Addis Ababa University, College of Health Sciences

School of Allied Health Sciences

Department of Medical Laboratory Sciences

Performance Evaluation of TB Smear Microscopists at External Quality Assessment Rechecking Laboratories in Ethiopia

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Advisors: Mr Kassu Desta (BSc, MSc, Assistant Professor)

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Research Thesis submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University for Partial Fulfillment to the Requirements for Degree of Masters in Clinical Laboratory Sciences specialty in Diagnostic and Public Health Microbiology.

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Addis Ababa, Ethiopia

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Date: September, 2015

Addis Ababa, Ethiopia

Research Project Submission Form

Name of investigator	Habtamu Asrat Alaba
Full title of the research project	Performance Evaluation of TB Smear Microscopists at EQA Rechecking Laboratories in Ethiopia
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Declaration

I the undersigned agreed and accepted all responsibilities for the scientific and ethical conduct of the research project. I have provided timely progress report to my advisors and seek the necessary advice and approval from my advisors in the course of the research. I have communicated to my advisors and all stakeholders involved in the study including any source of funding for this research. I declare that, this thesis is my own original work.

Name of the student: Habtamu Asrat Alaba

Signature: _____

Date: _____

Approval of the Advisors

Name of advisor: _Mr Kassu Desta (BSc, MSc, Assistant Professor)

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Approval of the Examiners

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I. Acknowledgment

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III. List of Tables

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V. List of Abbreviations

AFB:	Acid Fast Bacilli
AFS:	Acid Fast Stain
ANSV:	Annual Negative Slide Volume
CDR:	Case detection rate
DOTS:	Directly observed Treatment, Short course chemotherapy
DRC:	Democratic Republic of Congo
DTCs:	District Tuberculosis Centers
EPHI:	Ethiopian Public Health Institute
EQA:	External Quality Assessment
FMoH:	Federal ministry of health
HBCs:	High TB burden countries
HFN:	High False Negative
HFP:	High False Positive
HIV:	Human Immunodeficiecny Virus
HPF:	High Power field
LFN:	Low False Negative
LFP:	Low False Positive
LQAS:	Lot Quality Assurance Sampling
LT:	Laboratory Technologist/ Lab Technician
MC:	Microscopy Center
NRL:	National Reference Laboratory
NPV:	Negative predictive value
OSE:	On-Site Evaluation

PPV:	Positive predictive value
PT:	Proficiency Testing
QE:	Quantification Error
RBRC:	Random Blinded Re-checking
RRL:	Regional Reference Laboratory
SNNPR:	Southern nations, nationalities and peoples' region
SPR:	Slide Positivity Rate
TB:	Tuberculosis
WBCs:	White Blood cells
WHO:	World Health Organization
ZN:	Ziehl-Neelsen

VI. Operational Definitions

AFB Microscopy: Sputum smear microscopy for acid fast bacilli by Zeihl Neelsen technique.

Blind Rechecking: A process of collecting routinely examined sputum smear slides using lot quality assurance sampling (LQAS) technique without the knowledge of their results from smear microscopic centers, re-reading and providing feedback on AFB smears microscopic examination performance of the centers by regional reference or designated EQA centers.

Controller: Term used to describe the Regional Laboratory Supervisor or any technician responsible for rechecking slides.

EQA rechecking laboratory: Laboratories that are officially assigned to conduct blinded rechecking. It can be regional laboratories, sub-regional laboratories, hospital laboratories and health center laboratories.

External Quality Assessment (EQA): A process that allows participant laboratories to assess their capabilities by comparing their results with those in other laboratories in the network (RRLs and NRLs) through on-site evaluation of the laboratory, panel testing and blinded rechecking.

Feedback: Process of communicating results of EQA to the original laboratory, including suggestions for possible causes of errors and remedies.

High False Negative (HFN): A 1+ to 3+ positive smear that is misread as negative.

High False Positive (HFP): A negative smear that is misread as 1+ to 3+ positive.

Low False Negative (LFN): A scanty (1- 9 AFB / 100 fields) smear that is misread as negative.

Low False Positive (LFP): A negative smear that is misread as a scanty (1-9 AFB / 100 fields).

Lot Quality Assurance Sampling (LQAS): A statistical method recommended by World Health Organization for identifying the number of routine slides of each microscopy center to be rechecked.

Major error: This type of error is considered the most critical since it has the highest potential impact on patient management, and can result in an incorrect diagnosis or improper management

of a patient. Major errors may indicate gross technical deficiencies, and include both High False Positive and High False Negative errors.

Microscopy Center (MC): A laboratory located at a health center or hospital which provides smear microscopy services to an approximate population of 50,000.

Minor error: In clinical practice, these errors may have some impact on patient management. However, for the purpose of evaluating laboratory performance, this type of error is considered less serious, because of inherent limitations in consistently detecting a few AFB that may be unequally distributed within a smear. The frequency of minor errors may indicate technical deficiencies.

Onsite Evaluation (OSE): Is the EQA in which standard OSE checklist according to the National EQA guideline will be used to assess the overall AFB microscopy service quality and 5 positive and 5 negative slides will be randomly selected from the stored slides and re-checked by the researcher at the facility level to identify their smearing, staining, smear reading skill and to check their microscope quality.

Panel Testing: Sending stained and/or unstained smears from the NRLs to the RRLs and RRLs to MCs to check proficiency in AFB smear microscopy reading and reporting.

Quantification Error (QE): Difference of more than one grade in reading a positive slide between examinee and controller. This is considered as a minor error that generally has no impact on case management.

Regional Reference Laboratory (RRL): Regional or intermediate level laboratory existing usually in the Regional headquarters. The RRL may exist as part of the state public health laboratory if no RRL is available in the Region.

Scanty (Low Positive): Term used in this document to describe 1-9 acid-fast bacilli per 100 fields. These results are reported to the physician as exact number of AFB seen.

TB Smear Microscopist: A professional who is responsible to perform routine AFB smear microscopy and to conduct blinded rechecking at EQA rechecking laboratories.

Abstract

Background: Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It is the second leading cause of death among infectious disease worldwide. According to FMoH 2013/14 report, the TB case detection rate was 53.7% which is below the target set for the year (81.0%). Quality assured sputum smear microscopy is one of essential components of DOTs to detect TB cases. The quality of AFB smear microscopy often dependent on the strength of national TB program that support, train, and monitor the performance of individuals working in the laboratories.

Objective: To assess the performance of TB microscopists at EQA rechecking laboratories in Ethiopia.

Methods: A cross sectional study was conducted on 81 EQA rechecking laboratories in all regions of Ethiopia from April–July, 2015. Panel slides were prepared and validated at the National TB Reference Laboratory (NRL), Ethiopian Public Health Institute (EPHI). Validated panel slides and customized onsite evaluation (OSE) checklist were used to evaluate the performance of microscopists at EQA rechecking laboratories and the laboratories. Data were captured, cleaned and analyzed by SPSS version 20. Chi square test and kappa values were used for comparison purpose. *P value* < 0.05 was considerable to statistically significant.

Results: A total of 389 laboratory professionals form 81 TB EQA rechecking laboratories were participated in the study. Out of 389 study participants; 263 (67.6%) were male, 268 (68.9%) were from hospitals and 241(62%) had greater than five years' work experience on TB smear microscopy services. About 201 (51.7%) participants were BSc degree holders and 319 (82 %) participants were trained in TB smear microscopy in-service training. The overall performance of professionals scored ≥ 80% was 328(83.3%). The overall sensitivity and specificity in detecting TB bacilli were 84.5% and 93.1 %, respectively. The overall percent agreement of participant readers with reference readers were 87.1 (kappa=0.72) which was good agreement. Eighty (20.6%) participants correctly read all ten slides, 156 (40.1%) got 90-95%, 88 (22.6%) participants scored 80-85% and 65 (16.7%) participants scored below 80%. There were 806 (20.7%) total errors which account 143 (3.7 %) major errors and 663 (17%) minor errors. Of 143 major errors; 89 (2.3%) were HFN and 54 (1.4 %) were HFP errors. Of 663 minor errors; 334 (8.6 %) were LFN, 26 (0.7%) were LFP and 303 (7.8%) were QE. Overall achievements of 81 facilities during onsite evaluation were 85.6% with minimum score of 14.8 % and maximum performance of 98.8%. Greater than 80% of rechecking labs had appropriate facility and safety practice for TB bacilli detection.

Conclusion: The overall performance of participants in reading showed good agreement with the reference readers. Overall performance of facilities during onsite evaluation was 85.6%. Overall errors were 20.7% and majority of them were minor errors and the presences of these errors are alarming for TB control program and emphasis should be given for the EQA program. TB suspected cases may be misdiagnosed and detection rate could be reduced falsely with high risk of transmission. Even though gaps were noted on rechecking facilities, there was promising performance of those facilities selected for rechecking services during decentralization.

