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## The Results of Three Years Surveillance on Sputum Smear Microscopy in 285 District and Regional Tuberculosis Laboratories of Iran

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### ABSTRACT

**Background:** The study was designed to assess routine smear microscopy in 285 tuberculosis (TB) public health laboratories of Iran.

**Materials and Methods:** Over one year period (1999), the information regarding infrastructure, specimen processing (smearing, staining, smear reading), and safety points of TB laboratories were collected. Thereafter, in two consecutive years (2000 & 2001), the accuracy of smear reading was investigated by sending a set of 6 blinded slides to each laboratory. In total, 1710(X2) slides were prepared, of which 855 were positive (having varying degree of positivity), and 855 were negative.

**Results:** We found that 68.4% of TB laboratories were using commercially prepared staining kits of inferior quality and 72% of TB technicians examined, each slide for less than 7 minutes whereas the recommended standard time is 15-20 minutes. The results of blinded smear reading in the first round demonstrated 71% of accuracy. However, after 3 days of smear microscopy training for personnel in 60 poorly performing laboratories, we observed a substantial improvement in the quality of microscopy. The number of false positive reports dropped from 20 to 0 ( $p < 0.05$ ), and the number of negative reports declined from 40 to 5 ( $p < 0.05$ ). Overall, out of 237 participating laboratories in the second round, 217(91%) laboratories could correctly detect positive and negative smears.

**Conclusion:** This study demonstrates the importance of quality control and correct performance of smear microscopy. It also emphasizes the need for implementing strict and ongoing quality control for all laboratory procedures. (*Tanaffos* 2003; 2(5): 29-36)

**Key words:** Smear Microscopy, TB Laboratory, Quality Control

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## INTRODUCTION

Despite advance in molecular and imaging technology, the main diagnostic tool for pulmonary tuberculosis (TB) detection is microscopic observation of acid-fast bacilli (AFB) in the stained sputum (1,2). The technique is inexpensive, easy to perform and, able to detect the most infectious subset of TB patients (3). However, the main problem facing smear microscopy is its low sensitivity which can seriously limit both the extent and quality of its application. Previous studies showed that the smear sensitivity may have been very depending on smearing, staining and smear reading (4,5). For this reason, the World Health Organization (WHO) recommended that quality assurance (QA) should be an integral part of any national tuberculosis program (6). The recommendation includes systematic cross-checking of slides prepared by local diagnostic laboratories and/or examining the technical ability by sending blinded smear for microscopy reading (6,7,8). Currently, a variety of quality assurance systems are in operation (7) which ultimately result in better and timely diagnosis and treatment of pulmonary TB cases. Based on the data provided by WHO, the case detection rate in the most of developing countries is far less than 70% (9). It means that a considerable number of TB patients are missed in the early stage of disease. Each of these patients may develop more extensive disease and themselves become new vectors of transmission. Inaccurate diagnostic procedures and deficiencies in recording or reporting are important reasons behind the low case detection rate (10,11,12). Accordingly, the present study was undertaken to evaluate the laboratory infrastructure and their routine performance. According to the recommendations of WHO/Eastern Mediterranean Regional Office (EMRO), with cooperation and support of Iranian

National Tuberculosis laboratories (INTL), Iranian Centre for Diseases, and preventive Control (ICDC), the accuracy of smear reading in 285 TB laboratories of Iran was investigated.

## MATERIALS AND METHODS

### Supervisory Visit

The National Tuberculosis Laboratory Network in Iran consists of three levels of laboratory. The peripheral laboratories perform direct sputum microscopy; the intermediate laboratories perform culture and microscopy. The central level is responsible for training, evaluation of the peripheral and intermediate levels, and performing a variety of laboratory services. In the year 1999, the TB laboratories were visited by two senior officers from Iranian National Tuberculosis Program (INTP), and the following issues were investigated: the general laboratory facilities, reagents, materials, equipment, safety measures, waste disposable, procedures for collection of sputum, smear preparation techniques, microscopy reading, laboratory request forms, laboratory register, storage of slides for quality control, and work load.

