Outbreak, Surveillance and Investigation Reports
Field Epidemiology Training Program, Bureau of Epidemiology
Department of Disease Control, Ministry of Public Health, Thailand
Tel: +6625901734-5, Fax: +6625918581, Email: osireditor@osirjournal.net, http://www.osirjournal.net

The Proficiency Level of Microscopists Detecting *Mycobacterium tuberculosis* at Government Health Clinics in Three Selected States of Malaysia, 2009-2010

Fadzilah Kamaludin[1,*], Ganeswrie R[2], YK Loe[1], Teh CH[3], Suzana H[3]

1 Institute for Public Health, Ministry of Health, Malaysia

2 Sultanah Aminah Hospital, Johor Bahru, Johor, Malaysia

3 Tuberculosis and Leprosy Sector, Disease Control Division, Ministry of Health, Malaysia

* Corresponding author, email address: fadzilah@moh.gov.my

Abstract

Sputum smear microscopy is the cornerstone of diagnosing infectious tuberculosis. The presence of microscopic errors may misclassify or misdiagnose cases as non-cases, or vice versa. Substandard performance will compromise the efforts to detect tuberculosis and complicate measures to control. This study aimed to determine the proficiency of microscopists at three selected government health clinics in Malaysia. A cross-sectional study was conducted in 2009-2010. Three states were selected based on their high sputum positivity rate. All microscopists were enrolled and instructed to stain and grade a set of seven predetermined densities of mycobacilli slides. Two independent raters assessed their readings. A total of 100 microscopists and 700 slides were tested. 88.2% of slides were in agreement, with sensitivity of 83.8%, specificity of 94.3%, positive predictive value of 95.2% and false negativity rate of 18.7%. From the low positive slides, 27.5% were graded as negative. Two-third of microscopists achieved the accepted grading proficiency and 37% were scored as good staining. There was a need to intensify training on smear microscopy if the gateway for diagnosing TB in Malaysia was smear microscopy.

Key words: proficiency testing, *Mycobacterium tuberculosis*, Kappa agreement, microscopist

Introduction

In Malaysia, when a patient presents at a health clinic with chronic cough, fever and loss of appetite, the medical doctor will rely on the patient's clinical symptoms, results of sputum smear and chest X-ray findings to make a diagnosis of tuberculosis (TB). Care of TB patients starts with a quality assured diagnosis by identifying *Mycobacterium tuberculosis* in clinical specimens and microscopy of sputum smears.[1]

The cornerstone of the diagnosis of TB is direct microscopic examination of appropriately stained sputum specimens for tubercle bacilli. Grading of the positive smears gives a broad indication on severity of disease and response to therapy.[2] Positivity of these smears is highly dependent on quality of staining, microscope and number of microscopic fields examined. The technique is simple and inexpensive, and detects people with infectious TB which are responsible for most TB epidemics.[3]

In 2010, the World Health Organization (WHO) reported 8.8 millions of TB incident cases (range 8.5-9.2). While the incidence rate has been falling since 2002, the WHO emphasized that laboratory strengthening was needed to be accelerated.[4] As the incidence of TB declined, fewer cases were detected correspondingly and fewer specimens were tested. Thus, maintaining the proficiency in sputum microscopy would become more difficult.

The WHO recommended the countries to implement an External Quality Assessment (EQA) in their TB laboratories to improve their efficiency and reliability of the smear microscopy. There are three components in EQA which included on-site evaluation, blinded rechecking and panel testing.[5]

Since inception of the National Tuberculosis Control Program (NTCP) in 1961, TB ranked as second amongst the reported infectious diseases in Malaysia. The notification rate in 2009 was 64.0 per 100,000 populations and the rate has been plateaued for the past 15 years. As a routine practice of microscopists in TB laboratories, they submitted 100% of their positives slides and 10% sampling of the negatives slides to be read by their supervisors. In 2010, the NTCP embarked on the EQA program using a blinded

rechecking system to replace the routine practice of microscopists in TB laboratories (External quality assessment for National Tuberculosis Program, Ministry of Health, Malaysia. 2011, unpublished report).

