



Evaluation of Laboratory Professionals on AFB Smear Reading at Hawassa District Health Institutions, Southern Ethiopia

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Abstract:

Background: *Mycobacterium Tuberculosis (TB) detection through quality assured laboratories is an essential element of the World Health Organization (WHO) STOP TB Strategy. For this quality assurance of acid fast bacilli (AFB) stained sputum smear microscopy is essential as AFB Microscopic diagnosis has remained the routine laboratory method for the identification of TB in Ethiopia. This study intended to assess the performance of laboratory professionals in detecting TB bacilli at Hawassa and Hawassa Zuriya health institutions.*

Methods: *A cross-sectional study design was employed on a total of 67 laboratory professionals working in public health facilities. A standardized pre-validated panel slide and questionnaires were distributed to laboratory professionals along with on-site evaluation by using standard questioner. A total of ten slides per panel given for each professional. Each panel of slides includes 4 negative slide and 6 positive slides of different bacterial density (3 with 1-9 AFB/100 fields, one with 1+ one with 2+ and one with 3+). Agreement in detecting of TB bacilli Sensitivity, specificity and predictive values of readings were assessed using SPSS version 20.0*

Results: *Onsite evaluation showed that about 89% of laboratories has no separate area for TB work as well as 78% have no adequate ventilation very far from the standard Laboratory safeties. Nine participants (13.4%) correctly reported all panel slides. A total of 13.74%(92/670) error was reported that included major errors of 1.04% (5HFN; 2 HFP) and minor errors of 12.70% (17 LFN and LFP each, and 51QE). The sensitivity, specificity, positive predictive values (PPV) and negative predictive value (NPV) of participants in detecting TB bacilli as compared to the reference reading were 94.52%, 92.91%, 95.23% and 92.67% respectively.*

Conclusion: *laboratory safeties are deficient on most laboratories and there are a lot of technical problems observed during on-site observation. Agreement of the participants with reference reading in the detection of TB bacilli was very good according to national guide line. Though low major error were reported, training and supervisory activities are still important for successful TB control programs.*

Keywords: *TB lab examination, Panel testing; Onsite evaluation, southern Ethiopia.*

Abbreviations: *AFB: acid fast bacilli; PT: proficiency test; DOTS: Directly served Therapy strategy; APHL/CDC: Association of Public Health Laboratories/Center for Disease Control and Prevention; DOTS: directly observed treatment strategy; EQA: external quality assessment; IUATLD: International Union against Tuberculosis and Lung Disease; NTP: National tuberculosis Program; SOP: standard operating procedure.*

1. BACKGROUND

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), infects almost the third world population and kills around two million people world wide each year. About 80% of the global TB burden occurs in low-income countries, where pulmonary disease and its transmission are most serious public health problems [1].

Ethiopia is one of the 22 high burdened countries and TB remains one of the leading causes of mortality. As to the 2014 WHO report, the rate of all forms of TB is 211 per 100,000 of the population. About 13% of all new TB cases are also HIV co-infected. Moreover, Ethiopia is one of the high TB/HIV and multidrug resistant TB (MDR TB) burdened countries [2].

Ethiopia implements the STOP TB strategy of world health organization (WHO) starting from 2006 [3]. One of the components of the directly observed therapy strategy (DOTS) recommended by the WHO for diagnosis of TB is based on laboratory results of sputum smear microscopy. Hence correct reading of sputum smears is critical in case finding and management for further progress of TB prevention program. In addition the DOTS strategy also recommends quality control of smear microscopy be an integral part of national TB control programmes [2, 3 and 4].

Error in reading sputum microscopy may result in failure to detect persons with active tuberculosis and in reverse unnecessary anti-TB treatment for non-TB cases which predisposes the development of drug resistant tuberculosis (MDR-TB). Therefore quality assurance (QA) system for the sputum microscopy is important to minimizing the false positive and false negative results in the laboratory services [5, 6].