### Smear Preparation for Positive and Negative Slides

In two consecutive years, 1710 (X2) slides were prepared and sent to 285 TB laboratories. Each laboratory received a box containing 6 blind smears of which three were positive (with varying degree positivity) and 3 were negative.

### Negative Smears

Briefly, 10uL loopfuls of *Streptococcus sanguis*, *Corynebacterium diphtheriae*, and *Bacillus cereus* cultures (ATCC) were placed into three tubes containing 5ml of brain-heart infusion broth and incubated at 37°C for 24h (16). A 0.1ml aliquot of each was then inoculated into each of the three vials

containing 9.9 ml of diluting fluid (Becton Dickinson Microbiology Systems; Cockeysville, MD). One ml of each of these three 1:100 dilutions was added to 100 ml of 1.23% mucin phosphate buffered saline solution (pH=6.0) to provide background material. The suspension was mixed on a magnetic stirrer for 30 minutes, and 50µL aliquots were pipetted onto slides and spread to cover an area of approximately 1X2 cm.

### Positive Smears

One loop of *Mycobacterium tuberculosis* (H37 RV) was subcultured into the 10ml of Dubos-Davis broth containing 0.05% Tween 80. The subculture was incubated at 37°C on a rotary mixer until the optical density corresponded to a 1Mc Farland nephelometer standard (6,13). At that point, the subculture was sonicated for 20s to break up the clumps, and different dilution (100UI, 500UI, 1 ml) was added to 1.23% mucin-phosphate buffered saline solution (pH 6.0). Finally, 1ml of each *S. sanguis*, *C. diptheriae* and *B. cereus* 1:10 dilutions (prepared as described above) was added. The suspension was mixed on magnetic stirrer for 30 minutes, and 50 µL aliquots were pipetted onto slides, and spread. All the positive and negative slides were coded, air-dried, heat-fixed, and stained by Ziehl-Neelsen (6,13) method. The stained slides were examined under oil immersion (100X) and were reported negative when no AFB was seen in at least 100 microscopic fields. The smear was reported positive (6) as follows: 1-9 AFB were seen in 100 microscopic fields (few bacilli), 10-99 AFB were seen in 100 fields (1+), 1-10 AFB per field in at least 50 fields (2+), and more than 10 AFB per field in at least 20 fields (3+). The coded slides were examined by two technicians, and their observation were cross-checked by a senior technician. Then, the slides were cleaned by xylene and kept in special boxes for transportation, each box

contained forms which had to be completed by receiving laboratory. The collected information was analyzed using t and t paired tests.

## RESULTS

### Supervisory Visit 1999-2000

In this study 39 intermediate (13.6%) and 246 peripheral (86.3%) TB laboratories were investigated (table 1)

**Table 1.** Show the results of site-supervisory visit in 285 TB laboratories of Iran during the year 1999

% of laboratory working on standard procedure	
<b>General Work Load</b>	5. Examine 100 field per slide: 285 (100%)
1. Sputum Container: 263(%92)	6. Grading the positive smear: 285 (100%)
2. Checking the sputum before processing the sample: 5(1.7%)	7. Reading the slide in less 7 minutes: 206(72%)
3. Labeling slide/sputum container: 285(100%)	<b>Safety point</b>
4. Wearing the lab coat: 195(68%)	1. Proper air- flow: 103 (36%)
5. Wearing the gloves: 180(63%)	2. U.V. Light: 118(41%)
5. Washing hands after work: 285(100%)	3. Biological safety cabinet for Intermediate laboratories: 39(100%)
6. Filtering the carboul fuchsin solution: 120(42%)	<b>work load</b>
7. Cleaning the microscopy lens after use: 270(94%)	1. Processing 100 specimens per month: 3 (1.0%)
8. Using disinfecting for cleaning the surface: 285(100%)	2. Processing between 125-175 specimens per month: 232 (81.4%)
Completion of register: 285 (100%)	3. Processing 250 specimens per month: 50(17.5%)
<b>Bacteriology Work</b>	
1. Smear air-dried: 285 (100%)	
2. Smear heat-fixed: 285(100%)	
3. Hot-carboul Fuchsin: 270 (94.7%)	
4. Cold carboul Fuchsin: 15 (5.6%)	

