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External Quality Assessment of AFB Smear Microscopy and Associated Factors in Selected Private Health Facilities in Addis Ababa, Ethiopia

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Declaration

As thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance, by Lemi Mosissa; entitled: 'External Quality Assessment of AFB smear microscopy and associated factors in selected private health facilities in Addis Ababa, Ethiopia'

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As members of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, we certify that we have read and evaluated the thesis prepared by Lemi Mosissa and examined the candidate. We recommended that the thesis be accepted as fulfilling the Thesis requirement for the degree of Master of Science in Clinical Laboratory Management and Quality Assurance Specialty Track.

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Acronyms:

AA	Addis Ababa
AAHB	Addis Ababa health bureau
AAHRL	Addis Ababa health research laboratory
AAU	Addis Ababa University
AFB	Acid fast bacilli
APHL	Amazonian public health laboratory
CAREC	Caribbean epidemiology center
CDC	Centers for disease control
DOT	Directly observed treatment
EPHI	Ethiopian Public Health Institute
EQA	External quality assessment
FN	False negative
FP	False positive
GLP	Good laboratory practice
HFN	High false negative
HFP	High false positive
IUATLD	International union against tuberculosis and lung disease
LFN	Low false negative
LFP	Low false positive
MC	Microscopy center
NPV	Negative predictive value
NTLCP	National tuberculosis and leprosy control program

NTP	National TB program
NOT	Health facilities those do not provide anti TB drugs for free i.e. not on DOT
NRL	National reference laboratory
OSE	Onsite evaluation
OAR	Overall agreement rate
PHF	Private health facilities
PPV	Positive predictive value
PTB	Pulmonary tuberculosis
QA	Quality assurance
QC	Quality control
QE	Quantification error
QI	Quality indicator
RBRC	Random blinded re-checking
RLS	Regional laboratory supervisor
RNTCP	Revised national tuberculosis control program
RRL	Regional reference laboratory
SAARC	South-Asian association for regional cooperation
SMLS	School of medical laboratory science
SPR	Slide positivity rate
ZN	Ziehl Nelson

Operational definitions:

- Blinded Rechecking: A process, which involves collecting, smears from the microscopy center for blinded re-reading at regional reference laboratory and providing feedback to the microscopy center for corrective actions.
- Controller: Term used to describe the Regional Laboratory Supervisor or any technician responsible for rechecking slides.
- 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.
- High False Negative (HFN): A 1+ to 3+ positive smears that is misread as negative
- High False Positive (HFP): A negative smear that is misread as 1+ to 3+
- Kappa value: a measure of agreement between two observers taking into account agreement that could occur by chance (expected agreement).
- Low False Negative (LFN): Previously called a scanty false negative.
  A low (1- 9AFB/100fields) positive smear that is misread as negative.
- Low False Positive (LFP): A negative smear that is misread as a low (1-9AFB/100 fields).
- Major error: error 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.
- Minor error: in clinical practice, these errors may have some impact on patient management.
- Onsite evaluation: A direct observation made to assess laboratories whether they are performing according to the written/expected protocols
- Panel testing: Sending stained and/or unstained smears from the NRLs to the RRLs and RRLs to MCs to check proficiency in reading and reporting.
- Quantification Error (QE): the difference of more than one grade in reading a positive slide between examinee and controller.
- Scanty): Term used in this document to describe 1-9 acid-fast bacilli per 100 fields
- Slide positivity rate (SPR): Proportion of positive slides among all those examined (diagnosis and follow-up) in a MC over in a defined period of time, usually one year.

Abstract

Background: Tuberculosis is still public health problem in sub Saharan African countries. In resource-limited settings, tuberculosis diagnosis relies on sputum smear microscopy, with low and variable sensitivities, especially in paucibacillary pediatric and HIV-associated TB patients. AFB smear microscopy laboratories present several weaknesses like overworking, insufficiently trained personnel, inconsistent reagent supplies, and poorly maintained equipment; thus there is a critical need for investments in laboratory infrastructure, capacity building, and quality assurance.

Objectives: The main objective of the study was to assess laboratory performance quality of AFB smear microscopy and its associated factors in selected private health facilities in Addis Ababa, Ethiopia

Material and Methods: A cross-sectional study conducted in 33 selected private health facilities of which 7 hospitals, 2 NGO health centers, 23 higher clinics and 1diagnostic laboratory that provide AFB smear microscopy services in Addis Ababa from January to April 2014. A total of 283 stained smears were randomly collected for rechecking. 320 slides of panel testing were sent to 32 microscopy centers to evaluate reading, staining and reporting performance of individuals. Check lists were used to assess quality issues of laboratories. Data were captured, cleaned and analyzed using SPSS version 16.0; X^2 tests, kappa values were used for comparison purpose. P value <0.05 was considered to be statistically significant.

Results: Among the 32 participant laboratories, 2 scored 100%, 15 scored 80-95% & the remaining 15 scored 50-75% of overall proficiency test with 10 (3.15) major error and 121 (37.8%) minor error. The sensitivity, specificity, PPV and NPV of panel reading by microscopy centers were 89%, 96%, 96%, and 90 % respectively. Out of 283 randomly selected slides, the overall false reading for blinded rechecking was 3.9% with overall agreement of 97.5 % and sensitivity of 88.4 % and specificity of 99.3%. Of 283 rechecked slides 71.6 % were graded as good evenness, cleanness, thickness, size, staining and labeling having minimum and maximum score of evenness 161(56.9 %) and labeling 257 (90.8 %) respectively; having significant difference in slide quality of X^2 (p value <0.05). On-site evaluation indicated problems in terms of infrastructure, standard operating procedure, reagent quality; equipment maintenance, data management and training issues. Most of the health facilities had poor microscope maintenance (53.5%) and inventory management (25.0 %) system.

Conclusion: Microscopy centers scored a panel test score of 75.5% which is below acceptable minimum score of 80%. In blinded rechecking 3.9% of overall error was committed. In the onsite checklist, SOP, reagent quality, equipment maintenance, data management & lack of updated training on AFB microscopy techniques were mentioned as a major bottle neck for quality performance.

Key words: EQA, panel testing, onsite evaluation, blinded rechecking, major error, minor error

1. Introduction

1.1 Background

Tuberculosis (TB) is an infectious and transmissible disease caused by M. *tuberculosis* and occasionally by M.bovis; these two are the main pathogenic species within the M. tuberculosis complex. It remains a major cause of human morbidity and mortality, with an estimated 9 million new cases and 1.3 million deaths per year, with most cases occurring in low-income countries. (1)

In resource-limited countries, sputum smear microscopy is the best choice among diagnostic methods. The technique is simple, inexpensive, and efficient in detecting those cases of pulmonary tuberculosis that are most infectious. Since its yield is highly dependent on execution of the technician or even auxiliary personnel performing smear. In high HIV prevalence countries, patients may have disseminated disease with fewer AFB in their sputum. Smears may be negative or may require even more careful screening to identify low numbers of AFB. (2)

For many countries with a high burden of TB, direct smear microscopy remains the most cost-effective tool for the laboratory diagnosis of patients with infectious TB (smear-positive pulmonary disease). However, if the laboratory diagnosis is unreliable, then patients with infectious TB may not be diagnosed, resulting in ongoing transmission of disease in the community and more severe disease in the individual. Alternatively, patients without TB may be treated unnecessarily. Therefore, quality assurance of AFB smear microscopy is essential. (3)

Accuracy and reliability of laboratory testing are critical to the success of TB control programs. It must be monitored to ensure the quality of the overall process, to detect and reduce errors, and to improve consistency between testing sites. The minimum number of acid-fast bacilli necessary to produce a positive smear result has been estimated to be 5000-10,000/ml of sputum at concentration below 1000 AFB per ml of the sputum, the chance of observing the bacilli in a smear is less than 10%. (4)

Tuberculosis control strategy needs to be integrated with clinicians, laboratorians, and TB-control officials, effective public-private partnerships requires prompt, complete, and accurate communication among the laboratory systems. (5) Quality assurance of sputum microscopy includes laboratory arrangement & administration, equipment, specimens and request forms, reagents, stains, staining, smear examination and reporting. Sputum microscopy is recommended in current TB control strategies due to its attractive technology for public-health programs, provides visual evidence of TB's bacterial burden, is specific enough that no confirmatory testing is needed, only tiny amounts of material are examined, as little as 0.2 ml, hence bacteria must be presented in high concentrations to be visible, typically over 10,000 AFB per ml, takes about 3-5 minutes of technician's time. (6)