The main objective of the study was to determine the proficiency level for detecting *Mycobacterium tuberculosis* from sputum smears by the microscopists at peripheral laboratories in government health clinics.

Methods

A cross-sectional study was conducted in 2009-2010. Three out of 14 states in Malaysia with the highest TB burden and sputum positivity rate were selected. All the microscopists reading TB smears in the health clinics were enrolled. The study protocol was approved by the Medical Research Ethics Committee. A self-administered questionnaire was given to the microscopists to determine the level of working experience, training received, workload, quality of microscope and staining used. Medical officers in the health clinics were examined for red-green color vision defect and vision acuity. A set of seven unstained slides with predetermined densities of *Mycobacterium tuberculosis* was given to each microscopist to stain and grade the slides.

The slides were prepared using the Smithwick and Stratigos technique.[6] The positive slides were prepared from fresh sputum specimens which were not more than two days old with bacillary load of +2 or more acid fast bacilli (AFB), and were divided into three main groups: high positive slides (3+ mycobacillary load), moderate positive slides (2+ mycobacillary load) and low positive slides (1+ and scanty mycobacillary load). The negative slides were prepared from fresh sputum specimens with no bacillary load and were used as diluents for the positive slides. The set of seven unstained slides comprised of three negative slides and four positive slides, which included two low positive slides, one moderate positive slide and one high positive slide. To minimize the bias, the slides were blinded and randomly placed in slide boxes before transporting to the participating microscopists. The microscopists were required to stain using their own Ziehl-Neelsen (ZN) staining materials, record the grading and return the stained slides within two weeks (excluding delivery time) to the reference TB laboratory. The slides were then blinded and scored by two independent reference microscopists (raters). An inter-rater Kappa agreement with 95% confidence interval was conducted to determine the strength of agreement between the raters.

The raters used a point system to assess staining quality of each slide independently. The slides were categorized into acceptable and not acceptable quality. Points were given according to staining characteristics of the slides: two points for magenta red AFB, two points for blue background, one point for pink AFB, one point for very light blue background, one point for presence of scum and one point for very dark background. Slides with score point of three and four were categorized as acceptable, and slides with score point of two and below as not acceptable (Figures 1-5). If there was any discrepancy in staining assessment between two raters, the slides were re-assessed by a consultant reference microbiologist and the result was taken as final.

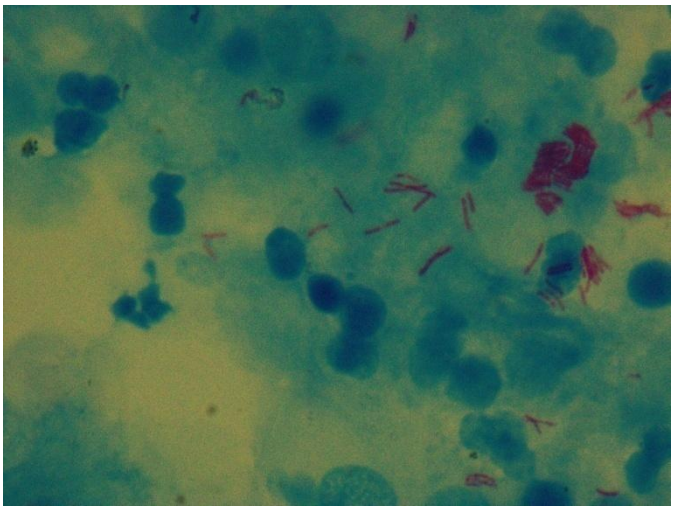

Figure 1. Slide with acceptable staining from sputum smear for *Mycobacterium tuberculosis*

