Original Article

PROFICIENCY TO READ SPUTUM AFB SMEARS BY SENIOR TUBERCULOSIS LABORATORY SUPERVISORS UNDER TRAINING AT A REFERENCE LABORATORY IN INDIA

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(Received on 11.5.2004; Accepted on 3.8.2004)

Summary:
Background: A national reference laboratory imparting training on sputum AFB smear microscopy to fresh Senior Tuberculosis Laboratory Supervisors (STLS).
Aim: To assess the proficiency of STLSs under training to read sputum AFB smears.
Methods: Each of 342 trainees read the same set of 15 to 20 Ziehl Neelsen stained smears in a blinded fashion on day–1 and on day-15 of the training programme. The smear results were matched with the original results.
Observations: The sensitivity, specificity, positive predictive value and negative predictive value of smear reading were 75%, 88%, 93%, 63% and 94%, 99%, 99%, 89% respectively on day-1 and day –15.
Conclusion: The sensitivity to read sputum AFB smears by fresh STLSs with little or no experience increased from 75% to 94% during the carefully planned training programme; the specificity increased from 88% to 99%. The study highlights the importance of training in improving the microscopy results. ***[Indian J Tuberc 2005; 52:11-14]***

Key words: Pulmonary Tuberculosis, STLS, AFB sputum smears

INTRODUCTION

Thousands of laboratory technicians are being trained in sputum AFB microscopy, all over the world, in order to improve the quality of diagnostic services for TB patients. The results of sputum AFB microscopy are known to be influenced by various factors including the proficiency to read smears by microscopists[1]. With the inherent limitations of sputum AFB microscopy, the highest sensitivity achievable by microscopists in reading smears can be 95%. Several documents recommend the need for training the laboratory technicians to improve the quality of sputum AFB microscopy[1,2]. However, there is no documented evidence on the performance of fresh trainees in a training programme. The present study documents the proficiency to read AFB sputum smears by STLSs under training at Tuberculosis Research Centre (TRC), a national reference laboratory.

MATERIAL AND METHODS

Trainees and material

A total of 342 STLSs were trained in 42 batches with an average of 8 per batch and most of them were new staff holding diploma in medical laboratory technology. A fixed schedule for 15 working days and modules were used for the participatory training[3,4]. Each trainee had access to a binocular microscope (Olympus, CH-30, Japan) during training period.

Panel slides

Direct sputum smears were prepared from sputum samples collected from pulmonary tuberculosis patients attending TRC Clinic. They were stained by hot Ziehl Neelsen staining method and read by TRC laboratory technicians. Before the

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start of training programme, a senior laboratory technician at the reference laboratory selected the required number of smears and arranged them in sets of 15 to 20 slides each. A total of 4870 slides, which included 3258 (67%) positive slides with different grades, were used.

Proficiency in reading

On day-1, each trainee was given a set of smears for reading. The facilitators collected the smear results in a sheet. On day-15 of the training, each trainee again examined the same set of slides. Trainees were not told about the identity of the smears and were blinded to the smear results. Care was taken to prevent bias in reading by collecting the results in sheets immediately.

Analysis of data

For 283 participants, the smear results were available both at the start and at the end of the training and were included in the analysis. The errors as defined in the International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines were identified[1]. The percentages of different types of errors were calculated for smears read on day-1 and day-15. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of smear reading by trainees were calculated. The agreement in reading between the trainees and the reference readings was studied using kappa statistics.

RESULTS

Comparison of results of smear read on day-1 and day-15 with the reference results is shown in Table 1. On day-1, the sensitivity, specificity, PPV and NPV of reading smears were 75%, 88%, 93% and 63% respectively. The agreement of smear results read on day-1 with the reference readings was poor (kappa = 0.57).

Table 1: Comparison of results of the same smears read on day-1 and day-15 by trainees with reference results

Read on	Results from Trainees *	Reference results *						
		Scanty	**1+**	**2+**	**3+**	**All Positive**	**Negative**	**Total**
Day-1	Scanty	119	322	71	9	521	65	586
	1+	63	430	307	45	845	74	919
	2+	6	89	244	116	455	16	471
	3+	2	34	191	384	611	36	647
	All Positive	190	875	813	554	2432	191	2623
	Negative	261	432	113	20	826	1421	2247
	Total	**451**	**1307**	**926**	**574**	**3258**	**1612**	**4870**
Day-15	Scanty	289	405	54	3	751	23	774
	1+	62	636	317	35	1050	1	1051
	2+	2	140	334	131	607	0	607
	3+	1	27	214	405	647	0	647
	All Positive	354	1208	919	574	3055	24	3079
	Negative	97	99	7	0	203	1588	1791
	Total	**451**	**1307**	**926**	**574**	**3258**	**1612**	**4870**

* Neg = no AFB in 100 fields; scanty = 1-9 AFB in 100 fields; 1+ = 10 - 99 AFB in 100 fields; 2+ = 1 to 9 AFB per field in at least 50 fields; 3+ = more than 10 AFB per field in at least 20 fields.

Table 2: Percentages and types of errors for trainees on day-1 and day-15

	Major errors		Minor errors		
Read on	High false-positive	High false-negative	Low false-positive	Low false-negative	Quantification error
Day-1	8% (126/1612)	20% (565/2807)	4% (65/1612)	58% (261/451)	5% (167/3258)
Day-15	0.06% (1/1612)	4% (106/2807)	1% (23/1612)	22% (97/451)	4% (122/3258)

On day-15, the sensitivity, specificity, PPV and NPV were 94%, 98%, 99% and 89% respectively. The agreement of smear results read on day-15 with the reference results was near (kappa = 0.9). The sensitivity of reading increased from 75% on day-1 to 94% on day-15.

