lot quality assurance sampling of sputum acid-fast bacillus smears for assessing sputum smear microscopy centers; *J clin Microbiol2005*; 43(2):913-915.
5. Mundy C.J.F, Harries A.D, Banerjee A, Salaniponi F.M, Gilks C.F, Squire S.B. Quality assessment of sputum transportation, smear preparation and AFB microscopy in a rural district in Malawi; *Int J Tuberc Lung Dis 2002*; 6(1): 47-54.
6. Managing the Revised National Tuberculosis Control Program in your area. Modules 1-4. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi. April 2005.
7. Mundy C, Ngwira M, Kadewele G, Bates I, Squire B, Gilks C. Evaluation of microscope condition in Malawi. *Trans Roy SocTrop Med Hyg 2000*; 94: 583–584.
8. Fujiki A, Giango C, Endo S. Quality control of sputum smear examination in cebu province. *Int J Tuberc Lung Dis.2002*; 6(1):39-46.
9. Nguyen TNL, Wells CD, Binkin NJ, Pham DL, Nguyen VC. The importance of quality control of sputum smear microscopy: the effect of reading errors on treatment decisions and outcomes. *Int J Tuberc Lung Dis.1999*; 3(6):483-487.
10. Selvakumar N, Prabhakaran E, Rahman F, Chandu NA, Srinivasan S, Santha T et al. Blinded rechecking of sputum smears for acid-fast bacilli to ensure the quality and usefulness of restaining smears to assess false-positive errors. *Int J Tuberc Lung Dis.2003*; 7(11):1077-1082.
11. Revised National Tuberculosis Control Program. Environmental and Bio-medical waste Management plan for RNTCP-II. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi. May 2005.
12. Martinez A, Balandrano S, Parissi A, Zuniga A, Sanchez M, Ridderhof J et al. Evaluation of new external quality assessment guidelines involving random blinded rechecking of acid-fast bacilli smears in a pilot project setting in Mexico. *Int J Tuberc Lung Dis.2005*; 9(3):301-305.
13. Selvakumar N, Prabhakaran E, Murty B.N, Sivagamasundari S, Vasanthan S, Govindaraju R, Perumal M, Wares F, Chauhan T, Santha T, Narayanan P.R. Application of lot sampling of sputum AFB smears for the assessment of microscopy centres; *Int J Tuberc Lung Dis.2005*;9(3):306-309.
14. Murthy BN, Radhakrishna S, Venkatasubramanian S, et al. Lot quality assurance sampling for monitoring immunization coverage in Madras City. *Indian Pediatr 1999*; 36: 555-559.
15. Deun AV, Chambuganj N, Hye A, Hossain A. Rapid fading of carbol fuchsin stained AFB smears under extreme conditions of temperature and humidity. *Int J Tuberc Lung Dis.1997*; 1(4):384-386.
16. Deun AV, Roorda FA, Chambuganj N, Hye A, Hossain A. Reproducibility of sputum smear examination for acid-fast bacilli: practical problems met during cross-checking. *Int J Tuberc Lung Dis.1999*; 3(9):823 -829.

17. Kantor IN, Laszlo A, Vazquez L et al. Periphery to center quality control of sputum microscopy and rapid fading of ziehl- nelson staining. *Int J Tuberc Lung Dis.2000*;4(9):885-889.

18. Managing the Revised National Tuberculosis Control Program in your area. Modules5-9. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare,Nirman Bhawan, New Delhi. April 2005.