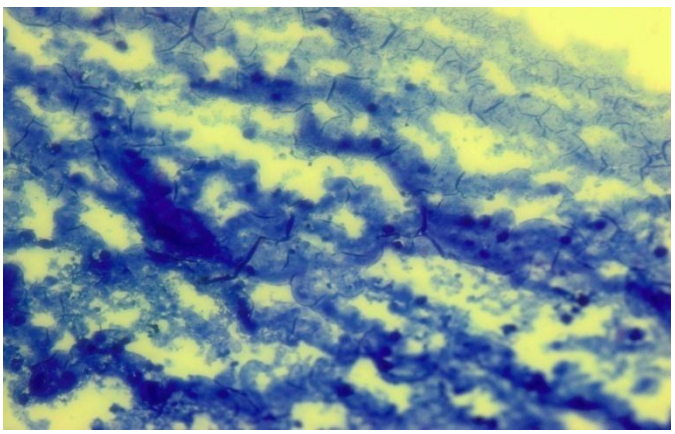

Figure 2. Slide with over-staining of methylene blue (poor staining) from sputum smear for *Mycobacterium tuberculosis*

Similarly to staining, the consultant reference microbiologist also addressed the discrepancies encountered for grading. Each slide was scored 10 marks for ability to differentiate positive and negative slides correctly, and five marks for any quantification error (QE). QE is defined as the

difference of at least two grades when reading the positive slides. A positive slide read as negative was scored as zero mark and vice versa. The criteria table from WHO/IUATLD was used for correctness in proficiency grading.[5] The total score of 80% and above was taken as the acceptable proficiency level.

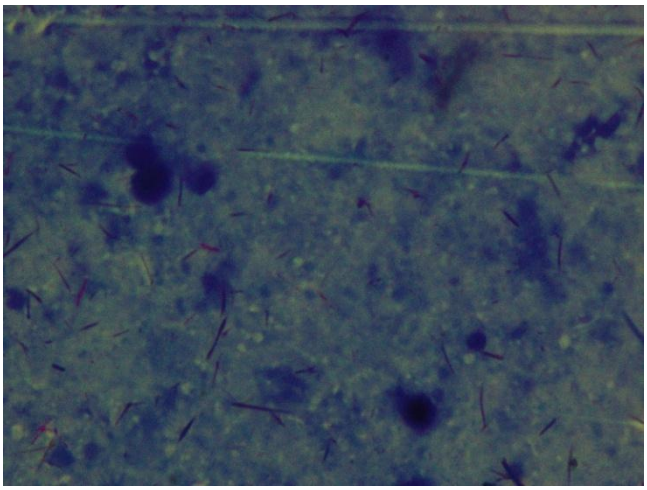

Figure 3. Slide with crystals (underestimation of AFB) from sputum smear for *Mycobacterium tuberculosis*

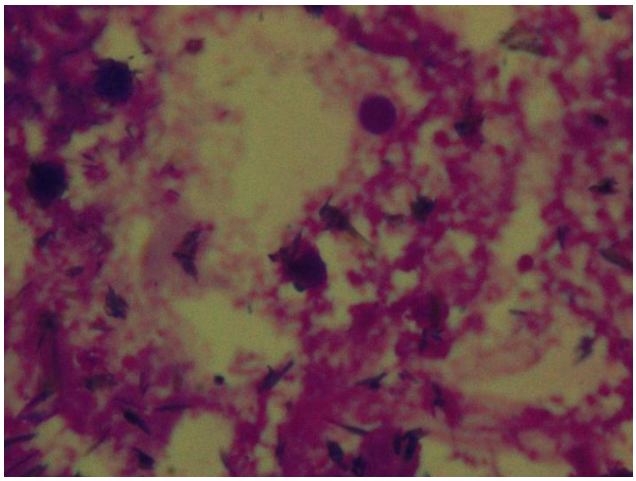

Figure 4. Slide due to over-staining from sputum smear for *Mycobacterium tuberculosis*

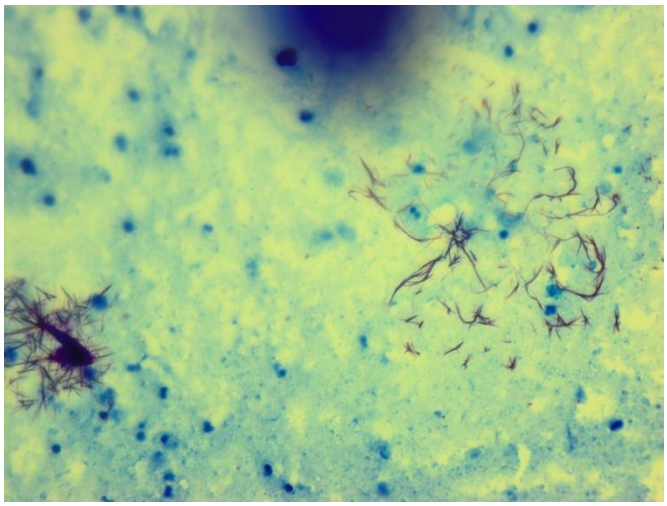

Figure 5. Slide with carbol fuchsin crystals looking like AFB from sputum smear for *Mycobacterium tuberculosis*