**Specimen Collection**

The laboratory technicians performed collection of sputum in all laboratories. In 275 laboratories (96.4%), the patients received the instructions on how to collect the specimens. However, in almost all of TB laboratories (98.2%) the responsible technicians did not visually check the quality of specimen, and if the obtained specimen was saliva, collection of sputum was not repeated.

**Specimen Processing**

Although, the TB laboratories were provided with WHO guideline, some laboratories were using different methods. In all TB laboratories, the smear was air-dried before heat fixing. 270 laboratories (94.7%) were using Ziehl-Neelsen by hot carboul fuchsin procedure and 15(5.2%) using cold carboul-fuchsin staining. 195 laboratories (68.4%) were using a commercially available staining kit whereas in the remaining 90 laboratories (31.5%) technical staff were preparing the staining solution themselves. Furthermore, we noticed that only 120 laboratories (42.1%) were filtering carboul fuchsin solution before using it. In all 285 laboratories (100%), the technical staff stated that they examine 100 or even more field per slide (positive or negative). However, 28% of TB technicians examined each slide for 15 to 30 minutes, and the remaining (72%) read each slide from 3 to 7 minutes.

**Work Load**

Our survey showed that; 1.0% of TB laboratories (n=3) were receiving 100 specimens per month, 81.4% of TB laboratories (n=232) were receiving between 100 to 200 specimens per month, and 17.5% of them (n=50) were receiving more than 200 specimens per month.

**Staff**

Overall, 407 technicians were working in 285 TB laboratories; 199 laboratories (69.8%) had only one, 50 laboratories (17.5%) had 2, and 36 (12.6%) had 3 technical staff. 72% of technicians stated that they were also performing other routine clinical laboratory works e.g. microbiology/hematology. During the

previous four years, all technicians had attended a training course organized by TB control managers.

**Infrastructure and Safety Point**

All 39 intermediate TB laboratories were equipped with a Biological Safety Cabinet (BSC) and centrifuges. However, none of the centrifuges were provided with a safety cap, and in only 6(15.3%) of intermediate laboratories the centrifuge was kept under the BSC. In most of peripheral TB laboratories (63%) no consideration of air-flow was observed. 260 laboratories (91%) were provided with U.V light. All of the laboratories (100%) were using house hold bleach including 5% phenol and/or Lysol as disinfectant for cleaning the work surface and decontaminating infectious items. In 180 laboratories (63%), laboratory technicians didn't wear gloves while preparing smears. However, all of them washed their hands with soap and water after specimen processing. Wearing a lab-coat was routine in 195 Laboratories (68%).

**Accuracy of Blinded Smear Reading First Round (2000-2001)**

In March 2000, 285 boxes containing a set of 6 blinded smears (3 positive & 3 negative) along with questionnaires were sent to 285 TB laboratories all over Iran. The laboratories were asked to send back their answers within 2 months of receiving the samples. 24.7% did not answer back and were excluded from study. In addition, the slides in 4 boxes (1.4%) were broken. Therefore, only 210 of TB laboratories (73%) were participated in the first round of smear reading test. Among these labs, 150 (71%) could correctly detect negative and positive slides. 20 laboratories (9.5%) have misread negative slides as positive. 40 laboratories (19%) reported +1 positive smear as negative and 15 laboratories (7.1%) had problems in grading the positive smears (Table 2).

The laboratories, which had more than one technical staff, sent their observations on separate forms and only in two laboratories (2.3%) the results, were different.