Most study in different part of the world has shown that during on site evaluation and panel testing laboratories didn't have a split area for TB examination with full safety problems in addition, overall error 13 - 25% were reported. For this most of literatures recommend that continues supervision and training programs are significantly improving the lab professionals in TB microscopic examination [12, 15, and 16]. According to the recommendations made by the national guideline, the quality control system for sputum smear microscopy for tuberculosis contain; internal quality control, quality improvement and external quality assessment which includes proficiency testing of professionals in TB microscope reading [4, 7]. There for the aim of this study is to evaluate the quality performance of laboratories in AFB smear microscopy in peripheral diagnostic centers in Hawassa & Hawassa Zuriya health institutions.

2. METHODS

2.1. Study Setting

Across sectional study was conducted on selected laboratories from October 2015 to January 2016. The study laboratories were include from Hawassa & Hawassa Zuriya governmental health institutions which are under Sidama Zone administrative of Southern nation and nationalities peoples region Which is found about 270 km away from the capital city, Addis Ababa. The study laboratories involve Hawassa referral hospital and Adare hospital in Hawassa, and seven governmental health center laboratories in Hawassa Zuriya. All health institutions are governmental and perform AFB TB examination as a routine test.

2.2. Data Collection and Panel Composition

Data were collected using on site evaluation check list and panel tests. All peripheral laboratories included in the study have employed AFB staining technique for detection of AFB in a sputum smear. Standardized panels containing 10 stained with varying AFB quantities was validated by the Hawassa University referral teaching hospital laboratory. This study panel slide composition with different grades of positivity, 3+,2+,1+,two smears for scanty and five negative in order to evaluate the ability of the lab professionals to properly grade positive slides. The composition of test panels standardized according to WHO manual and Ethiopia Federal Ministry of Health Guidelines [4, 8].

The result was expressed as major or minor error: Major errors were sub classified as; high false positive (HFP): If a negative smear misread as 1+ to 3+ positive, High False Negative (HFN): If a 1+ to 3+ positive smear misread as negative. Minor error included; Quantification error: when there is a difference of more than one Grade in a reading a positive smear between examinee and controller, Low False Positive (LFP): when a negative smear misread as low (1-9 AFB/100 field) positive and Low False Negative (LFN): when a low (1-9 AFB/100 field) read as negative result according to the International EQA classification (Table 1).

Table1. Evaluation and interpretation of errors between controllers and microscopist

Result of Technician	Result of Expertise				
	Negative	1-9AFB/100f	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
1-9AFB/100f	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

HFN, High False Negative; HFP, High False Positive; LFN, Low False Negative; LFP, Low False Positive; QE, Quantification Error

2.3.Scores for Grading

Regarding the score; Set of 10 panel testing slides, each carries 10 points, total possible score of 100. Committing major error (HFP and HFN) result in a score of 0 where as minor error (LFP, LFN and QE (QE = 2 grades difference)) result in scores of 5 points. In general assessment of performance was based on Table 1. Passing score was 80 points and poor performance was <80%. Interpretation of results was done based on International Union against tuberculosis and lung disease (IUATLD) WHO recommended grading of sputum microscopy results using [4, 8].

On-site evaluation of each laboratory was performed using the national on-site evaluation checklist to assess the different aspect of working environment inside TB laboratory [9, 10].

2.4.Data Analysis

Data processing and statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software (Windows version 16.0). The percentages of different types of errors were calculated for smears microscopy center. The sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) of smear reading by microscopic center laboratory technician was calculated. Chi square test is used to see any association with different variable. The agreement in reading between the peripheral diagnostic centers and the reference laboratory readings was done using kappa statistics and p value less than 0.05 was considered to be statistically significant. Data and materials supporting this study finding presented in the main paper or additional supporting files whenever possible.

2.5.Ethical Consideration

There search proposal was evaluated by the research and ethics committee of Department of Medical Laboratory Science and reviewed and cleared by Institution of Review Board (IRB) of Hawassa University. Prior to data collection, written consent was obtained from the heads of each laboratory personnel. Moreover, written consent was also obtained from laboratory professionals who participated in interview and panel testing. Confidentiality was maintained by coding each facility starting from data collection to analysis.

2.6. Results

A total of 67 laboratory professionals responded to the questionnaires with a response rate of 100%. Forty four (65.7%) of them were from health center and the rest 23(34.3%) were from hospital. about50% of participant responded that they got TB microscopy service training previously and almost 42% of participant laboratory have not involved in EQA. Thirty six (53.7%) of participant were BSc degree and the rest 31(46.3%) were diploma by qualification. The males were 45(67.2%) (Table2).