Key words: AFB, Quality of AFB microscopy, Rechecking laboratories, EQA, and Level of agreement

1. Introduction

1.1. Background

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can also affect other sites as well (extra pulmonary TB). The disease is spread in the air when people who are infected with pulmonary TB expel bacteria. TB remains a major global health problem, responsible for ill health among millions of people each year [1-3]. It is the second leading cause of death among all infectious disease worldwide next to the human immunodeficiency virus (HIV) [1, 3]. According to 2014WHO global TB report, there were 9.0 million new TB cases and 1.5 million TB deaths (1.1 million among HIV-negative people and 0.4 million among HIV-positive people) in 2013. One quarter of the global cases and deaths were in the African Region [1].

In the 2014 WHO report, Ethiopia ranks 10th in TB incidence among 22 high TB burden countries (HBCs) [1, 4]. Total TB case notification in Ethiopia were 131, 677 and out of reported cases, bacteriologically confirmed pulmonary TB were 43, 860; clinically diagnosed pulmonary TB were 45, 464 and extra pulmonary TB were 42, 353. The TB case detection in all forms was 62 % [1].

According to 2013/14 Ethiopian Ministry of health report, a total of 116,633 TB cases (all forms) were reported with a TB case notification rate of 133 per 100,000 populations. Out of the reported 116,633 cases, 34.0% were smear positive pulmonary TB, 34.8% were smear negative pulmonary TB, and 31.2% were extra pulmonary TB. The TB case detection rate was 53.7%, which is below the target set for the year (81.0%) [5]. TB is the fifth causes of death among ten top diseases in Ethiopia next to lower respiratory infections, cancer, diarrheal diseases and malaria [6].The prevalence of smear-positive TB among persons aged 15 years and above was 108/100, 000, whereas the prevalence of bacteriologically confirmed TB in the same age group was 277/100, 000 [7].

In most low- and middle-income countries, smear microscopy remains the foundation of TB diagnosis, despite its relatively low sensitivity and its inability to distinguish between drug-resistant and drug-susceptible strains. Microscopy is also remained essential to monitor TB treatment and a microscopy network with adequate population coverage and high quality

performance is therefore critical [1, 8].In 2013/14 FMoH report, Ethiopia has more than 3471 public health facilities (156 hospitals and 3,315 health centers) which perform TB smear microscopy [5]. Bright field sputum smear microscopy (conventional Ziehl-Neelsen microscopy) is widely available, simple to perform, inexpensive, and requires simple laboratory facilities [1, 8].

Therefore, one of the national TB control strategy recommended by WHO is to pursue high quality Directly observed treatment short course chemotherapy (DOTS) expansion and enhancement by early case detection and diagnosis through quality assured laboratory [9]. The availability and quality of AFB smear microscopy are dependent on national TB programs that support, train, and monitor the testing performance of individual laboratories [10]. Quality assurance of microscopy remains a critical activity of all laboratory networks, and a comprehensive external quality assessment (EQA) program should be implemented that includes on-site evaluation, random blinded rechecking, and panel testing [1, 8, 10].

In Ethiopia, laboratories whose annual negative slide volume (ANSV) is above 300 and Slide positivity rate (SPR) is equal to or above 2.5% are eligible for blind rechecking program. Blind rechecking is performed four times a year and sample slides are collected blindly by woreda TB focal person or by TB supervisor using Lot quality assurance sampling (LQAS) method based on the national guideline [11].There are selected TB rechecking laboratories in the country mandated by the regional health bureaus that act as controllers to reread the blindly collected slides from smear microscopy centers and this research will evaluate the performance of those rechecking laboratories.

1.2. Statement of the Problem

Developing countries in Africa are burdened with high rates of TB and are struggling to provide good quality microscopy service. Inadequate management and support of TB programs and weak laboratory networks are among the reasons that are hampering progresses against the disease [12].TB case detection rate (CDR) is the percentage of newly notified TB cases including relapses to estimated incident cases (all forms). In 2009, 2012/2013 and 2013/14, Ethiopia has targeted CDR to be above 70% (in 2009), 82.7% (in 2012/2013) and 81% (in 2013/2014), respectively. However, the CDR in 2012/2013 and 2013/2014 was 58.9% and 53.7%, respectively, which was far from the targets [13, 14, 15, 5]. Low CDR is often associated with lack of; effective program awareness, active cough identification and quality assured routine diagnosis (like sputum quality, reagent quality, knowledge and capacity of professionals). In Ethiopia, the determinants that are associated with low CDR were not studied well but it is likely to be associated with the above mentioned factors. Therefore, in the present study we were dealing with TB microsopisits performance as part of the factor, quality assured routine diagnosis.

External quality assessment (EQA) programs are needed to ensure that smears are performed properly and results are interpreted correctly and all microscopy centers achieve an accepted level of performance. Effective EQA program requires dedicated and qualified staffs for rechecking of smears. The implementation of EQA for microscopy has the advantage not only of strengthening laboratory networks but of improving diagnostic quality. Although it is expected to recheck a blinded random sample of smears, many countries have either not fully implement rechecking or still use unblinded rechecking and the results of which are ineffective and misleading [12].

Federal Ministry of Health and Ethiopian Public Health Institute decentralized EQA programs to Regional Reference Laboratories and have guided the regions to decentralize further to Sub-regional laboratories and EQA rechecking laboratory levels. This decision was passed with the assumption of that all microscopic centers in the different regions would have a chance of participating in EQA program and improved coverage of EQA can be achieved. The mandate of conducting a rechecking program was given to EQA rechecking laboratories by Regional Health Bureaus (RHB). Following RHBs endorsement, EQA rechecking laboratories have the right to

perform TB EQA blind rechecking by collecting slides from the microscopy centers in their catchment areas. However, this scheme was not clearly set criteria in terms of managerial and technical capacity of the clinical laboratories while selected as EQA centers/ EQA rechecking laboratories. Performance or technical capacity of professionals and the ability of rechecking laboratories were not clearly stated. Therefore, this research product gives a baseline data about the capacities and performance of those TB rechecking laboratories and microscopists.

1.3. Literature Review

A cross-sectional study was conducted in Ethiopia in Southern Nations, Nationalities and Peoples' Regional state (SNNPR) from October 2000 to June 2002 to see the quality of sputum microscopic examinations for AFB. Two thousand two hundred and nine slides [54% (1,184) positive and 46% (1,025) negative slides] were collected from the peripheral laboratories and re-read by regional laboratory. The overall false reading was 3.2% and the overall agreement with regional laboratory was 96.8% showing the nearly perfect level of agreement [16].

Similar study was conducted in Ethiopia in the Eastern Amhara region to see the performance of laboratories on AFB microscopy from October 2010 to April 2011. Seven hundred ninety nine stained smears were randomly collected for rechecking and a set of ten panel slides were sent to 21 microscopic centers to evaluate reading, staining and reporting performance of individuals. Out of 799 randomly selected slides, the overall agreement was 98.4% and the overall false reading was 1.6 %. Panel test scores were 100 %, 80-95 %, 60 % performed by 9, 11, 1 laboratories, respectively. The finding showed that there was acceptable performance in majority of microscopic centers [17].

A prospective cross-sectional study was conducted in Ethiopia Southern Nations, Nationalities and Peoples' Regional state (SNNPR) to evaluate the quality of TB smear microscopic examination from April 23 to June 26, 2012. Eighty one participants were selected, 11(13.6%) correctly reported all panel slides, 70 (86.4%) missed at least one slides. A total of 29.75% (241/810) errors were reported that include major errors of 2.22% (13 HFN; 5 HFP) and minor errors of 27.5% (25 LFN; 60 LFP and138 QE). The sensitivity and specificity of participants in detecting TB bacilli as compared to the reference reading were 91.97 and 80.0 %, respectively. Overall agreement of participants with the reference reading on TB detection was 95.18% (Kappa = 0.73). Agreement of the participants with reference reading in the detection of TB bacilli was good [18].

Similar study was conducted in west Amhara to see the quality of sputum smear microscopy in Public-Private Mix Directly Observed treatment laboratories in July 2013. 370 AFB panel slides were distributed and the result showed that 3.5% false reading and 96.5 % agreement with reference reader (Kappa = 0.92). Moreover, the consistency of reading scanty bacilli slides was

lower (93%) compared to 1+, 2+ and 3+ bacilli. Based on panel testing results, PPM-DOTS site laboratories showed good agreement with the reference laboratory [19].

Another cross-sectional study was conducted in Tanzania to assess the quality of sputum smear microscopy for AFB detection and 600 randomly selected slides from peripheral laboratories were blindly rechecked by intermediate and central laboratories. The overall agreement in reading was 89.2%. The finding showed that there were poor performances and activities should be done to improve the quality of the microscopy service [20].

In a related study done in Ghana to assess the situation of TB microscopy centers in the country and a total of 114 laboratories were visited between 2000 and 2001 to assess the smear preparation and reading ability. The overall reading agreement rate in reading was 73%. There were 13% false-negative rates and 14% false-positive rates. Most of the false results were high false-negative and false-positive. The study indicated that the need to improve the quality of TB laboratory services and to establish quality assurance system in Ghana [21].