An essential component of an EQA program that is to obtain a realistic picture of the conditions and practices in the laboratory is on site supervision. This is aimed at problem solving, provides continuing education and motivates staff to improve performance. (7) Onsite evaluation allows observation of worker performance under actual conditions, including condition of equipment, laboratory safety, adequacy of supplies, specimen collection, and the process for smearing, staining, reading, recording and reporting stained smears can be reviewed during the visit. When problems are detected, solutions can be suggested and potentially implemented immediately. (8)

Reliable laboratory results are essential not just for the initial diagnosis of patients, but also for proper categorization to follow their progress during treatment, including keeping them informed of the decision to start the continuation phase, and to declare them cured at the end of treatment. If laboratory diagnosis is unreliable, microscopy errors can result in unnecessary treatment for suspected cases. The purpose of a quality assurance system is the improvement of efficiency and reliability of smear microscopy services. (9) In Ethiopia, there is a problem of reading slides properly and unsatisfactory reporting (below acceptable score <80%) results, reporting both major & minor errors. (10)

1.2 Statement of the problem

Despite strong commitment from the international community to fight major infectious diseases, weak laboratory infrastructure remains a huge rate-limiting step. Some major challenges facing laboratory systems in resource limited settings include dilapidated infrastructure; lack of human capacity, laboratory policies, and strategic plans; and limited synergies between clinical and research laboratories. Together, these factors compromise the quality of test results and impact patient management. (11)

Laboratory services in developing countries have historically been grossly neglected and underfunded. Diagnostic and surveillance capacity is therefore a major bottleneck for scaling up management and control of the highest-burden infectious diseases, inadequate and unsafe laboratory infrastructure, insufficient and underfunded country-level strategic plans for laboratory, strengthening, vastly inadequate numbers of skilled technical staff, slow diagnostic tool development and delayed, extremely slow, technology transfer, insufficient and uncoordinated technical assistance at country level to strengthen laboratories. (12)

In resource-poor countries, many smear microscopy laboratories are single room and understaffed with poorly maintained microscopes, and some of these laboratories lack consistent sources of electricity and clean water. There are few opportunities for the training of staff and little staff capacity to handle high volume workloads. (13) Missed diagnosis of AFB positive patient (false negative) or delayed diagnosis like death or advanced, disabling disease, incorrect diagnosis as AFB-positive (false positive) like drug side effects, social and economic consequences. (14)

The technical challenges of working with sputum relates to its viscosity, in-homogeneity and variability of specimens from patient to patient. Furthermore, the relatively low concentration of target bacteria also presents a diagnostic challenge. (15)

It is important to understand the limitation of smear microscopy in the detection of AFB. The directly observed treatment (DOTS) detection rate remains low, at 34%, compared with WHO target of 70% detection. (16) The reduced sensitivity is related to problems associated with the stringent requirements of the test and technicians used to handle large amounts of smears, can be expected to score high in the test. Because of high workload and fatigue, they often make more mistakes in their routine more compared to laboratories processing moderate number with regular positives. (17)

Despite the simple and inexpensive sputum smear microscopy, quickly detecting infectious cases of pulmonary TB; sputum specimens from patients with pulmonary TB especially those with cavitary disease often contain sufficiently large numbers of AFB to be readily detected by microscopy. It is relatively insensitive as at least 5,000 bacilli per milliliter of sputum are required for direct microscopy to be positive. (18) Microscopy is usually the first line test performed on specimen with a turnaround time of less than 2 hours. (19)

Effective EQA programs are labor-intensive and complex, requiring dedicated staff for onsite supervisory visits and to recheck results for a relatively large workload of smears. Although international guidelines recommend rechecking a blinded random sample of smears, many regions and countries have either not fully implemented rechecking or still use unblinded rechecking. The implementation of EQA for microscopy has the advantage not only of strengthening laboratory networks but also of improving diagnostic quality. (20)

1.3 Significance of the study

Although the private sectors play an increasingly important role in healthcare in developing countries, it remains a new area of study and innovation. Private sector presents an opportunity for sustainable scale-up of healthcare services. Over the past decade there is a trend of fast development in the private hospital sector in Ethiopia. Lack of basic data on the factors affecting the growth of private health care provision in the country and no studies are available on this issue in Ethiopia. The aim of this study was to get some preliminary insights on the factors affecting the quality of TB microscopy laboratory and its professional proficiencies. They are facing problems like availability of trained manpower, escalation of costs, and are vulnerable to imperfections in the existing market structure.

The technical quality of care provided by private providers is often poor; Shortcomings in private sector TB care include: failure to test sputum, reliance on x-ray diagnosis alone, use of incorrect drugs or drug dosages, and failure to educate patients.

Its practices have been under-researched and the available evidence reveals serious technical weaknesses in the services supplied by many for-profit providers.

This proposed study was aimed to create pressure and inspire health facilities as well as laboratory professionals towards standardized TB laboratory that scales up the diagnostic efficiencies, improvement of laboratories by their logistic management and laboratory set up. In addition to these, it is essential to grow public interest in the quality of health care, increasing public expectations from all health care providers, besides these; makes laboratory conscious of quality, increase client confidence, builds staff morale, improves laboratory performance and competitiveness, establish inter-laboratory comparability; in agreement with a reference standards.

Therefore, this study was conducted to assess the quality of sputum smear microscopy detection of tuberculosis by proficiency testing, blinded slide rechecking and on-site evaluation in Addis Ababa to identify any gap in providing direct smear microscopy examination of AFB services and information to decision makers and program managers that could improve TB case detection and control program at large. This kind of assessment is also considered as an excellent exercise for standardizing laboratories for accreditation purpose at national & international levels.

1.4 Literature Review

A study conducted in America by Pan American health organization, smear microscopy quality assurance consensus shows that common sources of false results of smear microscopy are caused at pre laboratory under administrative issues; with false negatives caused by specimen quality, patient identification, specimen labeling and transport condition; whereas false positive is caused by specimen container, specimen labeling and patient identification. False negative results may also be caused by administrative or technical issues, specimen handling and registration or smear preparation, stain formulation, staining technique, microscope performance, and smear examination are some of the factors caused by technical aspects of the laboratory. (21)

A study conducted in Southeast Asian countries shows that quality-assured smear microscopy services are available through the laboratory networks in all 11 WHO member states of the region. Laboratory staff from several member states were trained in the management of TB laboratories, quality assurance, at an inter country workshop held at Bangkok in 2010. (22)

A study done in Thimphu, Bhutan on the report on AFB smear panel testing of district hospital laboratories for the panel testing shows that performance of laboratories, who have reported back the results, were found unsatisfactory. (23)

A study conducted in Indonesia by Probandari to assess causes of missed opportunity for standardized diagnosis and treatment shows that 9 to 53% of tuberculosis cases and 4-18% of sputum smear positive tuberculosis cases in hospitals that participated were not served with standardized diagnosis. (24)

According to the data analyzed from 300 consecutive TB cases reported in California in 1998, laboratory reporting to the specimen submitter were delayed for 26.9% of smear-positive patients and 46.8% of smear-negative patients. Delays were associated with the type of laboratory that performed the testing and with delayed transport of specimens. Referral laboratories (public and private) had longer median reporting time frames than hospital and health maintenance organization laboratories. (25)

A study conducted in India to assess the proficiency of technician on reading of AFB smear results had sensitivity, specificity, PPV and NPV of reading smears of 94%, 98%, 99% and 89% respectively. The percentages of HFP and HFN were 0.06% and 4% respectively. (26)

A study conducted in Haiti by Baker M. to assess implementation of an EQA program for AFB smear microscopy in Haiti shows that measurement revealed proficiency average of 60%. Most centers had one or more major errors, predominantly false negative. The use of panel testing and onsite corrective activities allows for an unbiased representative evaluation of the overall quality of smear microscopy. (27)