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Table2. Frequency distribution of characteristics of laboratory professionals, Hawassa Zuriya, Southern Ethiopia, 2016 (N=67).

Variable		Frequency (%)
Sex	Male	45 (67.2)
	female	22 (32.8)
Qualification	Diploma	31(46.3)
	degree	36(53.7)
Age	21-30	34 (50.7)
	31-40	27(40.3)
	>40	6(9)
Service year	<2 years	13(19.4)
	2-5 years	30(44.8)
	6-10 years	18(26.9)
	>10 years	6(9)
Previous training	Yes	34(50.7)
	no	33(49.3)
EQA involvement	Yes with feed back	16(23.9)
	Yes but no feed back	23(34.3)
	no	28(41.8)
Institution	Health center	44(65.7)
	Hospital	23(34.3)

3. ON-SITE EVALUATION

Regarding on-site evaluation, 44.5% of laboratories have SOP for AFB smear microscopy using ZN technique also about 89% of the study sites have no separate area for TB work. Expired laboratory reagents, and lack of routine maintenance of microscopes were among the problems identified in the peripheral laboratories. Moreover, all laboratories didn't include control smears during staining procedures. Furthermore, 100% laboratories did not filter reagents before use, 66.7% of them did not prepare slides with appropriate thickness, and 33.4% had unacceptable background staining (Table 3).

Table3. On-site evaluation visits of the selected health institution medical laboratories from October 2015 to January 2016

On-site evaluation	Number of Laboratory (%)
Laboratory safety	
SOP for AFB smear microscopy using ZN technique	4/9(44.5)
No separate area for TB work	8/9 (88.9)
No adequate ventilation	7/9(77.8)
No biohazard waste bin with a lid	7/9(77.8)
Improper use of PPE	8/9(88.9)
Laboratory reagents	
Expired reagents or no label	3/9(33.4)
Not filtered before use	9/9(100)
Filtered once a month	6/9(66.7)
Microscope	
Inadequate light source	2/9(22.3)
Objective not cleaned after slide examination	8/9(88.9)
No routine care/daily maintenance	7/9(77.8)
Smearing and staining procedures	
Background staining not acceptable	3/9(33.4)
Background material doesn't represent sputum	4/9(44.5)
Inappropriate smear thickness	6/9(66.7)
Inappropriate smear size	6/9(66.7)
Report with grading	2/9 (22.3)
Include control smears	
Daily	0/9 (0)
New batch of reagents	6/9(66.7)
Never	3/9 (33)

There was no statistically significant association between the proportion of errors made by the participants in the detection of TB bacilli and their sex, experience, Qualification, previous training, and EQA involvement. Professionals found in health center and those serving less than 2 year are likely there is statistically significant association with the proportion of errors they made (Table 4).

Table 4. Relationship between score of participant with selected demographic characteristics, Hawassa and Hawassa Zuriya, Southern Ethiopia, 2016 (N = 67).

Variable		Passed >80/100 (%)	Failed <80/100 (%)	Chi-square	Degree of freedom	P-value
Sex	Male	36 (80)	9(20)	0.66	1	0.797
	female	17(77.3)	5 (22.7)			
Qualification	Diploma	25(80.6)	6(19.4)	0.08	1	0.773
	degree	28(77.8)	8(22.2)			
Age	20-30	23(67.6)	11(32.4)	5.849	3	0.050
	31-40	24(88.9)	3(11.1)			
	>40	6(100)	0(0)			
Service year	<2 years	7(53.8)	6(46.2)	7.66	2	0.050
	2-5 years	26(86.7)	4(13.3)			
	6-10 years	14(77.8)	4(22.2)			
	>10 years	6(100)	0(0)			
Previous training	Yes	38(82.6)	8(17.4)	1.093	1	0.296
	no	15(71.4)	6(28.6)			
EQA involvement	Yes with feed back	23(88.5)	3(11.5)	2.38	1	0.29
	Yes but no feed back	16(76.2)	5(23.8)			
	no	14(70)	6(30)			
Institutions	Health center	12(52.2)	11(47.8)	15.36	1	0.0153
	Hospital	41(93.2)	3(16.8)			
	Total	53(79.1)	14(20.9)			

The type of error committed by the professionals as displayed in Figure 1, it is mainly the minor error like quantification error, Low false negative and Low false positives which account 92.3% of all error and the rest 7.7% are the major errors.