A study conducted in Kinshasa, Democratic Republic of Congo to evaluate the impact of external quality assessment guidelines for resource poor settings. A total of 741 slides were collected from the peripheral laboratories by using EQA guideline and rechecked by the national reference laboratory, there were 77 (10.4%) discrepant results. Discrepant slides were sent to supranational reference laboratory, 67 (87%) of these discrepant results were attributed to the peripheral laboratory and 10 (13%) were attributed to the national reference laboratory. The study showed that blind rechecking allows an unbiased and representative evaluation of the quality of sputum microscopy and identification of underlying problems [22].

Retrospective study was conducted in Taiwan to evaluate the quality of sputum smear microscopy in nine laboratories. Rechecking of 981 readable slides in 2005 identified 3 (0.3%) high false-negatives, 3 (16.7%) low false-positives and 26 (2.8%) low false-negatives; after training provided the corresponding errors were 3 (0.3%), 8 (28.6%) and 12 (1.3%) for the 972 slides rechecked in 2006.The study showed that technical training and EQA improved the quality of sputum smear microscopy services [23].

A study was done in India to assess the proficiency of Senior TB Laboratory Supervisors and district level Laboratory Technicians in sputum smear microscopy by using proficiency panel testing and on-site evaluation from January 2005 to June 2009. High level performance in ZN smear grading was found in district laboratory staffs with overall agreement level of more than 98%. The study showed that laboratory supervisor's proficiency should be quickly assessed by different quality assurance systems of sputum smear microscopy. The proficiency of district laboratory staff readers' was high level of precision and excellent consistency [24].

A study was done in Mexico to evaluate the implementation of proficiency testing in conjunction with a rechecking system for external quality assurance in tuberculosis laboratories in 2001. Different types of errors were encountered during the panel testing process and most common type of errors was quantification errors, followed by low false negatives (with 4.1% HFN, 0.9 % HFP, 5.7% LFN, 2.4% LFP and 12.3% EQ) [25].

A study was conducted in India to assess the facilities by using on-site evaluation method with three rounds from January 2005 to December 2010. The Gujarat district tuberculosis centers (DTCs) achieved an overall score of 86% (820/957) during the initial on-site evaluation visits which consistently improved to 88% (842/ 957) and 92% (885/957) during the two follow-up on-site evaluation visits along with sustenance and improvement in many important laboratory parameters [26].

1.4. Significance of the Study

This study result provides significant and tangible information for different stakeholders about the performance of smear microscopists in the laboratory and the capacity of the laboratory so that improvement projects can be planned and corrective measures can be taken. In addition, recommendations that indicate the necessity of implementation of continuous competency assessments are given to make the testing service and EQA program sustainable and reliable.

Therefore, evaluating the performance of microscopists in TB detection can have significance importance for tuberculosis control activities and help to identify area of improvement for better and effective tuberculosis control program.

2. Objectives

2.1. General objective

- To evaluate the performance of TB smear microscopists involved in EQA rechecking and EQA laboratories in Ethiopia.

2.2. Specific objectives

- To assess the detection and quantification capacity of smear microscopists with their work experience and educational level
- To compare the performance of smear microscopists working at different health facilities
- To assess TB rechecking laboratories using on-site evaluation checklist

Hypothesis

We hypothesized that, the overall performance of microscopists in TB EQA rechecking laboratories in Ethiopia is greater or equal to 80 %.

3. Methodology

3.1. Study Design

A cross sectional study was conducted from April to July 2015 in TB EQA rechecking laboratories in Ethiopia.

3.2. Study Area and Setting

The study was conducted in the TB EQA rechecking laboratories all over the country which include all regional laboratories, sub regional laboratories, selected hospitals (for rechecking) and selected health centers (for rechecking).

Ethiopia has an estimated 94 million populations [1] and the country is administratively structured with nine regional states and two city administrations. Its geographical diversity and topographic features range from the highest peak Ras Dashen, 4,550 meters above sea level to down the Afar valley, 110 meters below sea level. The climate varies with the topography, from as high as 47 degrees Celsius in the Afar Depression to as low as 10 degrees Celsius in the highlands. The total surface area of the country is about 1.1 million square kilometers [27].

The study included 33 sites from Amhara region, 28 sites from Oromia region, 8 sites from Tigray region, 5 sites from southern nations nationalities and peoples region, two sits from Benishangul gumuz region, one site each from Afar region, Harari region, Somali region, Gambella region, Dire dawa city administration and Addis Ababa city administration.

3.3. Population

3.3.1. Source Population

The source population was all laboratory professionals working in the TB EQA rechecking laboratories all over the country.

3.3.2. Study population

The study population was all TB smear microscopists participated in TB slide rechecking and all EQA rechecking laboratories in the country.

3.4. Sampling and Sample size Determination

There were 125 TB EQA rechecking laboratories (12 regional laboratories & 4 sub-regional laboratories, 75 hospitals and 34 health centers) in the country which this study took as study

populations. Stratified random sampling technique was used to select study participant facilities. The strata were regional laboratories, sub-regional laboratories, hospitals and health centers. All regional and sub-regional laboratories were included in the study due to small in number. After stratification, hospitals and health centers were selected based on the number of their catchment microscopy centers (MCs) and those facilities that covered ≥5 MCs were selected for the study. 65% of the study population (82 facilities and smear microscopists working in these facilities) which consisted of all regional & sub regional laboratories, 46 (61%) of hospitals and 20 (59%) of health centers were included in the study. Adequate and representative sample were taken from hospital and health center populations.

3.5. Participant Inclusion and Exclusion Criteria

3.5.1. Inclusion Criteria

All smear microscopists at TB EQA rechecking laboratories who are engaged in TB slide rechecking and all TB EQA rechecking laboratories.

3.5.2. Exclusion Criteria

Smear microscopists at TB EQA rechecking laboratories who were not volunteer to participate in the study, those who were not available during study time and TB rechecking laboratories providing rechecking service less than one year during the study period.

3.6. Variables of the study

3.6.1. Dependent variable

AFB smear microscopy performance of laboratory professionals and the status of TB EQA rechecking laboratories.

3.6.2. Independent Variables

Educational background, work experience, place of work (Regional laboratory, Sub-regional laboratory, Hospital or Health center) and in-service training on TB smear microscopy were independent variables. .

3.7. Methods of Data Collection

3.7.1. Panel Testing

Panel testing was done by preparing stained slides from the reference laboratory and distributed to participant laboratories in this study to assess reading and interpretation proficiency of professionals (smear microscopists) [10-11].

3.7.1.1. Validation of Panel slides

Panel slides were prepared in the National TB reference laboratory and each dilution panels validated by six different readers [10]. The readers had above five years' work experience in AFB smear microscopy and their educational qualification were BSc and above. The detail panel slide preparation and validation procedure is indicated in **Annex-1**[10-11].

3.7.1.2. Volume of panel slides

Two sets of validated panel slides (one panel set was included as a reserve in case of breakage) were prepared for each of 82 rechecking laboratories and every volunteer smear microscopists working in the rechecking laboratories were read a set of panel slides turn by turn. Verbal instruction was given for microscopists to read independently. A total of 1640 panel slides were prepared in national TB reference laboratory (492 negative slides, 492 scanty (1-9/100fields) slides, 328 slides graded 1+, 164 slides graded 2+ and 164 slides graded 3+) and one microscopist was read a set of 10 panel slides.

3.7.1.3. Panel slides composition and Interpretation

A set of 10 stained and validated panel slides were given for each microscopist and 50-70 minutes were allowed to complete the reading [10-11]. The panel composition and load of bacilli based on their grading were 1 slide 3+, 1 slide 2+, 2 slides 1+, 3 slides 1-9/100 fields and 3 negative slides [10].

The results were expressed as correct, minor error or major error. Major errors were classified as high false positive (HFP) if a negative smears misread as 1+ to 3+ positive and high false negative (HFN) if a 1+ to 3+ positive smears was misread as negative. Minor errors were classified as quantification error (QE) when there was a difference of more than one grade in a reading of positive smear between examinee and controller, low false positive (LFP) when a

negative smears was misread as scanty (1-9 AFB/100 field) and low false negative (LFN) when a scanty (1- 9 AFB/100 field) was misread as negative (Table 3.1) [10, 11, 28]. Ziehel-neelsen reagent preparation and staining procedures is indicated in **Annex-2** [29].

Table 3.1: Evaluation and interpretation of errors between controllers and microscopists

Result of Microscopist	**Result of Controller (NTRL)**				
	Negative	**1-9AFB/ 100 fields**	**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

3.7.1.4. Scores for Grading

A set of 10 panel slides, each slide worth 10 points, total possible scores were 100 (for 10 slides) and the passing score were 80 % and above. Committing major errors like high false positive (HFP) and high false negative (HFN) worth zero point whereas minor errors like low false positive (LFP), low false negative (LFN) and quantification errors (QE) worth 5 points [8, 10, 11]. Panel slide result and demographic information collection form is attached in **Annex-3.**

3.7.2. On-site Evaluation (OSE)

In this study, onsite evaluation was performed using standardized checklist for observation of overall processes under actual conditions and facility set up [10-11].On-site evaluation (OSE) checklist is attached in **Annex-4.**

3.8. Data Quality

For panel test; the standard stained smears with known grades of bacilli were prepared and validated by National TB reference laboratory. On-site evaluation for facility setup of TB EQA rechecking laboratories were assessed by using standard checklists and experienced TB microscopists. Before data collection period proper one day training/ orientation were given by principal investigator for data collectors to avoid bias.