### Accuracy of Blinded Smear Reading Second Round (2001-2002)

The technical staff of 60 TB laboratories who had misread a slide and 15 laboratories which had problems in grading the positive smears were called for 3 days of smear microscopy training in their respective intermediate laboratories. The training was performed by a senior mycobacteriologist from NTP. A second round of blinded smear testing in these laboratories was undertaken. All laboratories were able to detect negative smear correctly, and the number of laboratories which misread +1 positive smear as negative was reduced to 5 from 40. Moreover, only 2 out of 15 laboratories continued having a problem with grading of positive smears.

The remaining 150 laboratories had similar results as the previous year. In addition, 27 laboratories participated for the first time in the second round, and the following results were obtained: 4 laboratories had false positive, 15 had false negative, and 3 had problems with smear grading. Overall, 237 laboratories were participated in the program, out of which 234 (98.7%) correctly detected negative smears, 20 laboratories (8.4%) misread positive slide as a negative, and 5 laboratories (2.1%) had a problem in grading positive smears. 48 (16.8%) laboratories did not participate in the first or second round of blinded smear testing (Table 2).

**Table 2.** The results of smear proficiency test in second round (2001-2002) clearly shows remarkable increase in smear reading. The p value was statistically significant.

	Year 2000- 2001	Year 2001- 2002	Comparison of result in both years: p value
<b>Total number of TB laboratories:</b>	285	285	
Total number of TB laboratories not responded:	71 (24.9%)	48 (16.8%)	p<0.05
Total number of broken boxes	4(1.4%)	3(1.0%)	
Total number of laboratories which were participated in the program	210 (73.6%)	237 (83.1%)	
Total number of TB laboratories which could correctly detect positive smear	150/210 (71%)	217/237 (91%)	
Total number of TB laboratories which reported positive smear as negative (false negative)	40(19%)	20(8.4%)	p< 0.05
Total number of TB laboratories which graded positive smear wrongly	15(7.1%)	5(2.1%)	
Total number of TB laboratories which could correctly detect negative smear	150/210 (71%)	237/237 (100%)	p< 0.05
Total number of negative smear reported as positive (false positive)	20 (9.5%)		

## **DISCUSSION**

Although microscopic examination of sputum is the main diagnostic tool for TB detection in most developing countries, the concept of smear quality control of smear examination has not been widely implemented. For this reason INTP decided to introduce WHO guidelines in TB laboratory networks (in the year 1998), and the present study was undertaken to evaluate their implementation. A variety of technical deficiencies were identified, and measures were instituted to correct them. These measures appear to have improved the practice in the laboratories with substantial improvement in the quality of the work done. One of the problems identified was the inadequacy of a commercial kit being used. In the similar study, Somoskovi et al. (14) identified unexpected microscopy errors in a TB laboratory in the United States where commercially prepared acid fast staining kit was used. Proper training with constant supervision can increase the quality of smear microscopy (10,15,16). Recently, Aziz and Bretzel (11) showed that 37.5% of Uganda laboratories did not filter the carboul-fuchsin solution while 31.3% were filtering the solution once in every one to three months. For this reason, a majority of their smears contained large number of granules and crystals. Previous cross-checking in 30 local laboratories near Tehran city (17) revealed that 21.7% of reported smears were false negative while 6.7% of them were false positive. Although we don't know the real percentage of false positive or negative smear reports in the country, we expect that the number is substantial as a high proportion of the laboratories did not routinely filter the reagents before using them. This error along with the very short amount of time given to each smear examination certainly leads to inaccurate microscopy results (6,16,18). Our study also pointed out a

striking lack of safety standard in the laboratories that will likely require structural changes to correct them. This must have a high priority in the strengthening of the laboratories as the personnel working in them are at risk correct safety procedures are not followed. The ability to improve the skills of the laboratory personnel to correct technical errors identified by the survey is probably the most important observation of the study. This depends critically upon targeted retraining of those with deficiencies as well as routine supervision of all functioning laboratories to support the personnel working there. In conclusion, the accurate performance of AFB microscopy is imperative for the early recognition of TB patients and the important epidemiological figures such as incidence and prevalence of TB are derived from microscopy results. Therefore, reliable microscopy results are prerequisite for an efficient implementation of TB control program. We conclude that an important factor in increasing case detection is an efficient smear microscopy service and this can be maintained only with regular supervision, targeted retaining and a program of quality assurance.