The Kappa analysis and data analysis were conducted using SPSS version 15.0 (SPSS Inc., Chicago III).

Results

All 61 health clinics in the three selected states were involved in this study and some clinics have more than one microscopist. All the 122 microscopists registered at the 61 health clinics consented for the study, but only 100 (82.0%) were eligible for the proficiency testing. Two microscopists with red-green color visual defect and 20 microscopists without a complete set of seven slides were excluded. Age range of the microscopists participated in the study was 23-58 years old (Table 1). After excluding the sets with missing and broken slides, total 100 panel slides were tested. The raters scored a total of 400 positive and 300 negative slides independently. The Kappa agreement for grading and staining among the raters was 0.86 (95% CI = 0.8-0.9) and 0.66 (95% CI = 0.6-0.7) respectively.

Table 1. General characteristics of the microscopists reading sputum smears for *Mycobacterium tuberculosis* at government health clinics in 3 selected states, Malaysia, 2009-2010 (n=100)

Variable	Percent
Age range	23-58 years
Experience of 5 years or more in TB work (majority)	48
Maximum smear of 5 or less proceed per day (majority)	48
Good condition of microscope	97
Yearly servicing of microscope	74
Source of staining	
- Self-prepared	6
- Commercially prepared	37
- Centrally prepared	57
Attended in-service training on TB	80

A total of 618 (88.3%) slides were in agreement with the final raters' readings (Table 2). False positivity rate was 4.8% and false negativity rate was 18.7%. Sensitivity of grading from the microscopists compared to that of the raters was 83.8%, with specificity 94.3% and positive predictive value 95.2%. Of 300 predetermined negative slides, 5.6% were graded as positive by the microscopists. Among 400 predetermined positive slides, 16.3% were graded as negative and 12.5% were found to have QE (Table 3). The low positive slides had the highest incorrect reading (27.5%) whilst the moderate positive slides had the highest QE (29.0%) (Table 4). Sixty seven percent of the microscopists were scored as 80% or above proficiency level for grading and only 37% had achieved good proficiency level for staining (Table 5).

Table 2. Performance of the microscopists (n=100) and the raters reading sputum smears for *Mycobacterium tuberculosis* at government health clinics in 3 selected states, Malaysia, 2009-2010

Item				Performance
Performance between raters				
Kappa agreement for grading				0.86 (95% CI = 0.8-0.9)
Kappa agreement for staining				0.66 (95% CI = 0.6-0.7)
Performance between raters and microscopists				
Microscopists' reading		Raters' grading		
		Positive	*Negative*	*Total slides*
	Positive	335	17	352
	Negative	65	283	348
	Total slides	400	300	700
Overall agreement for grading*				88.3%
False positive**				4.8%
False negative***				18.7%
Sensitivity				83.8%
Specificity				94.3%
Positive Predictive Value				95.2%

* Number of negative and positive slides with consistent reading between raters and microscopists

** Grade as negative by rater, but grade as positive by microscopist

*** Grade as positive by rater, but grade as negative by microscopist

Table 3. Performance of the microscopists as graded by the raters reading sputum smears for *Mycobacterium tuberculosis* at government health clinics in 3 selected states, Malaysia, 2009-2010

Predetermined slide	Correct (10 marks)			Incorrect (0 mark)			Quantification error (5 marks)			Total
	Number	Percent	95% CI	Number	Percent	95% CI	Number	Percent	95% CI	
Negative	283	94.3	91.1-96.7	17	5.6	3.3-8.9	Not related	Not related	Not related	300
Positive	285	71.3	66.5-75.6	65	16.3	12.8-20.3	50	12.5	9.5-16.2	400
Total	568	81.1	78.0-83.9	82	11.7	9.5-14.4	50	7.1	5.4-9.4	700