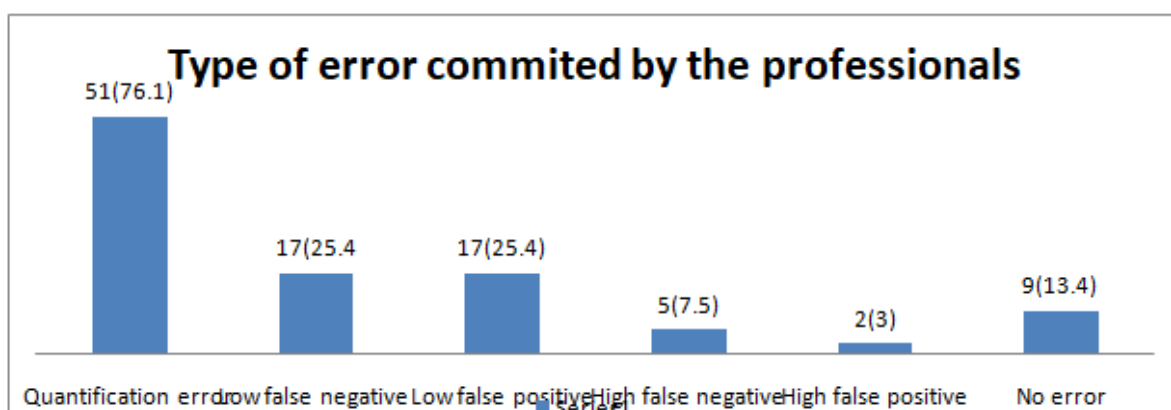


Figure 1. Type of errors committed by study participants Hawassa and Hawassa Zuriya, Southern Ethiopia, 2016

Out of 67 laboratory professionals involved in the panel test, 13.74% (92/670) were reported wrongly that includes major errors of 1.04% (5 HFN; 2 HFP) and minor errors of 12.7% (17 LFN; 17 LFP, and 51 QE). Among the 4 negative slides 2 (0.29%) of the participants made major errors (HFP) and among the 6 positive slides 5 (0.75%) of participants made major error (HFN). On the other hand, 2.53% of participants made minor errors (LFP) on 4 negative slides. The same amount (2.53%) participant also made minor errors (LFN) on the 1-9 AFB/100 field slides on all positive slides 7.61% minor error (QE) reported (Table 5).

Table5. Type of errors of participant in detecting Tb bacilli by health institution, (N=67)

Health Institutions	Major error		Miner error			Total error No (%)
	HFP No (%)	HFN No (%)	LFP No (%)	LFN No (%)	QE No (%)	
Hospitals	1(0.23)	3(0.68)	14(3.2)	12(2.7)	29(6.6)	59(13.40)
Health center	1(0.43)	2(0.87)	3(1.3)	5(2.17)	22(9.62)	33(14.34)
Total	2(0.29)	5(0.75)	17(2.53)	17(2.53)	51(7.61)	92(13.74)
	7(1.04)		85(12.70)			

HFN, High False Negative; HFP, High False Positive; LFN, Low False Negative; LFP, Low False Positive; QE, Quantification Error

Agreement of the overall participants with the reference reading on TB detection was 92.67% (Kappa = 0.80). The lowest agreement on detection was found among working in hospital 90.2% agreement with reference reading (Kappa = 0.76) (Table 6).

Overall, the sensitivity, specificity, positive predictive values (PPV) and negative predictive value (NPV) of participants in detecting TB bacilli as compared to the reference reading were 94.52%, 92.91%, 95.23% and 91.88% respectively. Agreement with reference was 92.67% (Kappa = 0.80) on detection of TB bacilli. Assessment across institutions as shown in Table 4, it is almost similar within institution.

Table6. Overall sensitivity, specificity, predictive value and agreement of participants in detecting TB bacilli by health institution. at Hawassa and Hawassa Zuriya, Southern Ethiopia, 2016.