3.9. Data Entry, Storage and Management

All data were recorded on a logbook during the study period and the data were stored in a CD-RW and USB drive as a backup. The logbook and CD were stored in lockable shelves and only the investigator has an access to the files. Cross-checking and data cleaning were done.

3.10. Data Analysis

All data were entered to excel sheet and transported to SPSS version 20.0 for analysis. The percentages of agreements, differences and the different types of errors were calculated for EQA rechecking laboratories. The sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) of smear reading by TB EQA smear microscopist was calculated. Chi square test were used to see any association with different variables. The strength of an agreement between participant readers and the reference readers were assessed using kappa statistics and the results were interpreted as poor agreement (if K<0.20), Fair agreement (if K= 0.20 - 0.39), Moderate agreement (if K=0.40 – 0.59), Good agreement (if K= 0.60 – 0.79) and Very good agreement (if K= 0.80 – 1.00) [30].

3.11. Ethical Consideration

For panel preparation, leftover samples were collected from federal hospitals anonymously. All information of each TB EQA rechecking laboratory were kept confidential and used only for the study purpose and for improvement of AFB microscopy. The research proposal was evaluated and approved by the research and ethical committee of department of medical Laboratory Science and reviewed and cleared by Institution of Review Board (IRB) before the start of fieldwork.

Addis Ababa University School of Allied health sciences, Department of Medical Laboratory Sciences wrote support letter to Ethiopian Public Health Institute for the purpose of panel slide

preparation and batch validation. Ethiopian Public Health Institute wrote official letter for participant sites for their good office. Confidentiality was maintained during data collection and written informed consent was also obtained from each study participants **(Annex 5& 6)**.

3.12. Dissemination of Results

The first copy of the finding was submitted to Addis Ababa University Department of Medical Laboratory Sciences and used for references for students. The other copies of the study were given to Ethiopian Public health institute, Ministry of Health and regional health bureaus and the information used for program revision and help to plan intervention activities for identified gaps.

Finally findings will be presented in different scientific seminars, scientific conferences, workshops, and published in reputable scientific journals.

4. Result

4.1. Study Participants

From a total 82 rechecking laboratories selected for the study, one facility was excluded from the study by exclusion criteria during data collection period. A total of 389 laboratory professionals (2 to 13 laboratory professionals per rechecking laboratory) from 81 TB EQA rechecking laboratories were participated in the study. Out of 389 professionals; 263 (67.6%) were male participants, the majority of the study participants (268 (68.9%)) were from hospitals, 241 (62%) of participants had greater than five years' work experience on TB smear microscopy services, 201 (51.7%) were BSc degree holders and 319 (82 %) participants took TB smear microscopy in-service training. Table 5.1 below summarizes the demographic characteristics of study participants.

Table 5.1: Demographic characteristics of laboratory professionals in TB EQA rechecking laboratories in Ethiopia (**N= 389**), April- July, 2015.

Variable	**Frequency**	
	Number	**Percent**
Sex		
Male	263	67.6
Female	126	32.4
Place of Work		
Regional & sub-regional Laboratory	62	15.9
Hospital	268	68.9
Health Center	59	15.2

Work Experience		
< 2 years	23	5.9
2- 5 years	125	32.1
>5 years	241	62.0
Educational Background		
Diploma	169	43.4
Degree	201	51.7
MSc	19	4.9
TB Smear Microscopy in-service training		
Yes	319	82.0
No	70	18.0

4.2. Panel Testing

Based on the national and WHO TB EQA guidelines [10-11], performance of participants in proficiency testing should be ≥ 80% to get a passing score. Among 389 participants the overall performance of professionals scored ≥ 80% was 328(83.3%). The performance of participants based on their place of work showed that, higher number (231 (86.2%)) of hospital participants achieved the passing score (≥ 80%) than other facility participants. On the other hand 21 (91.3%) professionals with less than 2 years' work experiences scored ≥ 80% which was higher than those professionals whose work experience were above 2 years'. The number of participants scored ≥ 80% was higher in MSc than others and the number of participants who perform ≥ 80% was slightly higher in those participants who didn't take TB smear microscopy in-service

training. In general there were no statistically significant associations between performances of participants in TB bacilli detection and their sex, work experience, educational background, place of work and TB smear microscopy in-service training. The relationship between participant scores and demographic characteristics is summarized in table 5.2.

Table 5.2: Relationship between demographic characteristics and participant scores in TB EQA rechecking laboratories in Ethiopia (**N=389**), April –July, 2015.

Variable	Passed ≥ 80% #(%)	Failed < 80% #(%)	Chi-square	Degree of Freedom	P-Value
Sex					
Male	221 (84.0)	42 (16.0)	0.319	1	0.572
Female	103 (81.7)	23 (18.3)			
Place of Work					
Regional & sub-regional Laboratory	49 (79)	13 (21)			
Hospital	231 (86.2)	37 (13.8)	5.650	2	0.059
Health Center	44 (74.6)	15 (25.4)			
Work Experience					
< 2 years	21 (91.3)	2 (8.7)			
2- 5 years	107 (85.6)	18 (14.4)	2.207	2	0.332
>5 years	196 (81.3)	45 (18.7)			
Educational Background					
Diploma	136 (80.5)	33 (19.5)			

Degree	171 (85.1)	30 (14.9)	1.945	2	0.378
MSc	17 (89.5)	2 (10.5)			
TB Smear Microscopy in-service training					
Yes	265 (83.1)	54 (16.9)	0.061	1	0.805
No	59 (84.3)	11 (15.7)			
Overall performance					
	324 (83.3)	65 (16.7)			

A total of 3890 validated slides were read by study participants and the overall sensitivity and specificity in detecting TB bacilli were 84.5% and 93.1 %, respectively. The overall percent agreement of participant readers with reference readers were 87.1 (kappa=0.72). Percent agreement of health center readers with reference readers were 83.1% (kappa=0.64) slightly lower than hospital and regional laboratory readers (Table 5.3).

Table 5.3: Overall sensitivity, specificity, predictive values and agreements of participants with reference readers in detecting TB bacilli, April- July, 2015.

Health Facility Laboratory Professionals		Reference Readers		Total	Sensitivity (%)	Specificity (%)	PPV	NPV	Percent Agreement	Kappa
		Positive	Negative							
Reg. & sub-reg. Lab	Positive	376	16	392						
	Negative	58	170	228	86.6	91.4	95.9	74.6	88.1	0.73
	Total	434	186	620						
Hospital	Positive	1598	51	1649						
	Negative	278	753	1031	85.2	93.7	96.9	73.0	87.7	0.73
	Total	1876	804	2680						
Health Center	Positive	326	13	339						
	Negative	87	164	251	78.9	92.7	96.2	65.3	83.1	0.64
	Total	413	177	590						
Overall	Positive	2300	80	2380						
	Negative	423	1087	1510	84.5	93.1	96.6	72.0	87.1	0.72
	Total	2723	1167	3890						

From a total of 389 study groups; 80 (20.6%) participants correctly read all ten slides and score 100%, 156 (40.1%) got 90-95% which means they committed one major error or two minor errors, 88 (22.6%) participants scored 80-85% which means they committed 3-4 minor errors or 2 major errors or 1 major & 1 minor errors or 1 major & 2 minor errors and 65 (16.7%) participants scored below 80% which means they had errors more than4 minor errors or 2 major errors or 1 major & 2 minor errors. Figure 5.1 showed summarized data on concordance of participants with national laboratory.