The complexity and cost of setting up and maintaining quality-assurance system few laboratories, almost exclusively those that are tertiary or privately owned, can provide evidence that their results are accurate. (28)

A study conducted in Manaus, Brazil by Francisco D. Vieira revealed that there was 0.27% transcription errors on readings of sputum smear microscopes, which were considered highly significant, since they created false-negative results. Subsequently, considering that the distinct categories of positivity present differences only in the number of AFB and that these differences are not considered diagnostic discrepancies, these categories were grouped, and reliability results were obtained for three categories (negative, inconclusive, and positive) and two categories (negative and positive). These are highly significant errors (FN or FP) in the transcription of the results of readings of sputum smear microscopes are considered serious errors by the APHL/CDC. This type of error can even result in legal action against the responsible analyst and the respective institution. A false-positive result causes human suffering and incurs financial costs, whereas a false-negative result in incurring costs on society, causes harm to the patient due to the delay in diagnosis, and makes physicians lose faith in the services offered. (29)

A study conducted by Codlin to assess sputum smear microscopy quality in private laboratories in Karachi Pakistan Indus hospital TB control program clinic shows that only two of seven private laboratories correctly classified all the specimens submitted, with four laboratories correctly classifying less than half of submitted specimens. For several laboratories, only the MTB negative specimens were correctly classified; and only three laboratories accurately classified all the MTB spiked specimens, which were submitted. Another three microscopists misclassified all the MTB spiked specimens as smear-negative, thus provided the patients with false negative results. According to this study, TB sputum smear microscopy services are highly variable across private laboratories and are often of extremely poor quality. Engagement, capacity building and rigorous monitoring of standards at private laboratories are of vital importance for the control of tuberculosis. (30)

Another study done in Thimphi, Nepal, on the panel testing of sputum smear microscopy of national tuberculosis reference laboratories in SAARC region shows that 778 panel test slides were dispatched to and read by the participating laboratories with percentage agreement of 98%. The percentage agreement of individual participating laboratories varied from 87.50 to 100%. 14 minor errors and 1 major error (HFP) were also observed with sensitivity and specificity ranging from 90.90 to 100% and 83.33 to 100%, respectively. (31)

A cross-sectional study conducted in Gujarat, India on the proficiency panel testing and a reliable tool in EQA of sputum smear microscopy services indicates that there was high level of concordance in ZN smear grading that found between microbiologist and district laboratory staff. The readers reported overall consistency level of more than 98% in ZN grade agreement during the IRL, EQA, and OSE visits. The tendency to over-grade the panel slides were much higher (more than 22%) as compared to under-grade (less than 2%) them in correct slides. HFP error was not observed in this study. Proficiency panel testing is highly replicable and reproducible tool for quick and reliable assessment of proficiency of the staff and can be made more effective by raising the proportion of lower grade positive slides in panel set of each reader. Overall agreement level of more than 98% in ZN suggests high level of precision and excellent consistency during both the IRL, EQA, OSE rounds. (32)

A study conducted in Kinshasa by A. Van to evaluate knowledge and skills of laboratory technicians shows shortages of materials such as distilled water, lens tissue, and disinfectant and the unavailability or the poor condition of the necessary equipment including wire loops, staining racks, a biohazard waste bin, and a microscope. Other common problems involved poor microscope care, improper smear preparation, poor staining or reading techniques, incorrect data recording and slide storage, and a lack of feedback from the NTP. In this study blinded rechecking was conducted among 741 slides collected from the peripheral laboratories and reviewed by the NRL with 77 (10.4%) discrepant results. According to the SRL results, 67

(87%) of these discrepant results were attributed to the peripheral laboratory and 10 (13%) were attributed to the NRL. Overall, 26% of the discrepancies were detected and most (80%) of the discrepancies detected by restaining were minor errors. (33)

A study conducted in Kano Nigeria by Sarkinfada shows an increased concordance rate and decreased false positivity and false negativity rates of AFB microscopy results. (34)

A study conducted by Estifanos B, Mohammed A, and Bernt L in Southern Ethiopia shows that overall false reading was 3.2% and among the 95 discordant slides that were re-examined at the NRL, 74% were found to be in-agreement with the regional laboratory readings while 26% were in agreement with the peripheral readings. 1% of the slides had poor smearing and/or staining quality. Thus, the slides were re read by the RRL team, and 4.5% of them were found to be discordant. The discordant slides were sent to the NRL where four of the slides could not be read due to fading or poor staining. The false positive reading of 3.2% exceeds the recommended cut-off point of 2%. The result showed variations across the zones. (35)

Another cross-sectional study conducted by Gemechu Y. in graduating classes of three universities of the year 2012 in medical laboratory technology (Addis Ababa, Gondar & Jimma Universities) to evaluate the AFB bacilli smear microscopy proficiency shows 19.1% major errors & 81% minor errors. The result indicated 14.5%, major error (11.3% HFN, 1.6% HFP and 1.6%) both (HFN and HFP); according to this study, there were a total of 64.5% minor error with 8.1% LFP, 25.0% LFN, 11.3 QE while others reported multiple error LFP and QE 15.5%, LFN & QE 3.2%, and 1.6% reported LFP and HFP. (36)

2. Objectives

2.1 General objective:

- To assess laboratory performance of AFB smear microscopy and associated factors in selected private health facilities in Addis Ababa

2.2 Specific objectives:

- To assess quality performance of PHF labs using PT & blinded rechecking
- To determine Sen, Spec, PPV, NPV of AFB smear microscopy grading between the MCs and the reference laboratory.
- To assess overall situation of the AFB microscopy centers using onsite checklists.

Hypothesis: The overall performance of AFB smear microscopy status of private health facilities in Addis Ababa is above 80 %.

3. Methods and Materials

3.1 Study Setting

The study was conducted in selected private health facilities in Addis Ababa that provide AFB microscopy service.

3.2 Study design:

A cross-sectional study was conducted in Addis Ababa private health facilities that provide AFB smear microscopy diagnosis services in Addis Abba.

3.3 Study population:

Source population: All private health facilities in Addis Ababa

Sample population: Laboratories providing AFB service and laboratory professionals working at the source population and involved in this study were used as the sample population.

3.4 Inclusion and exclusion criteria:

3.4.1 Inclusion criteria: hospitals and higher clinics which provide AFB microscopy laboratory service

3.4.2 Exclusion criteria: Medium level and specialty clinics were excluded from the study

3.5 Sample size:

3.5.1 Sampling procedures:

For the determination of sample size, previous prevalence (87.1%) was used which was conducted on the proficiency test of three universities' medical laboratory technologists in 2012 and the study was conducted at 95% confidence interval and allowed error to be 5%

3.5.1.1 Sample size determination for assessment of laboratory facilities

Study facilities were stratified according to type of health facilities as hospital or higher clinic laboratories. Individual laboratory participants from each stratum were selected based on proportion to size using stratified random sampling method.

According to health and health related indicators released from federal ministry of health there are 35 private hospitals in Addis Ababa, among these 11 are on DOT program and a report from Addis Ababa health bureau 25 provide anti-TB drugs officially.

$n = \underline{z\ (a/2)^2\ p\ (1\text{-}p)/}\ d^2$

Sample size =n

Level of significance =0.05

Marginal of error (d) = 5%

P = 50%

Z a/2 = Z-score at 95% confidence interval; which is 1.96

$$n = \frac{z\ (a/2)^2\ p\ (1\text{-}p)}{d^2}$$

$n = (1.96)^2\ 0.5(1\text{-}0.5)$ = 384, and from this 384 population, the finite population correction actor was used o reach on the final sample size. Since the calculated sample size from the above formula was 384, the total population was 36 (11+25); therefore, using the finite population correction factor to estimate final sample size (nf) from target population (N), the sample size was reduced according to the following formula. Nf= n/1+n/N, nf= 384/1+384/36, 384/ (420/36) = 384/11.66 nf = 33 hence, the final sample size was 33 health institution whether hospital or clinics.