Health institution	Participant reader	Reference reader		Total	Sensitivity	Specificity	PPV*	NPV**	Agreement	Kappa
		Positive	Negative							
Health center	Positive	131	4	135	94.93	95.65	97.03	92.63	95.37	0.84
	Negative	7	88	95						
	Total	138	92	230						
Hospital	Positive	249	15	264	94.32	91.47	94.31	91.47	90.20	0.76
	Negative	15	161	176						
	Total	264	176	440						
Total	positive	380	19	399	94.52	92.91	95.23	91.88	92.67	0.80
	Negative	22	249	271						
	Total	402	268	670						

PPV*= Positive predictive value

NPV** = negative predictive value

According to IUATLD/WHO recommended grading of sputum smear microscopy results, 53(79.1%) of the participants were rated as passed, 14(20.9%) were failed. Among 44 participants who worked at hospital, 41(93.2%) were passed, 3(16.8%) were failed. About 82.6% of participants who had TB microscopy training were passed (Table 4).

4. DISCUSSION

It is well known that grave deficiencies can occur in the laboratory when insufficient attention is given to the quality of the work product. Many countries including Ethiopia, however, have no complete laboratory EQA program or do not provide sufficient administrative support and attention [8, 11].

Although new diagnostic technologies' were available, still microscopic examination of sputum smear used in Ethiopia. Therefore, the skill of laboratory personals on AFB examination seriously affects the case management of TB. Consequently, proficiency testing in sputum smear microscopy was essential for a successful TB control program [4].

The overall error in this study was more than 13.74 %. About 80 % of participants are in acceptable passing score which is 80% [9]. The Overall sensitivity, specificity, PPV and NPV of participants in detecting TB bacilli were 94.52%, 92.91%, 95.23% and 91.88% respectively. These findings were in agreement with our previous finding done in Hawassa town southern Ethiopia (12), also with findings in another place in Ethiopia [13- 16], and elsewhere in the world (17-20). Unlike these studies, our findings reveal comparatively high specificity in detection of TB bacilli indicates that there were very few false positive results.

The agreement on detection of TB bacilli compared to reference reading was 92.67% ($\kappa = 0.80$) which is defined as good agreement based on the Kappa index interpretation (21). The overall agreement in the current study was almost comparable with others study in Ethiopia (12, 14, 15, and 16). However, it is higher than a study conducted in Malaysia which reported 88% agreement (19).

Different type of Errors was reported in all levels of health institution almost in similar fashion. Major error were reported by 7(1.04%) of participants, among these 5(0.75 %) were HFN. Even though the major error committed by the participants is lower than national cut-off point, the possible reason for major error could be due to proficiency in identifying AFB. It is recognized that major errors primarily alter the disease classification and management of a patient. The finding we got is almost similar with others report from Ethiopia, and Tanzania [12, 15, 17, 23 and 24]. But quit very small compared to finding to other similar studies in Ethiopia (22) The main drive of an EQA programmer is to identify this error which suggests that Patients with TB case were not treated on time because of diagnosis miss, resulting in suffering due to the disease; further spread of TB to the population, in addition Patients may lose confidence in the health services or a particular laboratory.

The leading error among the minor Error (7.61%) was quantification error and we can say about 76% of all type of error is QE. This shows that lab professionals miss to detect low AFB count from patient sample. Though quantification errors are minor importance, as it doesn't influence case management, this type of error can differentiate the skill of laboratory personals. The possible reason might be the laboratory personals not perform reading in all part of the fields on the other handwork environment and training level of the laboratory professionals also differ. Our finding seems similar with those previously explained studies in type as well percentage of miner error [12, 15, 16, 17 and 24].

5. CONCLUSION

Laboratory safeties are deficient on most laboratories and there are a lot of technical problems observed during on-site observation. Agreement of the participants with reference reading in the detection of TB bacilli was very good compared to national guide line. Though low major error were reported, training and supervisory activities are still important for successful TB control programs.

AUTHORS' CONTRIBUTIONS

All authors participated in proposal writing, data collection, analysis, interpretation and critical review of the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY STATEMENT

All data used in this manuscript can be shared by all researchers. Data sharing allowed to verify the results of an article, replicate the analysis, and conduct secondary analyses.

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