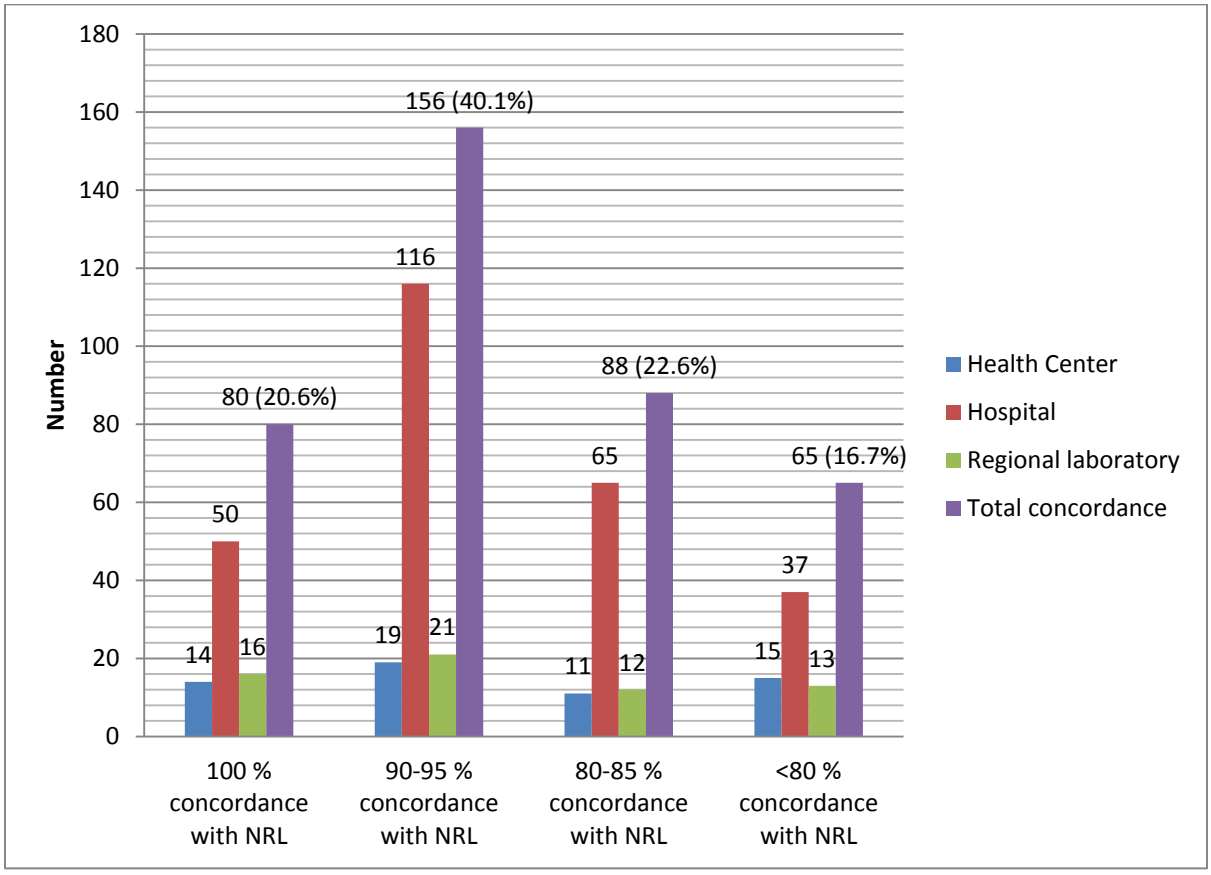

Figure 5.1: Concordance of participants with reference readers (NRL) in detecting TB bacilli in TB EQA rechecking laboratories in Ethiopia (**N=389**), April- July, 2015.

From a total of 3890 examined slides, there were 806 (20.7%) total errors which account 143 (3.7 %) major errors and 663 (17%) minor errors. Of 143 major errors; 89 (2.3%) were high false negative and 54 (1.4 %) were high false positive errors. Of 663 minor errors; 334 (8.6 %) were low false negative, 26 (0.7%) were low false positive and 303 (7.8%) were quantification errors (Table 5.4).

Table 5.4: Type of errors committed by participants during detecting TB bacilli in TB EQA rechecking laboratories of different institutions in Ethiopia (**N=3890 slides**), April- July, 2015.

Health Facility	Major error		Minor error			Total error
	HFN	HFP	LFN	LFP	QE	No. (%)
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Reg. & sub-reg. Lab (n=620)	11 (1.8)	11(1.8)	47 (7.6)	5 (0.8)	75(12.1)	149 (24)
Hospital (n=2680)	62(2.3)	31(1.2)	216(8.1)	20(0.7)	194(7.2)	523(19.5)
Health Center (n=590)	16(2.7)	12(2.0)	71(12)	1(0.2)	34(5.8)	134 (22.7)
Total	89 (2.3)	54 (1.4)	334 (8.6)	26 (0.7)	303 (7.8)	806 (20.7)
	143 (3.7)		663 (17)			

4.3. On-site Evaluation

A total of 81 TB EQA rechecking laboratories were assessed during on-site evaluation by using a standardized check list (Annex- 4). Of 81 facilities, the evaluation covered 15 regional &sub-regional laboratories, 46 hospital laboratories and 20 he alth center laboratories. Overall scores during ons ite e valuation w ere 85.6 % w ith m inimum s core of 14.8 % f or the pr esence o f equipment for panel testing slide preparation and maximum performance of 98.8% for presence of personal pr otective equipment's (PPE), Spirit l amp or B unsen bur ner, d isinfectants an d staining/ smearing equipment's (racks, loops, sticks). Greater than 80% of rechecking labs had

appropriate facility and safety practice for TB bacilli detection. Only 14.8 %, 72.8% and 76.5% of f acilities ha d e quipment f or preparation of proficiency t esting slides, distilled w ater and equipment f or preparation of r eagents (weighing balance, measuring cy linder), re spectively (Table 5.5).

Table 5.5: Summarized on-site a ssessment r esult o f T B EQA r echecking laboratories w ith standardize checklist in Ethiopia (**N=81**), April- July, 2015.

S. No	Item	Reg. & sub reg. Lab (n=15)		Hospital (n=46)		Health Center (n=20)		Total (N=81)	
		Frequency		Frequency		Frequency		Frequency	
1	**Facility and Safety**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**
	Separate area for TB laboratory work	14	93.3	40	87	16	80	70	86.4
	Separate tables for specimen receipt/smear preparation/ microscopy	15	100	42	91.3	15	75	72	88.9
	Uninterrupted power supply	12	80	41	89.1	13	65	66	81.5
	Uninterrupted running water supply	12	80	37	80.4	17	85	66	81.5
	Waste containers with lid	14	93.3	45	97.8	18	90	77	95.1
	Waste disposal by Autoclave/disinfection/buried	15	100	45	97.8	18	90	78	96.3
	General order/cleanliness	14	93.3	44	95.7	12	60	70	86.4
	Personal protective Equipments used and practices(aprons, hand wash, etc)	15	100	45	97.8	20	100	80	98.8
2	**Manuals, Standard Operating Procedure, Job Aids**								
	Standard operating procedure for smear preparation and staining	13	86.7	46	100	14	70	73	90.1
	Grading chart, TB smear microscopy job aids posted and used	12	80	45	97.8	18	90	75	92.6
	EQA Protocol and training manual available and followed	15	100	33	71.7	11	55	59	72.8
	Sufficient EQA forms	15	100	45	97.8	19	95	79	97.5

3	**Adequate stock and supply of Staining reagents / equipment**								
	Slides	14	93.3	42	91.3	20	100	76	93.8
	Lens Tissue	12	80	38	82.6	17	85	67	82.7
	Filter paper	13	86.7	42	91.3	18	90	73	90.1
	Spirit lamp or Bunsen burner	15	100	46	100	19	95	80	98.8
	Immersion oil	12	80	46	100	20	100	78	96.3
	Disinfectants	14	93.3	46	100	20	100	80	98.8
	Smearing/staining equipment (staining racks, loops, sticks etc)	15	100	45	97.8	20	100	80	98.8
	Slide boxes	14	93.3	44	95.7	20	100	78	96.3
	Auramin O	13	86.7	45	97.8	20	100	78	96.3
	Potassium permanganate	13	86.7	46	100	20	100	79	97.5
	Acid alcohol	13	86.7	46	100	20	100	79	97.5
	Distilled water	10	66.7	35	76.1	14	70	59	72.8
	Equipment for preparation of stains/ reagents such as balance (for weighing reagents), measuring cylinders etc	15	100	35	76.1	12	60	62	76.5
	Equipment for preparation of panel testing slides	7	46.7	5	10.9	0	0	12	14.8
	Number of functional Microscope	2-21		1-9		1-5		1-21	
4	**Internal Quality Control and External Quality Assessment**								
	Control smears are used for each new batch of stain	14	93.3	45	97.8	20	100	79	97.5
	Control smears are used for once every week for checking the quality of stain	8	53.3	43	93.5	20	100	71	87.7
	Laboratory participated in External Quality Assessment	11	73.3	44	95.7	20	100	75	92.6
	All MCs are visited at least once in every six month by your staff, as per their tour program	11	73.3	36	78.3	19	95	67	82.7

5. Discussion

This cross- sectional study was aimed to evaluate the performance of TB smear microscopists working at EQA rechecking laboratories and the status of the respective laboratories using panel tests for microscopists and standard questionnaires for on-site evaluation. In this study, the overall agreement of participants in reading the validated slides with reference readers was 87.1% (kappa=0.72) which was good agreement based on kappa statistics [30]. However, lower agreement was observed when compared with a different study conducted by Shargie *et al.,* in Southern Ethiopia which was 96.8% (kappa=0.936) [16] and also done by Hailemariam *et al.,* in Hawassa town, Ethiopia which was 95.18% (kappa=0.73) [18]. When compared with the study of East and West Amhara region, Ethiopia done by Mulat *et al* and Manalebh *et al.*, higher agreement than ours was observed which were 98.4% and 96.5% (kappa=0.92), respectively [17, 19]. The performance in the present study was slightly lower than similar studies conducted in different parts of Ethiopia. This probably due to quiet big number of laboratories and/or laboratory professionals was included in present study. It is more of a nationwide study and might be more representative than others.