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## REFERENCES

- Enarson DA, Rieder HL, Arnadottir , and Trebucq A. Management of tuberculosis: a guide for low-income countries. 5<sup>th</sup> ed. Paris: IUATLD. 2000.
- Gebre N, Karlsson U, Jonsson G, Macaden R, Wolde A, Assefa A, et al. Improved microroscopic diagnosis of pulmonary tuberculosis in developing countries. *Trans R Soc Trop Med Hyg* 1995; 89(2): 191-3.
- Murray PR, Elmore C, Krogstad DJ. The acid-fast stain; a specific and predictive test for mycobacterial disease. *Ann Intern Med* 1980; 92(4): 512-3.
- Huebner RE, Good RC, Tokars JI. Current practices in mycobacteriology: results of a survey of state public health laboratories. *J Clin Microbiol* 1993;31(4):771-5.
- Kubica, U.P. Correlation of acid fast staining methods with culture results for Mycobacteria. *Bull. Int. Union. Tuberc.* 1980; 55: 117-124.
- Laboratory Services in Tuberculosis Control. Microscopy. Geneva. Switzerland. World Health organization. 1998.
- Boulahbal F, Mazouni L, Chaulet P. Prospective study of the organization and supervision of the bacteriological diagnosis of pulmonary tuberculosis in a case finding network in Algeria. *Bull Int Union Tuberc* 1976; 51(1): 313-21
- Van Deun A, Portaels F. Limitations and requirements for quality control of sputum smear microscopy for acid-fast bacilli. *Int J Tuberc Lung Dis* 1998; 2(9): 756-65.
- Global Tuberculosis Control. Geneva. Switzerland, World Health Organization Report. 2003. Communicable Disease. WHO. 2003.
- Aber VR, Allen BW, Mitchison DA, Ayuma P, Edwards EA, Keyes AB. Quality control in tuberculosis bactriology. 1. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination. *Tubercle* 1980; 61(3): 123-33.
- Aziz M, Bretzel G. Use of a standardised checklist to assess peripheral sputum smear microscopy laboratories for tuberculosis diagnosis in Uganda. *Int J Tuberc Lung Dis* 2002; 6(4): 340-9.
- Netto EM, Dye C, Raviglione MC. Progress in global tuberculosis control 1995-1996, with emphasis on 22 high-incidence countries. Global Monitoring and Surveillance Project. *Int J Tuberc Lung Dis* 1999; 3(4): 310-20.
- Kent PT, Kubica GP. Public health mycobacteriology: a guide for a level III laboratory. Atlanta, GA: Centers for Disease Control. 1985.
- Somoskovi A, Hotaling JE, Fitzgerald M, O'Donnell D, Parsons LM, Salfinger M. Lessons from a proficiency testing event for acid-fast microscopy. *Chest* 2001; 120(1): 250-7.
- Kinyanjui MG, Githui WA, Kamunyi RG, Omwega MJ, Waiyaki PG, Muthami LN. Quality control in the laboratory diagnosis of tuberculosis. *East Afr Med J* 1991; 68(1): 3-9.
- Van Deun A, Roorda FA, Chambugonj 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): 823-9.
- TB detection, Microscopy. (I). Persian version of "laboratory services in Tuberculosis control. Microscopy. WHO." Center for Diseases and Preventive Control (CDC). 1998. Tehran. Iran.
- Nguyen TN, 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): 483-7.