Table 3.1: Participant private health facilities that provide AFB smear microscopy service in Addis Ababa that were included in the study, 2014 (n, 33)

S/N	Lab code	Facility Type	Number of staff	Lab code	Facility type	Staff number
1	LAB 01	Higher clinic	3	LAB 25	Higher clinic	4
2	LAB 02	Higher clinic	3	LAB 26	Higher clinic	3
3	LAB 03	Health center	3	LAB 27	Higher clinic	3
4	LAB 04	Higher clinic	3	LAB 28	Higher clinic	3
5	LAB 06	Higher clinic	3	LAB 29	Health center	3
6	LAB 07	Higher clinic	4	LAB 30	Higher clinic	3
7	LAB 08	Diagnostic lab	3	LAB 31	Higher clinic	3
8	LAB 09	Higher clinic	3	LAB 32	Higher clinic	2
9	LAB 10	Higher clinic	3	LAB 33	Higher clinic	5
10	LAB 15	Higher clinic	6	LAB 05	Hospital	6
11	LAB 16	Higher clinic	4	LAB 11	Hospital	3
12	LAB 17	Higher clinic	6	LAB 12	Hospital	9
13	LAB 20	Higher clinic	3	LAB 13	Hospital	8
14	LAB 21	Higher clinic	3	LAB 14	Hospital	4
15	LAB 22	Higher clinic	2	LAB 18	Hospital	4
16	LAB 23	Higher clinic	2	LAB 19	Hospital	5
17	LAB 24	Higher clinic	5	7 hospitals, 23 higher clinics 2 health center and 1 diagnostic laboratory		
Total		**119**				

3.5.1.2Sample size determination for interview of the health facilities and assessing providers' proficiency of AFB reading

$n = \underline{z (a/2)^2 \, p \, (1-p)/} \, d^2$

Sample size =n

Level of significance =0.05

Marginal of error (d) = 5%

$Z\ a/2$ = Z-score at 95% confidence interval; which is 1.96

$$n = \frac{z\,(a/2)^2 p\,(1-p)}{d^2}$$

$$n = \frac{(1.96)^2\ 0.871(1-0.871)}{(0.05)^2} = 173$$

Formula for calculating the sample size (n) when non-response rates are considered:

nf= n/(1+n/N), nf= 173/(1+173/384)→173/(557/384)→173/1.45= 119→ nf = 119

When 10% non-response rates considered, the sample size calculation were 119+12 (10% non response rate) =131 based on the above corrective method, minimum sample size was131.

Therefore, laboratory professionals from the selected health facilities were chosen randomly and interviewed by the data collector.

3.5.2 Data collection procedures:

Before data collection begins, letters of support from AAU, department of medical laboratory were obtained and submitted to the respective hospitals' administrations. Permission was secured from each hospital and clinic's management; data for this study was collected by direct observation during the onsite evaluation and standardized checklists, random blinded slide rechecking and panel testing were used.

3.5.3 Data collection and sampling technique

3.5.3.1. Panel testing: Preparation of AFB smear for panel testing: Panel testing smears were prepared by digesting with Sodium hydroxide (NaOH) and N-acetyl L-cysteine. These methods use AFB positive and negative sputum specimens collected from patients. NaOH and NALC are used as mucolytic agents to digest and homogenize the sputum.

The N-acetyl L-cysteine (NALC) method was described by international (WHO and NRL recommendation) procedures as described in the procedure below.

3.5.3.2Smear preparation procedure for panel testing [1](annex-27)

1. 3ml AFB positive sputum (>2+ AFB ZN direct smear)

   ↓
2. Fix with formaldehyde (1 drop/ ml of sputum at room temperature for 1 hour)

   ↓
3. Treatment with 4% N-acetyl-L-cysteine (NALC)

   ↓
4. Centrifuge at 3000x g for 20 minutes

   ↓
5. Suspended pellet with 1ml distilled water

   ↓
6. Estimation of positivity

   ↓
7. Dilution AFB + specimen with AFB – specimen at defined ratio

   ↓
8. Panel slide (AFB: 1+, 2+, 3+, scanty, Negative)

Fig 1: smear preparation procedure for panel slides

3.5.4 Sample size for panel testing:

A set of 10 slides were considered as acceptable number according to national reference laboratory guidelines for QA of smear microscopy for TB diagnosis; this set of slides consisted of five stained and five pre-fixed unstained smears. For this study sites, a total of 330 validated panel-testing slides including: negative (165) and positives (165) of different grade 3^+→33, 2^+→33, 1^+→33 and actual →66 respectively.

3.5.4.1 Panel composition and validation

Standardized panels containing 5 stained and 5 unstained slides with varying AFB quantities were validated by the national reference TB laboratory. Five stained and five unstained slides were randomly selected from each group, the latter was stained, read and quantified. 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 laboratory professionals to properly grade positive slides. Multiple

[1] Similar procedure is followed for the AFB smear negative sputum except the incubation time which is 10 minutes at 55-60^{o}c

3+ smears were not included.

Table 3.2 Evaluation and interpretation of errors between the microscopy centers and the controllers

Result of MC	Reference laboratory(controller) result				
	Negative	1-9 AFB/100fields	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
1-9 AFB/100fields	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

3.5.4.2 System for sending slides to the laboratories

Validated panels were distributed to participant microscopy centers without breakage or degradation of the slides. The time allotted for reading the 10 slides were 50 to 70 minutes according to national guideline. Classifying a smear as negative, international guideline recommends examination of 200/HPF that normally requires 10 minutes. No formal recommendations exist for positive smears, although less time is required to examine smears with high concentrations of bacilli. The results of the finding were reported by using national and WHO-IUATLD guidelines. The investigator collected the smear results onsite in a work sheet

3.5.4.3 Slide transportation system

Randomly collected slides were kept on table and examined onsite. After commencement of the re checking, we rearranged the slides to their original places.

3.5.4.4 Slide evaluation and interpretation

Smears were evaluated for appropriate smear size and thickness, quality of staining, cleanness and evenness as well as labeling by senior laboratory technologist (Annex IXI). The results of the finding were reported and interpreted according to national and WHO-IUATLD standard guidelines.

3.5.5 Sample size Determination for blind rechecking

3.5.5.1 Blinded rechecking

All routinely examined slides (283 processed slides) from January to April 2014 were rechecked onsite by experienced third person technologist and the discordant slides were brought to national reference laboratory for final result.

3.5.5.2 Slide Collection and selection

Well-trained laboratory technologist collected slides in order to ensure that a random sample obtained. To avoid bias, the technicians in study site were never performing the sampling. In order to eliminate selection bias, slides were randomly selected using the TB register logbook by an independent supervisor from list of specimens processed. This ensures that the technicians keep all slides regardless of result but slides were not selected from the slide box.

3.5.5.3 Discordant slides

Second controller confirmed discrepant results; the second controller was provided with the smear results but the identity of the readers was concealed. The results of the second controller were final.

3.5.5.4 Feedback for the blinded rechecking results

Feedback was given to the participating laboratories by principal investigator and blinded rechecking was discussed with the personnel's in the study sites and was completed soon at the spot.

3.5.6 Sample size for onsite evaluation

3.5.6.1 On-site evaluation

On-site evaluation of each laboratory was performed using the national comprehensive on- site evaluation checklist

3.6 Scores for grading

Set of 10 panel testing slides, each slide carries 10 points, total possible score = 100 (for 10 slides). Committing major error (HFP and HFN) results in a score of 0 where as minor error (LFP, LFN and QE) result in a score of 5 points. Passing score was 80 points. Generally the overall agreement rate (OAR) were considered excellent >95%, Satisfactory >90 % and poor performance < 80 %.

3.7 Dependent and independent variables

3.7.1. Dependent variables:

- Results of panel testing, random blinded rechecking and on-site evaluation
- Sensitivity, Specificity, Positive predictive value and Negative predictive value of AFB smear microscopy grading between the MCs and the reference laboratory

3.7.2. Independent variables:

- Work experience and educational level
- Availability and usages of SOP and EQA protocol
- Physical infrastructure and supply chain management
- Smear quality, stained and unstained smear size, thickness, quality of staining, cleanness and evenness, availability and use of laboratory reagents / equipments bio safety

3.8 Data Management:

The confidentiality of this data was kept secret and the result was disclosed by code. The codes of the hospitals were notified for the participant facilities prior to completion of the study.