Table 4. Performance of the microscopists graded by the raters using the predetermined positive slides from sputum smears for *Mycobacterium tuberculosis* at government health clinics in 3 selected states, Malaysia, 2009-2010

Positive slide	Correct		Incorrect		Quantification error		Total Number
	Percent	95% CI	Percent	95% CI	Percent	95% CI	
Low positive	69.5	62.6-75.8	27.5	21.4-34.2	3.0	1.1-6.4	200
Moderate positive	64.0	53.8-73.4	7.0	2.9-3.9	29.0	20.4-38.9	100
High positive	82.0	73.1-89.0	3.0	0.6-5.0	15.0	8.6-23.5	100

Table 5. Overall proficiency level of the microscopists for grading and staining quality from sputum smears for *Mycobacterium tuberculosis* at government health clinics in 3 selected states, Malaysia, 2009-2010

Proficiency level	Percent	95% CI
Proficiency level for Mycobacterium grading		
Scored ≥ 80%	67	56.9-76.1
Scored ≤ 79%	33	23.9-43.1
Proficiency level for Ziehl-Neelsen staining		
Scored ≥ 80% (good)	37	27.6-47.2
Scored 79-50% (fair)	40	30.3-50.3
Scored 49-0% (poor)	23	15.2-32.5

Discussion

In diagnosing the infectious pulmonary TB, sputum smear examination is the most important test to diagnose a person with persistent cough.[7] A number of newer TB diagnostic tools were becoming available, but the screening method in Malaysia was still microscopic examination of sputum smear. Consequently, the microscopists' knowledge on AFB morphology, their microscopic skills and staining technique greatly affect the patient care. Thus, reaching and maintaining an acceptable level of proficiency in sputum smear microscopy was imperative for a successful NTCP.

This study showed the false negative rate of 18.7% which, if translated into the working environment, indicated that some patients might not receive appropriate treatment and transmitted TB in general population. In addition, the study revealed that more errors occurred among the low positive slides when the staining was substandard. Poor quality of staining contributes to low detection of tubercle bacilli. This is especially true among the low positive slides. A study in India has shown that occurrence of false negative could be reduced from 58% before training the laboratory technicians on proper staining technique to 22% after training.[8]

Detecting TB at an early stage is of utmost importance and lacking the ability to detect bacilli in smears with low density could result in less effective TB control program. A person with untreated smear positive TB may infect 10-15 people per year, making the identification of these infectious patients as one of the key aspects of TB control.[9]

Sputum AFB microscopy may never reach 100% agreement in reading smears even between the experienced readers.[10] This can be seen from the Kappa agreement between the raters in this study, 0.86 (95% CI = 0.8-0.9) and 0.66 (95% CI = 0.6-0.7) for grading and staining respectively.

QE has no direct impact on treatment and monitoring, but it can indicate the general knowledge of the microscopist on AFB microscopy and skills of using the microscope. Correct quantification can at times be helpful to clinicians for decision making in difficult cases.[5] This study revealed that QE was high (29%) among the predetermined moderate positive slides which might indicate the possibility of microscopists not following the standard procedure for reading the smears. Consistently, under-reading of number of AFB can give useful indication to problem areas in the diagnostic process.

The microscopists were instructed to use their own ZN staining materials available in their laboratories to enable capturing the existing conditions in routine staining practice. In other words, the substandard staining performance might not be due to technique alone, but also might include other limiting factors such as the quality of ZN stains. The maximum number of ZN smears examined per a microscopist on average per day should not exceed 20. If more smears on average are read over a period of time, visual fatigue will lead to deterioration of reading quality. On the other hand, proficiency in reading ZN smears can only be maintained by examining at least 10 to 15 smears on average per week, i.e. a minimum of 2-3 examinations per day.[10,11] This could be one of the possible reasons for the poor performance because the microscopists might have experienced fatigue from other routine works. Inadequate number of smears read per day may also affect the ability to maintain proficiency. Hence, proficiency of microscopists can fluctuate from time to time depending on regular practice as well as other conditions such as fatigue resulting from reading many slides.