Our finding was also lower than the study done by Dave *et al.,* in India [24] and Basra *et al.,* in Tanzania [20] which was 98% and 89.2%, respectively. It was higher in reading agreement than the study done by Addo *et al.,* in Ghana (73%) and Rie *et al.,* in democratic republic of Congo (DRC) which was 74% [21-22]. In general agreement in reading was slightly lower than other similar studies; this might be due to panel slide compositions as we prepared a second degree of difficulty slides (three scanty and three negative slides) for this study [10].

In our study, the overall sensitivity and specificity were 84.5% and 93.1%, respectively. The finding by Hailemariam *et al.,* in Hawassa town, Ethiopia showed higher sensitivity (91.97%) but lower in specificity (80.0%) [18]. On the other hand, both sensitivity and specificity was higher in West Amhara, Ethiopia by Manalebh *et al.,* report which was 96.5 % and 96.4%, respectively [19]. Similarly, the study conducted by Mulat *et al.,* in East Amhara, Ethiopia showed higher sensitivity (88.4%) and specificity (99.3 %) [17]. Both sensitivity and specificity was 96.8% in southern Ethiopia by Shargie *et al.,* finding which was higher than our study [16]. Sensitivity and specificity was higher in the study conducted by Basra *et al.,* in Tanzania which was 88.5 % and 100%, respectively [20]. In this study sensitivity was lower and it indicates that

there were high false negative rates or patients with TB bacilli misdiagnosed as negative. Low in sensitivity has a consequence of TB patients not treated resulting on-going disease, disease transmission or death.

Out of 389 study participants, 80 (20.6%) correctly read all their panel slides (100% concordant with national reference laboratory) and 156 (40.1%) scored 90-95% (they committed at least one error). Hailemariam *et al.,* in Hawassa town, Ethiopia reported 13.6% were correctly read all panel slides which were slightly lower than our finding and 86.4% committed at least one error among 10 slides which were significantly higher than our finding [18].In the study done by Dave *et al.,* in India, 95% of the readers had reported with no errors [24] and this was excellent proficiency than the present study. Even though majority of participants (83.3%) scored acceptable performance (≥80%) there was an implication of weak achievement. Because study participants were from facilities who have a responsibility to recheck other health institutions slides and provide support to them. With this responsibility participants supposed to score better than the current findings.

In the present study, out of 3890 slides there were 806 (20.7%) total errors which account143 (3.7%) major errors and 663 (17%) minor errors. The false reading (false positive and false negative) was 503 (12.9 %). Of 143 major errors, 89 (2.3%) were high false negative (HFN) and 54 (1.4 %) were high false positive (HFP) errors. Minor errors were 334 (8.6%) low false negative (LFN), 26 (0.7%) low false positive (LFP) and 303 (7.8%) quantification errors (QE).In the present study LFN were higher followed by QE. False reading by Shargie *et al.,* in Southern Ethiopia, Mulat *et al.,* in Eastern Amhara and Manalebh *et al.,* in West Amhara, Ethiopia was 1.6% ,3.2% and 3.6%, respectively which was lower than our finding [16, 17, 18]. On the other hand, 29.75% errors were found by Hailemariam *et al.,* in Hawassa town, Ethiopia which was higher than ours and when we saw major errors, the finding was lower (2.22 %) and minor errors were higher (27.5%) than ours and QE were the most contributing error while in the present finding LFN were most frequent errors [18]. Fewer errors were observed in the study done by Dave *et al.,* in India and out of which QE were the frequent error with no HFP [24]. In similarly study conducted by Martinez-Guarneros *et al.,* in Mexico, there were errors with frequently occurring of QE (12.3%) followed by LFN (5.7%) [25] and by Rie *et al.,* in DRC with LFN

frequent errors [22]. In another study conducted by Wu *et al.,* in Taiwan, low false positive errors were much higher (28.6%) than the present study [23].

False negative error reports could lead to failure to detect persons with infectious TB, who may continue to spread infections in their communities, while false positives could lead to unnecessary anxiety, exposure of the patient to unwanted side effect of medication, and unnecessary expenditures [31]. In EQA, any major error (HFP or HFN) or any HFP with more than three LFN is not acceptable performance, but lower rates of minor errors can be acceptable due to the inherent problems with AFB smear microscopy. Larger numbers of minor errors may represent performance problems, and it may be useful to address these issues. Unlike QE, false negative and false positive errors have significant impact on patient management as well as the TB control program. Hence, improving the competency of professionals' through training, implementation of EQA, supportive supervision, mentoring and other interventions are critically important to reduce or avoid these types of errors [10].

In the present study, the overall achievements during onsite evaluation were 85.6%.The study conducted by Mulat *et al.,* in East Amhara, Ethiopia showed lower (69.2%) performance than our finding [17]. Comparative overall score (86%) were observed by Patel *et al.,* in India with the current study [26] but have higher score (100%) for presence of power supply, slides, lens tissue, spirit lamp or Bunsen burner. The most common problems encountered during Rie *et al.,* study in DRC were shortages of materials (distilled water, lens tissue, and disinfectants) and the unavailability or the poor condition of the necessary equipment (wire loops, staining racks, a biohazard waste bin, and a microscope) [22]. Laboratories internal quality control practice in this study was higher (87.7%) than the study done by Manalebh *et al.,* and Shiferaw *et al.,* in West Amhara, Ethiopia; which was 54% and 83.1%, respectively [19, 32]. In our study, 81.5% of facilities had uninterrupted power and water supply whereas Shiferaw *et al.,* in West Amhara reported lower supply of power and water which was 56.7% and 18.4%, respectively [32]. In general the current on-site assessment findings were promising and continuous support is needed to fill those identified gaps.

6. Limitation of the Study

Some microscopists in the TB E QA rechecking laboratories were not included in the study because they were not volunteer to participate. Slide rechecking was not conducted in the facilities due to shifting from Ziehl-Neelsen to fluorescent microscopy technique so that smearing and staining quality of slides in the facilities was not evaluated. Unstained slides were not sent to participant laboratories and we didn't see the quality of reagents they used for routine service.

7. Conclusion and Recommendation

7.1. Conclusion

The overall performance of TB EQA rechecking laboratories in reading showed good agreement with the reference readers (87.1%) and overall facility assessment results were 85.6%. Overall errors were 20.7% and majority of them were minor errors and the presences of these errors are alarming for TB control program and emphasis should be given for the EQA program. Most of TB suspected cases may be misdiagnosed and detection rate may be reduced with high risk of disease transmission.

This cleared that, though overall performances are acceptable, errors that should require corrections and follow up measures are indicated. As TB is a global threat these days, detection errors have serious consequences so that all steps to improve the diagnosis performance of the laboratories should be considered. Even though gaps were noted on rechecking facilities, there was promising performance of those facilities selected for rechecking services during decentralization.

7.2. Recommendations

- Although the overall agreement of participants with reference readers is good, improvement plan should be designed by responsible organization to score better than the current performances.
- Huge amount of minor errors were noted and continuous monitoring and support should be given by the responsible body to minimize errors and improve EQA activities in Ethiopia.
- Attention should be given for EQA rechecking laboratories to equip them by necessary materials for routine microscopy service and rechecking purpose.

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9. Annexes

Annex-1: Panel preparation and Validation procedure

NaOH Method

1. Materials Required

Note: Processing should be performed in a Biological Safety Cabinet.

- 50 ml plastic screw cap tubes
- 40% Formaldehyde
- 4% NaOH
- Vortex
- Water bath at 55-60°C
- Distilled water
- Centrifuge
- Slides

Positive specimen (fresh specimens, no more than 2 days old, are preferred)

Amount: 3 ml or more;

AFB load: >2+ AFB by Ziehl-Neelsen direct smear;

Color: White to light green; blood stained specimens should be avoided;

Thickness: Watery (less mucous) specimens are preferred to increase consistency.

Negative specimen (fresh specimens, no more than 2 days old, are preferred)

Amount: 5 ml or more;

Color: white to green;

Thickness: Watery (less mucous) specimens are preferred to increase consistency

Note: An AFB negative specimen with 20 or more white blood cells per field is preferred.

2. Preparation of AFB Positive Stock

a) Place 3 ml of AFB positive specimen into a 50 ml screw cap plastic tube. If volume of the specimen is more than 3 ml, aliquot it into separate tubes.

b) Add 1 drop (approx. 50 µl) of 40% Formaldehyde per 1 ml of sputum, vortex well.

c) Incubate for 1 hour at room temperature (25- 30oC).

d) Add 1 ml of 4% NaOH (if the sputum is too thick, add up to 2 ml of NaOH solution so that the final concentration of NaOH is always 1-2%).

e) Vortex thoroughly for 4-5 min.

f) Add up to 20 ml of distilled water, mix well.

g) Incubate in a water bath for 30 min. at 55-60oC, mix occasionally by inverting the tube during incubation. If there is no water bath available, boil a beaker of water, cool to 90-95oC and place the tube in the beaker for 20-25 min. It is important to maintain the incubation temperature in the 55-90oC range.

h) Add distilled water to a total volume of 40 ml, mix by inversion.

i) Centrifuge @ 3,000 x g for 20 min. at room temperature (25-30oC).

j) Decant supernatant carefully; add 0.5-1 ml of distilled water to re-suspend pellets. If initial sputum was aliquoted into portions, pellets from the same specimen are combined, prior to re-suspending.