3.9 Data Analysis:

Raw data was entered and analyzed using SPSS version 16.0 and results were explained using absolute numbers, percentages by discussing results in detail. It assessed the EQA of AFB smear microscopy using proficiency testing, blinded rechecking and onsite evaluation method by preparing panel slides and structured questionnaires. The questionnaire of this study included proficiency of personnel against AFB microscopy laboratories, including sample collection, smearing, staining, and reporting.

Kappa statistics were calculated to show the association between microscopy centers and the NRL as well as inter-laboratory comparability. Besides this, we calculated sensitivity, specificity PPV and NPV to show the test accuracy. We also calculated the X^2 and p-value to see the association between different factors.

3.10 Data quality assurance

To assure data quality, quality control for panel testing, smear preparation of sputum smear, microscopy AFB detection was accomplished starting from sample collection, smearing, staining and microscopy and reagent quality according to the national guideline. Each new batch of ZN reagents were checked by known unstained positive and negative control smears during on-site evaluation.

3.11 Ethical consideration

The ethical clearance was obtained from:

AAU College of Health Sciences School Medical Laboratory Sciences Departmental Research and Ethical Review Committee (DRERC), Addis Ababa Regional Heath Bureau Ethical Clearance Committee and the Ethiopian Public Health Institute (EPHI) Scientific and Ethical Review Office (SERO) Moreover, privacy and confidentiality were assured for all interviewees. The right of any individual not to participate or withdraw from the study at any time was fully respected. Data collection from each study subjects was started after verbal consent is reached. During data collection there were a high degree of confidentiality and informed consent was also being obtained from each study sites, no name and other identifier on the questionnaire. At the end of the study, feedbacks were given from panel, direct observation and random blinded re checking for each study site.

4. Result

4.1 Participant microscopy centers

Among the 33 private health facilities included in the study, 7 (21.2 %), were general hospital, 2 (6.06%) NGO health center, 23 (69.7%) were higher clinics and 1 (3.03%) diagnostic laboratory. (Table 4.2) All laboratories were willing to participate in the study except one higher clinic and three hospitals those refused to admit the research project to be carried out at their facility for unknown reason and we substituted them by higher clinics for the refused facilities.

4.2. Panel Testing

For the assessment of panel testing a total of 330 AFB smears of various grades of slides were used. The response rates of participant laboratories were 97 % because one health institution refused to read the panel slides after distribution. The results were given back within one day of panel distribution. Among these laboratories, 2 (6.25 %) could correctly detect negative and positive slides. Two laboratories 2 (6.25 %) have misread negative slides as 1+ to 3+. Eighteen laboratories (56.25 %) misread 1+ to 3+ as negative; 20 (62.5%) laboratories misread scanty as negative; five laboratories (16.6 %) misread negative as scanty and 25 (78.25 %) laboratories had problems in grading the positive smears, quantification errors. (Table 4.1)

Thirteen (40.6 %) of the 32 laboratories reported back unsatisfactory (below acceptable score < 80 %) results and the remaining 19 (59.4 %) had acceptable performance (passing score). However, 8 (25 %) of MCs have reported major errors (9 HFN and 1 HFP), 26 (81.25 %) MCs committed minor errors (33 LFN, 4 LFP and 49 QE) where as 7 participants microcopy centers (21.9 %) committed both major and minor errors with (9 HFN, 13 LFP and 20 QE).

One higher clinic had unsatisfactory scored (score of 50 %) with major and minor errors (3 HFN, 1 LFN, and 3 QE).The total score of microscopy center ranges 50 to 100 %. Two centers had scored 100 %; six had scored 90 %. (Table 4.1) About 131 (40.9 %) of panel slides were incorrectly interpreted by microscopy centers. HFN error observed in 7 (2.2 %), LFP in 4 (1.25 %), HFP in 1 (0.3 %), QE in 71 (22.2 %) and LFN errors in 46 (14.4 %) of panels. Over all agreement was very good with kappa value of 0. 87 (Table 4.1), among the total 131 (40.9%) error committed by all health facilities, 2 (6.6 %), 90 (28.3 %), 8 (2.5 %) and 3 (0.9 %) were committed by hospitals, higher clinics, health centers and diagnostic laboratory respectively. However there was no statistically significant differences in errors types and health facilities (p value > 0.05) (Table 4.1)

Table 4.1: Performance level of microscopy centers in Addis Ababa, 2014 (n=33)

Lab Code	HFN	HFP	LFN	LFP	QE	Total error	Total scores
LAB-01	0	0	4	0	2	6	70
LAB-02	3	0	1	0	3	7	50
LAB-03	0	0	3	0	1	4	80
LAB-04	0	0	0	1	2	3	85
LAB-05	0	0	2	0	2	4	80
LAB-06	0	0	1	0	2	3	85
LAB-07	1	0	2	0	4	7	60
LAB-08	0	0	0	0	2	2	90
LAB-09	1	0	2	0	4	7	60
LAB-10	0	0	0	0	5	5	75
LAB-11	0	0	1	0	2	3	85
LAB-12	0	0	3	0	2	5	75
LAB-13	0	0	0	0	0	0	100
LAB-14	0	0	0	0	3	3	85
LAB-15	0	0	0	0	0	0	100
LAB-16	0	0	3	0	2	5	75
LAB-17	0	0	3	1	1	5	75
LAB-19	0	0	3	0	2	5	75
LAB-20	0	0	1	0	3	4	80
LAB-21	1	1	2	0	4	8	50
LAB-22	0	0	0	0	2	2	90
LAB-23	0	0	1	0	1	2	90
LAB-24	0	0	1	1	2	4	80
LAB-25	0	0	0	0	2	2	90
LAB-26	0	0	0	0	2	2	90
LAB-27	0	0	1	0	3	4	80
LAB-28	0	0	2	0	4	6	70
LAB-29	1	0	0	0	3	4	75
LAB-30	1	0	3	0	2	6	65
LAB-31	0	0	3	0	1	4	80
LAB-32	1	0	3	0	0	4	75
LAB-33	0	0	1	1	3	5	75
Total	**9**	**1**	**46**	**4**	**71**	**131**	**2442**
Average	**2.8**	**0.3**	**14.4**	**1.3**	**22.2**	**40.9**	**75.6**

Hospitals on DOT program committed no major error but minor errors of LFP and QE which was 13 (4 %) errors whereas those not DOT program committed error frequency of less than this 7 (2.2 %).

But in higher clinics, more error committed in those institution of not DOT program with both major and minor errors 2 (0.6 %) and, 32 (9.7 %) respectively. While those higher clinics not on DOT committed more error in reading the distributed panel slides as major errors 7 (2.4 %) and 127 (39.6 %). Out of the two health centers, one was providing DOT program and committed 1 major error (0.5%) and 3 QE (0.9 %) where as the one not on DOT program committed no major error but 4 (1.3%) minor errors. One participant diagnostic laboratory committed no major but 2 (0.6 %) minor errors. (Table 4.2)

Table 4.2: Performance of AFB microscopy centers on panel slides in PHF in Addis Ababa 2014 (n, 32)

Health institution		**Major errors**		**Minor error**			**Total errors**
		HFN N (%)	**HFP N (%)**	**LFN N (%)**	**LFP N (%)**	**QE N (%)**	**Major + Minor**
Hospital	On DOT(n,5)	0	0	7 (2.2)	0	6 (1.9)	13 (4 %)
	Not on DOT	0	0	2 (0.6)	0	5 (1.6)	7 (2.2 %)
Higher	DOT	2 (0.6)	0	10 (3.1)	1 (0.3)	21 (6.6)	34 (10.6 %)
clinics	Not on DOT	6 (1.9)	1(0.5)	24 (7.5)	3 (0.9)	33 (10.3)	67 (20.9 %)
Health	DOT	1(0.5)	0	0	0	3 (0.9)	4 (1.3 %)
Center	Not on DOT	0	0	3 (0.9)	0	1(0.5)	4 (1.3 %)
Diagnostic laboratory		**0**	**0**	**0**	**0**	**2**	2 (0.6 %)