Table 2 gives the percentages of different types of errors for the same smears read on day-1 and day-15. The percentages of high false-positives and high false-negatives were 0.06% and 4% respectively on day-15.

DISCUSSION

It is very essential that every laboratory technician should be trained in sputum AFB microscopy. Hundreds of laboratories all over the world carry out training programmes for laboratory technicians before implementing DOTS strategy to treat pulmonary tuberculosis patients. However, there is no documented evidence on the performance of fresh trainees under training. It is also desirable to know the proficiency of smear reading by the laboratory technicians before they are assigned with the responsibility of sputum AFB microscopy. The present study documents the experiences of the authors responsible for training fresh STLSs at the reference laboratory in TRC.

During the training schedule, each trainee was given 120 to 150 AFB smears on 6 or 7 occasions for reading and grading. The number of errors committed by the trainees decreased gradually on subsequent occasions (data not shown) and the sensitivity of reading increased from 75% to 94%. In National Tuberculosis Programme, the managers expect that the microscopy technicians should have at least 85% sensitivity (relative to controllers). **The results of the study suggest that fresh trainees can achieve the expected level of 85% sensitivity during the 15 days' training programme recommended by World Health Organization (WHO) and IUATLD.**

The limitation of sputum AFB microscopy is that it is seldom possible to achieve 100% agreement in reading smears even between the experienced readers. As a result, at the beginning of the training programme, the occurrence of false-negatives was more frequent than the false-positive results. With the experience gained during the course of training, low false-negatives decreased from 58% to 22%. It is to be pointed out that smears used in the training programme were obtained from patients admitted and treated under various controlled clinical trials in TRC and they included diagnostic as well as follow up smears. With manufactured smears, suggested for panel-testing, trainees might have performed still better as they are likely to give consistent results for each grade[2]. It should be pointed out that the sensitivity of a laboratory goes up when more low-positive smears are identified, as their value is twice that of other positive slides identified correctly[5].

The observance of high level performance on day-1 (75% sensitivity) and on day-15 (94%

sensitivity) could be attributed to the quality of microscope, the quality of smears, experience gained during the training, the comfortable laboratory environment provided, the fear of being tested in a national reference laboratory, more than recommended 100 fields examined and to the evaluation process that was carried out immediately at the end of training. It has been reported that the smear reading capability of the Istanbul medical graduate students was less satisfactory with 40% of false-negatives and 26% of false-positives in reading smears[6]. It should be pointed out that in the field conditions in Mexico, the agreement achieved before and after a course of refresher training, ranged only from 65% to 67% and from 75% to 80%, respectively in a panel-testing programme[5]. Similar results were shown for a few intermediate laboratories in a panel-testing programme in India[7].

The results of the study support the statement that assessing proficiency of the technicians by panel-testing is a matter of careful technique and organization[8]. All said and done, the proficiency in reading smears during the training programme may not result in expected level of performance at the peripheral laboratories.

ACKNOWLEDGEMENTS

We gratefully acknowledge the contribution of Dr. M. Perumal, State TB Officer, District TB Officers and Medical Officers in the Peripheral Health Institutions in Tamil Nadu state; K. Rajaraman and other staff in Model DOTS project; faculty members in the Department of Mycobacteriology for their excellent technical support; Dr. Fraser Wares, Stop TB Unit, SEARO, WHO, New Delhi for his critical review of this manuscript. The training programme was supported in part by the funds from USAID through SEARO, WHO, New Delhi.

REFERENCES

1. Hans L Rieder, Martin Chonde T, Helge Myking, et al. The Public Health Service National Tuberculosis reference Laboratory and the National Laboratory Network. Minimum Requirements, Role and Operation in a Low-Income Country. IUATLD. 1998.
2. External Quality Assessment for AFB Smear Microscopy. PHL, CDC, IUATLD, KNCV, RIT. Association of Public Health Laboratories, Washington DC. 2002, 31.
3. Manual for Laboratory technicians. Revised National Tuberculosis Control Programme. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi, India. 1998.
4. Module for Senior Tuberculosis Laboratory Supervisor. Revised National Tuberculosis Control Programme. Central Tuberculosis Division. Director-General of Health Services. Ministry of Health and Family Welfare. New Delhi. 2001.
5. Martinez-Guarneros A, Balandano-Campos S, Solano-Ceh MA, Gonzalez-Dominguez, Lipman HB, Ridderhof JC, Flisser A. Implementation of proficiency testing in conjunction with a rechecking system for external quality assurance in tuberculosis laboratories in Mexico. *Int J Tuberc Lung Dis* 2003; **7**: 516-521.
6. Kilicaslan Z, Kiyan E, Erkan F, Gurgan M, Aydemir N, Arseven O. Evaluation of undergraduate training on tuberculosis at Istanbul Medical School. *Int J Tuberc Lung Dis* 2003; **7**: 159–164.
7. Paramasivan CN, Venkataraman P, Vasanthan JS, Rahman F, Narayanan PR. Quality assurance studies in eight state tuberculosis laboratories in India. *Int J Tuberc Lung Dis* 2003; **7**: 522-527.
8. Armand Van Deun. External quality assessment of sputum smear microscopy: a matter of careful technique and organization. *Int J Tuberc Lung Dis* 2003; **7:** 507-508.