Note: It is advisable to avoid specimens containing impurities (food remains etc.)

However if the impurities are still found in the sediment after it is dissolved in distilled water, filter the specimen through the gauze and re-centrifuge it.

3. Preparation of AFB Negative Stock

a) Distribute 3-4 ml aliquots of AFB-negative sputum into 50 ml screw cap tubes.

b) Note: Several good quality negative sputa can be pooled together and then split into 3 ml aliquots. Sputa should be checked for AFB prior to pooling.

c) Add 1 drop (approx. 50 µl) of 40% Formaldehyde per 1 ml of sputum, vortex well.

d) Incubate for 1 hour at room temperature (25-30oC).

e) Add 1 ml of 4% NaOH (if the sputum is too thick, add up to 2 ml of NaOH solution so that the final concentration of NaOH is always 1-2%).

f) Vortex for 2-3 min.

g) Add up to 20 ml of distilled water, mix well.

h) Incubate in a water bath for 10 min. at 55-60oC (Note: the negative specimen should be heated for a shorter period than the positive specimen to preserve white blood cells). If there is no water bath available, boil a beaker of water, cool to 90-95oC and place the tube in the beaker for 5-10 min.

This preparation is used as a diluent in the Dilution Procedure (step 5).

4. Evaluation of Positive Stock Preparations

a) If foam has formed on top of the stock solution, pipette the contents from beneath the foam into a fresh tube.

b) Using a standard microbiological loop make 2-3 test smears (approx. 1x2 cm in size) from the suspension for evaluation of the stock preparations.

c) Use a well leveled surface for drying the smears.

Positive stock: It is optimal to have concentration 50-60 AFB per microscope field

5. Dilution Procedure

a) Using negative preparation as a diluent make dilutions according to WHO Guidelines for AFB quantification:

0 AFB/100 fields: negative

1-9 AFB/100 fields: exact # of AFB required

10-99 AFB/100 fields: 1+

1-10 AFB/field: 2+

>10 AFB/field: 3+

b) Choose suitable AFB concentration on a case-to-case basis within suggested range. For better results, however, it may be recommended using 20 AFB/field for 3+ smears, 5 AFB/field for 2+ smear, 50 AFB/100 fields for 1+ smears, and 5 AFB/ 100

fields for “exact” smears.

c) Make 3-4 ml of each suspension in order to be able to generate sufficient amount of smears.

d) For easy calculations both AFB-positive and AFB-negative aliquots are measured in drops. Calibrate one typical disposable Pasteur pipette by measuring the number of drops in 1 ml of sputum suspension. Note: do not use water for calibration since the amount of drops may be different from sputum due to the lack of viscosity.

e) For calculation of the dilution factor use the following formula :

N = (DC / AC) * A

Where:

N - is amount of drops of positive sputum to be added.

DC - is desired AFB concentration.

AC - is actual AFB concentration.

A - is the amount of drops in a given volume that was estimated during calibration.

Example: AFB concentration in the stock suspension (AC) is 65 AFB/field and we have to prepare 4 ml (A = 60 drops) of 2+ suspension (DC=5 AFB/field).

In this case N = (5 AFB /65 AFB)*60 drops

N = 4.6 drops (approx 5 drops). So, 5 drops of the positive prep is mixed with 55(60 - 5 = 55) drops of the negative preparation.

Procedural notes:

1. It is important for reading and interpretation of results that appearance of the smears is more or less consistent, and that is why it would be beneficial to keep the amount of leucocytes as stable as possible in various dilutions. In order to achieve this, it is suggested to dilute negative sputum with distilled water (prior to adding NaOH) when the amount of leukocytes is relatively high and avoid dilution if the amount of

leukocytes is low.

2. It would also useful when making 1+ suspension to consider making two different concentrations: 50 AFB/100 fields for 1+ smear preparation and 15 AFB/100 fields for further dilution to "exact" count smear.

6. Prepare and Validate Batches of Slides

a) Using diluted stock preparations, prepare slide batches (50-100 slides per batch is recommended). **Note:** If laboratories are proficient in developing consistent slides, then developing many slides from fewer samples will help to save time. Heat fixed slides should last for months if stored in a cool/dry location.

b) The consistency of each batch of slides must be validated by selecting a sample of = 6 slides from each batch to be stained and read by different technicians to document consistency. Some samples that are produced and tested will not be of sufficient consistency and should be discarded.

Validation Log for AFB Panel testing slide batches can be used to record results for the test slides and determine if consistency standard is acceptable.

Number of Slides made:there should be a record to indicate how many slides were made from each sample to determine how many slides are available for test slide sets. It recommended that laboratories prepare 50-100 slides so that sufficient slides are available to put duplicate samples in test slide sets.

Date slides made:this is the date that the test slides were produced. The length of time that slides can be stored without affecting performance has not been determined, but we estimate that 4-6 months is practical with proper storage.

Slide test results (columns 1-6) each column represents the number AFB/100 fields for 6 separate slides selected for the sample and preferably read by 2-6 different technicians. For high positives (2+ or 3+) the technicians may estimate the number AFB/100 fields by selecting a sufficient number of representative fields. For low positives (exact count AFB/100 fields and 1+) and AFB negatives slides the technicians should read a minimum of 300 fields per slide and record the average number AFB/100 fields.

Average/Mean: average is computed from slide test results 1-6.

Standard deviation: the standard deviation is computed from slide test results 1-6.

$$\frac{\sqrt{n\sum x^2 - (\sum \mathrm{x})2}}{\mathrm{n}(\mathrm{n}-1)}$$

Consistency: The consistency column result is computed using the following formula:

Mean [M] minus 2 standard deviations [SD]

If M - 2 SD is > 0 then consistency is true (sufficient)

If M - 2 SD is < 0 then consistency is false (insufficient)

If the consistency is false—then there is too much variation in the number of AFB per slide and this sample is not of sufficient consistency to use in a PT test for a reliable evaluation of performance. This formula provides an objective evaluation of consistency, but the laboratory should still review and determine what is acceptable variation within a sample of slides.

Report Result This is the slide test result for all the test slides. This test result should be representative of the 6 slides tested and the sample should meet the consistency criteria.

7. Prepare Panel Testing Sets

Sets of slides with identical composition of positives and negatives can be made from the prepared batches of slides.

Annex-2: Ziehel-Neelsen reagent preparation and staining procedures

Required Materials

Reagents

- Basic fuchsin powder
- Phenol
- Distilled water
- Hydrochloric acid (HCl)
- Methylene blue powder
- Ethanol (95/96%)

Supply and Equipment

- Weighing balance
- Spatula
- Measuring cylinder
- Reagent bottle (Brown or amber)
- Funnel
- Stirrer
- Beaker
- Flask
- Filter paper
- Water bath (optional)

1% Carbol Fuchsin Preparation

The required reagents for preparing 1 liter of 1% Carbol Fuchsin staining solution are the following:

Chemicals	Amount	Grade
Basic fuchsin powder	10g	Certified
Ethanol (or methanol)	100ml	Technical
Phenol crystals*	50g**use colorless not tinted crystals*	Analytical
Distilled water	900ml	

NB: Phenol crystals/vapor is corrosive and toxic may cause burns. Therefore, prepare in a well-ventilated area.

- **Preparation**
  - Add 100ml of ethanol (or methanol) to a one liter glass flask
  - Add 50g of phenol crystals and dissolve
  - Add 10g of basic fuchsin and mix well until dissolved
  - Add distilled water to make one liter
  - Label the bottle "1% carbol fuchsin", date and initial
  - Store in a dark bottle in a cupboard at room temperature (expiry 12 months)

3% Acid Alcohol Preparation

The required reagents to prepare 1 liter of 3% acid alcohol are the following:

Chemicals	Amount	Grade
Fuming hydrochloric acid (HCl)	30ml	Technical

95% ethanol	970ml	Technical

- **Preparation**
  - ❖ Measure 970ml of 95% ethanol and transfer to flask
  - ❖ Measure 30ml of hydrochloric acid (HCI) and add the HCI slowly to the ethanol
  - ❖ Transfer the acid alcohol to a brown/ amber color bottle
  - ❖ Label the bottle : Name (**"3% HCI in ethanol")**, preparation and expiry date(expiry 12 months)
  - ❖ Store in a dark bottle in a cupboard at room temperature

Methylene Blue (0.1%) Preparation

The required reagent for preparing 1 liter of 0.1% Methylene Blue is below:

Chemicals	**Amount**
Methylene blue	1g
Distilled water	1000ml

- **Preparation**
  - ✓ Measure 1000ml of distilled water and transfer to flask
  - ✓ Measure 1gm of methylene blue powder
  - ✓ Add into the flask containing distilled water and mix well
  - ✓ Transfer the prepared **methylene blue** solution to a brown/ amber color bottle
  - ✓ Label th e b ottle : N ame (**"0.1% methylene blue")**, pr eparation a nd e xpiry da te (expiry 1 2 months)
  - ✓ Store in a dark bottle in a cupboard at room temperature

Staining Procedure

1. Place the slides on the slide rack with the smeared slide upward, their edges separated one finger apart and the numbers turned towards the operator. Do not stain more than 12 slides in one batch.
2. Filter Carbol fuchsin solution before adding it to the slides. And cover the whole surface of the slides with 1% carbol fuchsin.
3. Heat the slide gently until steam appears. Use cotton wool soaked in alcohol for flaming. Do not boil or dry the carbol fuchsin on the slide.
4. If the stain accidentally runs away, add more and heat again. Wait for 5 minutes.