Out of 320 panel slides distributed to the selected sites 50 % (160) were stained and 50 % (160) were unstained panel slides. About 5 (18.1%) of errors were reported from stained and while 73 (22.8 %) errors committed from unstained panel smears. There was no statistically difference in the frequency of errors and panel slides types (x^2 = P value > 0.05).The overall sensitivity, specificity, PPV and NPV of panel reading by microscopy centers were 89 %, 96 %, 96 %, and 90 % respectively. (Table 4.3)

Table 4.3: Comparison of stained & unstained panel-testing of PHF in Addis Ababa, 2014 (n, 32)

Types of panel slides	Major error		Minor error			Total error
	HFN	HFP	LFN	LFP	QE	
Stained panel slide	3 (0.9%)	1 (0.3%)	20 (6.3%)	0	34 (10.6%)	**58 (18.1%)**
Unstained slide	6 (1.9%)	0	25 (7.8%)	5 (1.6%)	37 (11.6%)	**73 (22.8%)**
Total	**9 (2.8%)**	**1 (0.3%)**	**45 (14%)**	**5 (1.6%)**	**71 (22.2%)**	131 (40.9%)

Both microscopy center and NRL were agreed on 202 (63.1 %) positive and 58 (18.1 %) negative readings but disagreed on the remaining 60 (18.8 %) of slides which comprises 54 (16.9 %) FN and 6 (1.9 %) FP results, Hence, the overall sensitivity, specificity, PPV, NPV and accuracy of private health facilities in panel testing shows 78.9 %, 90.6 %, 97.1 %, 51.8 % and 81.3 % respectively. The reading agreement has a kappa value of 0.54, which has moderate agreement with the NRL. (Table 4.4)

Table 4.4: A two by two table for panel testing that compares MCs to NRL, 2014 (n, 32)

		National reference laboratory (True value)		Test total
		Positive	**Negative**	
PHFs (Test value)	**Positive**	202	6	208
	Negative	54	58	112
True value total		256	64	320

Both hospital laboratory and NRL were agreed on 38 (63.3 %) positive and 12 (20 %) negative readings but disagreed on the remaining 10 (16.7 %) of slides which comprises 10 (16.7 %) FN and 0 FP results, this resulted in sensitivity, specificity, PPV, NPV and accuracy of 79.2 %, 100 %, 100 %, 54.5 % and 83.3 % respectively having a kappa of 0.61 this is substantial agreement. This shows more performance than higher clinics but less than those of health centers and diagnostic laboratories. Table (4.5)

Table 4.5: A two by two table for controller & MCs of hospitals in Addis Ababa, 2014, (n, 6)

		Controller result		
		Positive	**Negative**	**Total**
Microscopy center result	Positive	38	0	38
	Negative	10	12	22
Total		48	12	60

Both higher clinic laboratory and NRL were agreed on 144 (62.6 %) positive and 40 (17.4%) negative readings but disagreed on the remaining 46 (20 %) of slides which comprises 40 (17.4 %) FN and 6 (2.6 %) FP results, hence the panel testing had sensitivity, specificity, and PPV, NPV, accuracy of 78.3 %, 86.9 %, 96 %, 50 % and 60 % respectively with Kappa value of 0.78 showing substantial agreement. It showed lowest Kappa value of 0.51 as compared to other health facilities showing moderate agreement. (Table 4.6)

Table 4.6: A two by two table for controller & MCs of higher clinic, 2014 (n, 23)

		Controller result		
		Positive	**Negative**	**Total**
Microscopy center result	**Positive**	144	6	150
	Negative	40	40	80
	Total	184	46	230

Reading of health center and NRL were agreed on 12 (60 %) positive and 4 (20 %) negative readings but disagreed on the remaining 4 (20 %) of slides which comprises 0 FN and 4 (20 %) FP results, hence the panel testing had sensitivity, specificity, and PPV, NPV, accuracy of 100 %, 50%, 75%, 50 % and 60 % respectively with Kappa value of 0.78 showing substantial agreement. (Table 4.7)

Table 4.7: A two by two table for controller & MCs of health center, 2014, (n, 2)

		Controller result		
		Positive	**Negative**	**Total**
Microscopy center result	**Positive**	12	4	16
	Negative	0	4	4
	Total	12	8	20

Reading of diagnostic laboratory and NRL were agreed on 8 (80 %) positive and 2 (20 %) negative readings but disagreed results, hence the panel testing had 100% sensitivity, specificity, PPV, NPV, accuracy. It showed Kappa of 1.0, which is perfect agreement. (Table 4.8)

Table 4.8: A two by two table for controller and MCs of diagnostic laboratory, 2014 (n, 1)

		Controller result		
		Positive	**Negative**	**Total**
Microscopy center result	**Positive**	8	0	8
	Negative	0	2	2
	Total	8	2	10

The overall performance of microscopy centers by health facilities (hospital, higher clinic, health center and diagnostic laboratory) shows the sensitivity and specificity of 75-100 % and 50 – 100 % respectively. Health center laboratory and diagnostic laboratory showed more performance than higher clinic and hospitals laboratories with kappa values of 0.78, 1.0 0.51 and 0.61 respectively in which a perfect agreement is observed between microscopy centers. (Table 4.9)

Table 4.9: Sensitivity, specificity & predictive values of the MCs for panel slides by facility

Health facilities	Sensitivity	Specificity	PPV	NPV	Accuracy	Kappa value
Hospitals	79.2 %	90.6 %	100 %	54.5 %	83.3 %	0.61
Higher clinics	78.3 %	100 %	96 %	50 %	60 %	0.51
Health center	75 %	86.9 %	75 %	50 %	60 %	0.78
Diagnostic laboratory	100 %	50 %	100 %	100 %	100 %	1.0
Average (n,32)	**83.3 %**	**81.9 %**	**92.8 %**	**63.6 %**	**75.8 %**	**0.73**

4.3 Blinded re checking

Among the 33 participant laboratories, blinded rechecking was conducted only in 14 (42.4 %) of them; 5 (35.7 5) hospital, 8 (57.1 %) higher clinics and the remaining 1 (7.1 %) of them were health center. The remaining 19 (57.6 %) do not store slides and we couldn't conduct the blinded rechecking. (Table 4.10)

From 283 random blindly selected and rechecked slides, 14.1 % and 85.7 % were reported to be positive and negative by microscopy centers and 16.3 % and 80.6 % were reported as positive and negative by the controller respectively. (Table 4.10)

Comparison of the results derived from the microscopy centers with those of the controller showed that they were similar in 38 (13.4 %) cases for positive findings and in 236 (83.4 %) cases for negative findings. Considering the reading of the controller as a true value, there was 9 (3.2 %) discordant slides (2 FP and 7 FN) that results in specificity and sensitivity of 99.3 % and 84.4 % respectively. The PPV and NPV were 95 % and 97.1 %, respectively. There was no disagreement between microscopy center and controller with overall agreement of 97.5 %. Among the 9 discordant slides that was re-examined at facility level by the controller, 98.4 % were found to be in agreement with the microscopy center readings with kappa value of 0.87. Thus, the reading agreement between the microcopy center and controller is almost perfect. (Table 4.10)

Table 4.10: Blinded rechecking results between the MC & the controllers, Addis Ababa, 2014 (n, 14)

Microscopy Centers	Total no. of re checked	MC result		Rereading result		Discordant result	False results	
		Pos	Neg	Pos	Neg		FP	FN
LAB-07	20	1	19	2	18	1	0	1
LAB-11	27	7	20	7	20	2	1	1
LAB-12	25	4	21	5	20	1	0	1
LAB-13	13	3	10	4	9	1	0	1
LAB-18	23	3	20	4	19	1	0	1
LAB-19	17	0	17	0	17	0	0	0
LAB-20	18	2	14	2	16	0	0	0
LAB-22	18	2	16	3	15	1	0	1
LAB-25	34	3	31	3	31	0	0	0
LAB-27	13	3	10	4	9	3	1	2
LAB-28	10	3	7	2	8	1	1	0
LAB-29	26	3	23	3	26	0	0	0
LAB-32	19	1	18	1	18	0	0	0

LAB-33	20	4	16	5	15	0	0	0
Total	**283**	**40**	**243**	**46**	**228**	**11**	**3**	**8**