Choosing the number of slides for this study was considered as a good representation for the assessment indicator and concurrently not to add unnecessary burden to the existing workload of the technicians in the laboratories. A set of seven slides was taken as appropriate for the raters and other microscopists in various laboratories to process and examine per working day without losing the quality. Literature reviews have shown studies using slides ranging from 6-10 slides per set for panel testing with maintaining the necessary composite of mycobacilli density.[5,9]

To reduce burden for preparation of numerous slides, only one set of unstained slides per microscopist was used in this study as compared to other studies that used both stained and unstained slides. We could only determine two different proficiencies of the microscopists, namely, staining alone and staining with grading together as one entity. Hence, in this study, when a microscopist performed poorly for grading, we could not determine whether he was truly poor at grading which here referred to the cumulative process of staining with grading. In other words, if the same microscopist was given a different slide with good staining quality, his capacity to read the slide might be much better.

Scoring for staining is difficult as it is very subjective. However, we have attempted to identify the weakness in diagnostic process by reading the characteristics and the features presented after staining. Sending

unstained slides for test panels has the advantages of testing several aspects of the staining procedure conducted by the microscopist, including preparation of staining reagents, staining procedures, reading and reporting results.[5]

This panel testing was timely since the NTCP was at the early stages of conducting the EQA rechecking program for all states in Malaysia. The findings of this study could provide a quick assessment among the microscopists from the three states and the template would be useful if similar assessments were to be conducted in other states. In a northern province of South Africa, a proficiency testing was conducted with the aim to solve operational problems and developed an 'intervention plan' for corrective action. Before intervention, the correct result was 85.5% and with the intervention, the result was increased to 97.4%.[11]

Limitations

As with any study, limitations were inevitable and the results from this study might be biased as the microscopists were aware that they were being tested and thus, they would have dedicated more time and effort to get a better performance. Nevertheless, if poor proficiency was found, it implied that the real performance might be even poorer.

Findings and interpretations from this study were only applicable to the microscopists in the three states. These might not be generalized to other states as the resources, workload and manpower were different, and the selection was based on TB prevalence and laboratory burden.

Public Health Actions and Recommendations

We recommended that tests for vision and cataract should be incorporated into the health examination for microscopists aged 40 years and above. This would help to detect any vision impairment among microscopists that may affect their ability to read the ZN stained AFB slides. It would also be a good practice to screen the microscopists serving in TB laboratories for the red-green color vision defect.

We suggested training the microscopists to emphasize the importance of staining and preparation for staining. Regular quality control on staining reagents should be encouraged by using positive and negative control slides before a new batch of reagents is used in the laboratory. As carbol fuchsin and methylene blue may precipitate over time, it is essential that microscopists filter their stains when precipitation occurs as this may affect the quality of their staining.

This study was presented to the policy decision makers, and the eyes tests for vision and cataract among microscopists aged more than 40 years has been incorporated into the health screening for health staff in accordance with the Public Service Circular number 3/2003. As for the existing and new microscopists working in TB laboratories, they have been directed to be screened for red-green color vision defect using the Ishihara Chart which has been available in all health clinics.

Conclusion

This study revealed that only 67% of the microscopists had the acceptable proficiency level for grading and the false negativity was high (18.7%). The low-density predetermined positive slides had the highest percentage (27.5%) of incorrect reading. The aim of staining is to make the bacilli easily visible so that their presence can be detected. Only 37% of the microscopists had achieved good staining level. When there is low staining quality accompanied by low-density of mycobacilli, this further compounds the problem on visibility which results in false negative slides. This is of great concern for intermediate burden countries like Malaysia where most of the suspected TB cases may have low bacillary load at the onset of disease. Due to better health care facilities or better awareness, patients with suspected TB may seek treatment early, but might not be detected by sputum smear microscopy. This may be one of the reasons for late detection of cases and patients being diagnosed as moderate or severe form of pulmonary TB.