5. Tilt the slide to drain off excess stain. And rinse each slide individually in a gentle stream of running water (tap water or bottled water with a dropper) until all free stain is washed away. Do not splash adjust slides. And tilt the slide to drain off excess water.
6. Decolorize the smear using 3 % acid-alcohol for 3 minutes. If the slide is under decolorized after 3 minutes, further decolorize for 1-3 minutes.
7. Gently rinse each slide with water. Do not splash adjacent slides. And tilt each slide to drain off excess water.
8. Cover the slide with 0.1 % methylene blue for 1 minute.
9. Gently rinse each slide with water. Do not splash adjacent slides. And tilt and drain excess water.
10. Clean the opposite side of the smear using cotton wool soaked in alcohol.
11. Place the slide on the rack to dry in the air. Do not expose to sun to dry.

Annex-3: Panel slide result and Demographic information collection form

1. Facility Code:_____
2. Educational background: circle one (Certificate, Diploma, Degree, MSc or PhD)
3. Work experience: circle one (< 2 yrs, 2-5 yrsor> 5 yrs)
4. Sex: circle one (M / F)
5. Have you taken in service training on TB smear microscopy? Circle one (yes / No)

To be entered by laboratory personnel		**To be entered by supervisor**		Remark
Slide number	**Result**	**Expected result**	**Error type**	
AFS-01				
AFS-02				
AFS-03				
AFS-04				
AFS-05				
AFS-06				
AFS-07				
AFS-08				
AFS-09				
AFS-10				

Signature:_____Date:_____

Annex-4: On-Site Evaluation Checklist

Facility Code: _____

Adequate: refers to quantity and acceptable refers to quality. Both should be satisfactory to label as 'Y' and even if one of them is not satisfactory, label as 'N'.

S. No	Item	Adequate / Acceptable	Problems Identified
1	**Facility and safety**		
	Separate area for TB laboratory work	Y / N	
	Separate tables for specimen receipt/smear preparation/ microscopy	Y / N	
	Power supply	Y / N	
	Running water supply	Y / N	
	Waste containers with lid	Y / N	
	Waste disposal by Autoclave/disinfection/buried	Y / N	
	General order/cleanliness	Y / N	
	Personal protective Equipments used and practices(aprons, hand wash, etc)	Y / N	
2	**Manuals, Standard Operating Procedure, Job Aids**		
	Is there standard operating procedure for smear preparation and staining	Y / N	
	Grading chart, TB smear microscopy job aids posted and used	Y / N	
	EQA Protocol and training manual available and followed	Y / N	
	Is there sufficient EQA forms	Y/ N	
3	**Adequate stock and supply of Staining reagents / equipment**		
	Slides	Y / N	

	Lens Tissue	Y / N	
	Filter paper	Y / N	
	Spirit lamp or Bunsen burner	Y / N	
	Immersion oil	Y / N	
	Disinfectants	Y / N	
	Smearing/staining equipment (staining racks, loops, sticks etc)	Y / N	
	Slide boxes	Y / N	
	Carbol fuchsin	Y / N	
	Methylene Blue	Y / N	
	Acid alcohol	Y / N	
	Distilled water	Y / N	
	Equipment for preparation of stains/ reagents such as balance (for weighing reagents), measuring cylinders etc	Y / N	
	Equipment for preparation of panel testing slides	Y / N	
	Number of Binocular Microscopes (functional)	Y / N	
4	**Internal Quality Control and External Quality Assessment**		
	Control smears are used for each new batch of stain	Y / N	
	Control smears are used for once every week for checking the quality of stain	Y / N	
	Laboratory participated in External Quality Assessment	Y / N	
	All MCs are visited at least once in a six month by your staff, as per their tour program	Y / N	

Annex-5: Information Sheet

Background Information: My name is Habtamu Asrat and currently I am master's student in clinical laboratory sciences in diagnostic and public health microbiology track. I am doing a research undertaken by Addis Ababa University, School of Medical Laboratory sciences.

Aim of the study: The aim of the study is to evaluate the performance of TB smear microscopists working at EQA rechecking laboratories in Ethiopia. And to provide training based on their performance.

Benefits of the study participant: The study can give opportunity for participants to know their ability in smear microscopy. This study will help to identify gaps and will be used to improve the diagnosis outcomes. It will be also important for government to make decision on TB programs. Based on identified gaps, training will be provided for participants.

Risk and Complication of the participant: The study have no risk and complication in the participants.

Confidentiality: The information you provided will be kept confidential and will be used only for the study purposes.

Right to withdraw from the study: Participation in the study is voluntary and you have the right to participate or to withdraw any time and have the right to jump questions which is uncomfortable for you. However, your participation is important to full fill the study purpose.

I hope that you will be frank and honest in answering questions and reading 10 stained slides:
Do you agree to answer the following questions and read 10 slides to the best of your ability?
Yes ( ) No ()
If your answer is yes, please continue responding few questions and reading 10 stained slides
Thank you in participating with this important study.

Participant signature: _____ Date: _____

Annex-6: Information Sheet (Amharic Version)

መግቢያ ሀሳብ፡ እኔ ሀብታሙ አስራት እባላለሁ፤ በአዲስ አበባ ዩኒቨርሲቲ የህክምና ላቦራቶሪ ትምህርት ቤት ውስጥ የሁለተኛ ድግሪ ተማሪ ነኝ፡፡ በአሁኑ ስዓት የመመረቂያ ማሚያ ፅሁፌን እየሰራሁ እገኛለሁ፡፡

የጥናቱ አላማ፡ የጥናቱም አላማ ቲቢ ምርመራ ላይ የሚሰሩ የላቦራቶሪ ባለሙያወችን ብቃታቸውን መፈተሽ እና ባክቴሪያውን የመለየት አቅምን ስልጠና በመስጠት ማሳደግ ይሆናል፡፡

የጥናቱ ተሳታፊዎች ጥቅም፡ ቲቢ ምርመራ ላይ የሚሰሩ የላቦራቶሪ ባለሙያወች ብቃታቸው ምን ደረጃ ላይ እንዳለ ለማወቅ ይረዳቸዋል፡፡ ያጋጠሙ ክፍተቶችን በማየት ስልጠና በማዘጋጀት የላቦራቶሪ ባለሙያወች በስልጠናው እንዲሳተፉ እና ብቃታቸው ከፍ እንዲል ይጠቅማል፡፡ ቲቢ ፕሮግራም ላይ ለሚሰሩ ባለድርሻ አካላት ተጨባጭ የሆነ መረጃ የሚሰጥ ይሆናል፡፡

ሊያጋጥም የሚች ልጉዳት፡ በጥናቱ ላይ የሚሳተፉ ባለሙያወች ላይ የሚደርስ ምንም አይነት ጉዳት የለም፡፡

ሚስጥራዊነት፡ እርስዎ የሚሰጡን መረጃ በሚስጥር የሚያዝ እና ለጥናቱ አገልግሎት ብቻ የሚውል ይሆናል፡፡

በጥናቱ ላይ ያለ መሳተፍ መብት፡ በጥናቱ ላይ ለመሳተፍ ሙሉ በሙሉ በበጎ ፍቃደኝነት ላይ የተመሰረተ ነው፤ አለመሳተፍም ሆነ ጥናቱን በፈለጉት ስዓት ማቋረጥ ይችላሉ፤ ነገር ግን የእርስዎ በጥናቱ ላይ መሳተፍ የተፈለገውን ውጤት ለማግኘት በጣም ጠቃሚ ነው፡፡

በግልፅነት እና በታማኝነት ጥያቄዎችን እንደሚመልሱልን እንዲሁም የምንሰጥዎትን 10 ስላይዶች እንደሚያነቡልን ተስፋ እናደርጋለን፡፡

ጥያቄዎችን ለመመለስ እና የምንሰጥዎትን 10 ስላይዶች ለማንበብ ፍቃደኛነዎት?

አዎ ( ) አይደለሁም ( )

መልስዎ አዎ ከሆነ፤ እባክዎትን እነዚህን ጥቂት ጥያቄዎች በመመለስ እንዲሁም የሰጠንዎትን 10 ስላይዶች ማንበብ ይቀጥሉ፡፡

በዚህ ጠቃሚ ጥናት ውስጥ ስለተሳተፉልን በጣም እናመሰግናለን፡፡

የጥናቱ ተሳታፊ ፊርማ፡---------- ቀን፡----------