Out of the 283 re checked slides by random selection, 38 (13.4 %) positive and 236 (83.4 %) negative however, disagreed on 9 (3.2 %) meaning that 7 (2.4%) false negative and 2 (0.7 %) false positive results. The sensitivity and specificity of the microscopy centers whether they are on DOT or NOT (n, 14) for blinded rechecking ranges from 85-90 % and 90-100 % respectively. In general, the overall sensitivity, specificity, positive predictive value and negative predictive values of the blinded rechecking were 88.4 %, 99.3 %, 92.4 % and 98.9 % respectively. Moreover the overall kappa value found to be 0.87 and this shows us the reading agreement is almost perfect. (Table 4.11)

Table 4.11: 2x 2 tables for blinded rechecking at MC& controller Addis Ababa2014, (n, 14)

Microscopy center		**Controller value (True value)**		**Total**
		Positive	**Negative**	
Test value	**Positive**	38	2	40
	Negative	7	236	243
True value total		45	238	283

The test agreement among randomly blinded rechecked slides with almost perfect agreement consistent re reading results among microscopy centers. They show sensitivity, specificity, PPV, NPV and accuracy for microscopic centers by hospital, higher clinic, health center and diagnostic laboratory were 90.3 %, 99.3 %, 97 %, 98 %, and 97.6 %, respectively. All hospital microscopy centers had very good agreement with the controller having a kappa value of 0.93 which is almost perfect agreement.

Table 4.12: Reading agreement between microscopy centers for blinded rechecking slides for the private health facilities Addis Ababa, 2014 (n, 14)

Type of health facility	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**	**Kappa value**
Hospitals	83.3 %	98.8 %	95.2 %	96.6 %	95.4 %	0.86
Higher clinics	87.5 %	99.2 %	95.5 %	97.6 %	97.4 %	0.92
Health center	100 %	100 %	100 %	100 %	100 %	1.0
Average (n, 14)	**90.3 %**	**99.3 %**	**97 %**	**98 %**	**97.6 %**	**0.93**

Overall quality of smear was assessed during the random blinded re checking using visual assessment of stained smears. Among these an average of 203 (71.7 %), 220 (77.7 %), 190 (67.1 %), 161 (56.9 %), 185 (65.4 %) and 257 (90.8 %) slides have proper cleanness, size, thickness, evenness, staining and labeling qualities respectively. (Fig 2)

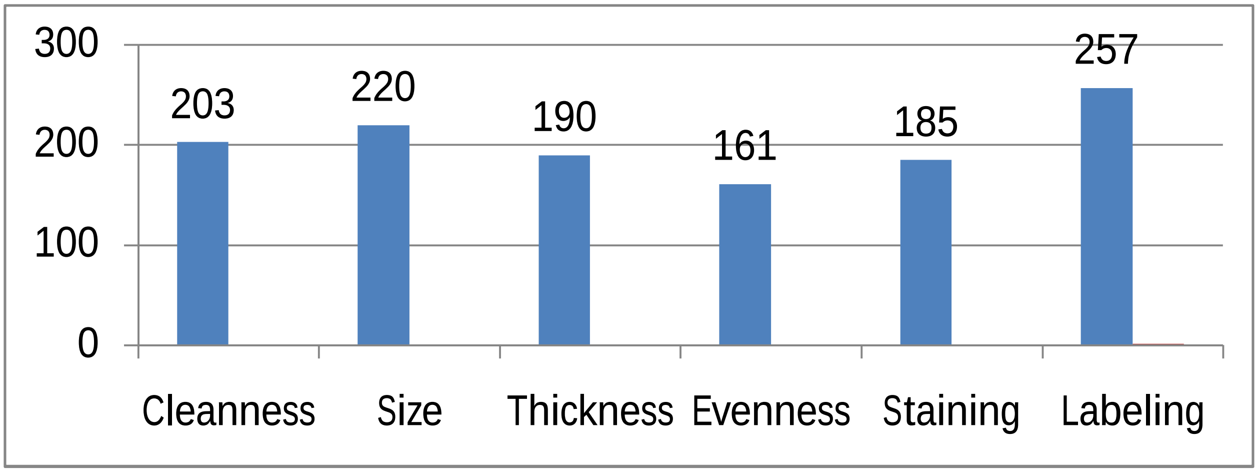

Fig 2: Quality of stained slides for the blinded re checked sites by controllers at the private health facilities in Addis Ababa, (n, 283)

Smear quality were assessed during the visual re-examination of the sampled smears classifying them as hospital, higher cleaning and health center with average of 221 (78%) had proper smear size, 188 (66.4 %) had proper thickness, 175 (61.8 %) had proper staining, 199 (73.3 %) had cleanness of smears and 161 (56.9 %) had evenness of smear. There was significant difference in slide quality among the 14 rechecked sites X^2 (p value <0.05). (Fig 3)

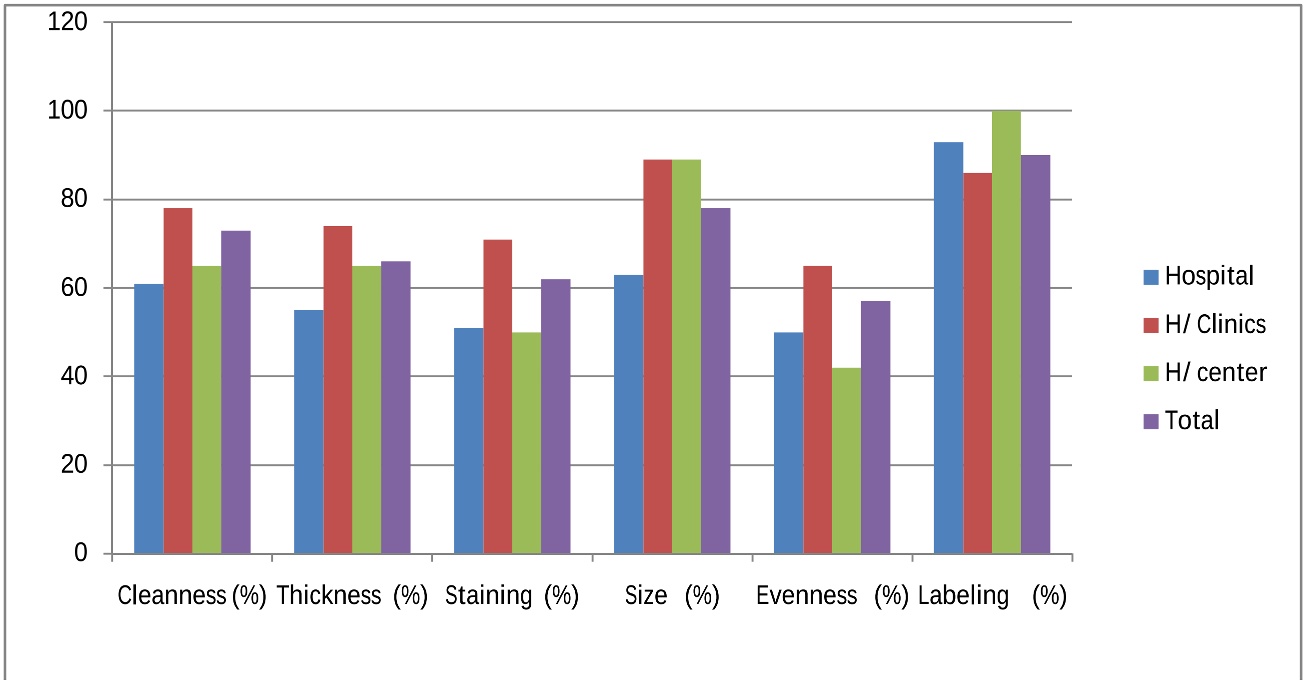

Fig 3 smear quality of MCs for random blinded rechecked slides in Addis Ababa, 2014 (n, 14)

4.4. On-site evaluation

A standardized checklist (adapted from the national EQA of sputum smear microscopy) was used to assess the status of infrastructure, SOP, reagent and equipment, maintenance of microscope, biosafety and waste disposal, training related to AFB quality assessment, data and supply management.