The optimum performance in diagnostic process is derived from skillful practice of a series of procedures that begins with sampling and carries through smearing, staining and grading. If there is a weak link in any of the procedures, then it is difficult to attain the desired performance. Identifying the problem area certainly makes the corrective action on easier tasks and ultimately, customizes training according to the needs of the diagnostic system.

Acknowledgements

The authors wished to express our sincere appreciation to the Director General of Health, Malaysia for allowing us to publish this paper and to the Deputy Director of Health (Research and Technical Support) for the encouragement and support in making this study a success.

We would also like to thank the State Directors, Hospital Directors, State TB Officers and laboratory staff for their co-operation and keen interest in the

study. Without their commitment and dedication, this study would not have come this far.

Suggested Citation

Kamaludin F, Ganeswrie R, YK Loe, Teh CH, Suzana H. The proficiency level of microscopists detecting *Mycobacterium tuberculosis* at government health clinics in three selected states of Malaysia, 2009-2010. OSIR. 2013 Sep;6(3):11-17. <http://www.osirjournal.net/issue.php?id=44>.

References

1. Ridderhof JC, van Deun A, Kam KM, Narayanan PR, Aziz MA. Roles of laboratories and laboratory systems in effective tuberculosis programmes. Bull World Health Organ. 2007 May;85(5):354-9. [cited 2013 Jan 3]. <http://www.who.int/bulletin/volumes/85/5/06-039081/en/ >.
2. Standard operating procedure for laboratory diagnosis of tuberculosis and *M. Avian* complex diseases in HIV positive patients. In: Kumari S, editor. Guidelines on standard operating procedures for laboratory diagnosis of HIV-Opportunistic infections. New Delhi: World Health Organization; 2001 Jun. p.11-26. [cited 2013 Jan 3]. <http://apps.searo.who.int/pds_docs/B0189.pdf >.
3. World Health Organization. Laboratory services in tuberculosis control part II: microscopy. 1998. [cited 2013 Jan 3]. <http://whqlibdoc.who.int/hq/1998/WHO_TB_98.258_(part2).pdf >.
4. Dawson D, Kim SJ, World Health Organization Regional Office for the Western Pacific. Quality assurance of sputum microscopy in DOTS programmes - guidelines for Pacific Island Countries. Manila: World Health Organization; 2003. [cited 2013 Jan 3]. <http://www.wpro.who.int/publications/docs/Quality_assurance_for_sputum_PIC.pdf >.
5. Ridderhof J, Humes R, Boulahbal F. External quality assessment for AFB smear microscopy. APHL, CDC, IUATLD, KNCV, RIT, WHO. 2002. [cited 2013 Jan 3]. <www.aphl.org/AboutAPHL/publications/Documents/External_Quality_Assessment_for_AFB__Smear_Microscopy.pdf>.
6. Smithwick RW, Stratigos CB. Preparation of acid-fast microscopy smears for proficiency testing and quality control. J Clin Microbiol. 1978 Jul;8(1):110-1.
7. Luelmo F. What is the role of sputum microscopy in patients attending health facilities? In: Frieden T, editor. Toman's tuberculosis: case detection, treatment, and monitoring - questions and answers. 2nd ed. Geneva: World Health Organization; 2004. pp. 7-13.
8. Selvakumar N, Kumaraswami, V, Gopi PG, Sivagamasundari S, Prabhakaran E, Vasanthan S, et al. Proficiency to read sputum AFB smears by senior tuberculosis laboratory supervisors under training at a reference laboratory in India. Indian J Tuber. 2005;52:11-4.
9. World Health Organization. Fact sheet on tuberculosis. March 2006. [cited 2013 Jan 3]. <http://www.who.int/mediacentre/factsheets/fs104/en/>.
10. World Health Organization. Laboratory services in tuberculosis control part 1; organization and management. 1998. [cited 2013 Jan 3]. <http://whqlibdoc.who.int/hq/1998/WHO_TB_98.258_(part1).pdf >.
11. Rawlinson J, Mogale J. Implementing proficiency testing for TB smear microscopy in the northern province, South Africa. Durban: Health Systems Trust; 2001.