In this study 7 hospitals (21.2 %), 23 higher clinics (69.7 %), 2 health centers (6.06 %) and 1 diagnostic laboratory (3.03 %) were investigated. Based on the on-site evaluation, the facilities were assessed for infrastructure, EQA, SOP's, internal quality control, reagents, safety and waste disposal practices, training status, microscope maintenance, data management and inventory management. Most of the health facilities had poor microscope maintenance (53.5 %) and inventory management (25.0 %) system.

Among the 33 participant laboratories only 6 (18.2 %) have separate area of AFB laboratory for specimen receipt and smear preparation. And 15 (45.5 %) have well ventilated window while 29 (87.8 %) have regular water supply but most institutions 31 (93.9 %) and 30 (90.9 %) have permanent electric power with backup generator respectively. (Table 4.15)

Table 4.15: Infrastructure of PHF Addis Ababa 2014 (n, 33)

Infrastructure	Frequency (Yes)	(%)
Separate area for AFB laboratory work	6	18.2
Separate tables for specimen receipt & smear preparation	6	18.2
Well ventilated laboratory (window)	15	45.5
Regular water supply	29	87.8
Permanent electric power	31	93.9
Backup generator	30	90.9

Nineteen (57.6 %) of laboratory have posted smear & staining procedure and grading chart, but only 9 (27.3 %) have arranged slides in slide box as per lab register and slide number. Twenty four (72.7 %) examines at least 100 fields before reporting negative, where as 5 (15.2 %) and 31 (93.9 %) respectively examines 20-50 and 50-70 fields to report negative results. (Table 4.16)

Table 4.16: Availability of standard operating procedures in laboratory

Available Standard Operating Procedure (SOP)	Frequency	%
Posted smear preparation & staining procedure & grading chart	19	57.6
Arranged slides in slide box as per lab register & slide numbers	9	27.3
At least 100 fields are examining before reporting negative result	24	72.7
Minimum 20-50 100 fields examined to report negative result	5	15.2
Minimum 50-70 100 fields examined to report negative result	31	93.9

All laboratory personnel in the microscopy centers were responsible for sputum collection, along with quality of the sample; smears were dried and fixed prior to staining. However, 22 (66.7 %) collects sputum in spot morning spot system for AFB microscopy by checking sputum quality. All the 33 health institutions use new slides for sputum smears but only 23 (69.7 %) of them label these slides with lab code, serial number and sequence identifier. Only one laboratory (3.0 %) filters carbol fuchsin before staining and 10 (30.3 %) filters monthly but 22 (66.7 %) of them do not filter at all. 28 (84.8 %) use functional binocular microscope with regular maintenance of microscope is given for 21(63.6 %) while 23 (69.9 %) have knowledge of repairing simple microscope problems. Eighteen (54.5 %) have spare parts like bulbs and only 11 (33.3 %) document their daily preventive maintenance which is cleaning microscope. Twenty five (75.8 %) of the facilities have standard waste container with lid and 32 (96.9 %) disinfect their working area. Only 11 (33.3 %) of the laboratories have adequate space for sputum processing. (Table 4.17)

Table 4.17: Reagents and consumable AFB smear microscopy laboratory items Addis Ababa 2014 (n, 33)

Collecting sputum for AFB microscopy at spot-morning-spot	22	66.7
Checking sputum quality	22	66.7
New slides used for sputum smears	33	100
Labeling with lab code, serial number and sequence identifier	23	69.7
Carbol fuchsin filtered always before use	1	3.03
Carbol fuchsin filtered monthly	10	30.3
Not filtered at all	22	66.7

EQA protocol is available and followed in 6 (18.2 %) and slides are collected by RRL for EQA from 7 (21.2 %). Five (15.5 %) responded as they participate in panel testing.

Among the 33 laboratory assessed on site, only 2 (6.06 %) use control smears at least once a week by preparing positive and negative slides for reagent quality control. Nine (27.3 %) use standardized laboratory registration and only 11(33.3 %) and 12 (36.4 %) have standardized request form and consistent reporting with complete registration book respectively. Concerning the supply chain managements, only 9(27.3 %) and 11 (33.3 %) have inventory system and plan their supplies respectively. Thirty (90.9 %) of laboratories obtain their reagents by direct purchase while the remaining were by AAHRB regional laboratory and distributed by pharmacist and laboratory professionals. No laboratory service interrupted due supply problems and no facility prepares reagents in its laboratory.

Among the participant facilities, 14 (42.4 %) were on DOT program. Based on the on-site evaluation, the facilities were assessed for standards of infrastructure, EQA, internal quality control, staining reagents, safety and waste disposal practices, SOP's, training status, microscopy maintenance, data management and inventory management Most of the health facilities had poor microscope maintenance 21 (63.6 %) and inventory management 9 (27.3 %)system.

4.5 Staffing

There were a total of 119 laboratory personnel in 33 private health facilities. Among these 50 (42 %) were female and the remaining was male laboratory professionals working in the institutions. one (0.8 %) MSc, 43 (36.1 %) BSc, 49 (41.2 %) diploma and the remaining 26 (21.8 %) were level IV laboratory technicians. Out of this, 18 (15.1 %) laboratory personnel at BSc level took additional training for AFB. On the other hand, 19 (16 %) diploma level took AFB related training and the remaining 7 (5.9 %) were level IV technicians for TB. The age group in years 20-25, 26-30, 31-35, 36-40 & >41 years and professional levels were 30 (25.2 %), 52 (43.7 %), 23 (19.3 %), 11 (9.2 %), & 3 (2.2 %) and 10 (8.4 %), 20 (16.8 %, 15 (12 %), 41 (34.5 %), 31 (26.1 %) and 2 (1.7 %) were expert medical laboratory technologist, senior medical laboratory technologist, junior medical laboratory technologist, junior medical laboratory technician , senior medical laboratory technician, chief medical laboratory technician respectively. (Table 4.18)

Table 4.18 Age group and professional level distribution of study participant Addis Ababa (n, 119)

Age group in years	**Frequency**	**%**
20-25	30	25.2
26-30	52	43.7
31-35	23	19.3
36-40	11	9.2
>41	3	2.5
Professional level		
Expert Medical Laboratory Technologist	10	8.4
Senior Medical Laboratory Technologist	20	16.8
Junior Medical Laboratory Technologist	15	12.6
Junior Medical Laboratory Technician	41	34.5
Senior Medical Laboratory Technician	31	26.1
Chief Medical Laboratory Technician	2	1.7

Thirty one (26.1 %) were working in hospitals, 79 (66.4 %) in higher clinics, 6 (5.04 %) in health center and 3 (2.58 %) working in diagnostic laboratory. The work experience is dominated by 1-5 years with 71 (59.7 %), 30 (25.2 %) were 6-10 years, 4 (3.4 %) of them were 16-20 while the remaining 11 (9.2 %) were having greater than 21 years of experience. Twenty five (21 %) were laboratory heads, 11 (9.2 %) quality officer and the remaining 83 (69.7 %) of respondents were laboratory experts. (Table 4.19)

Table 4.19: staff composition and distribution in the private health facilities 2014 (n, 119)

Working Organization	**Frequency**	**%**
Hospital	31	**26.1**
Higher clinic	79	**66.4**
Health Center	6	**5.04**
Diagnostic laboratory	3	**2.5**
Work Experience in years		
1-5	71	**59.7**
6-10	30	**25.2**
16-20	4	**3.4**
>21	11	**9.2**
Position in this organization	3	**2.5**
Laboratory head	25	**21.0**
Quality officer	11	**9.2**
Laboratory expert	83	**69.7**

Facility based AFB related training was given for 78 (65.5 %) among these trained personnel, 21 (17.6 %) working in hospital, 54 (45.4 %) in higher clinics, 3 (2.5 %) in health center and no personnel trained from the diagnostic laboratory. (Table 4.20)

Table 4.20: Distribution of laboratory professionals with AFB related training in 2014 (n, 33)

	No. of HF	No. of staffs	MSC n (%)	BSc n (%)	Diploma N (%)	Level IV n (%)	Total
Hospital	7	39	0	12 (17.6)	7 (8.8)	2(1.7)	**21 (17.6 %)**
Higher clinics	23	71	1	13(10.9)	23 (19.3)	17(14.3)	**54 (45.4 %)**
Health center	2	6	0	1(16.6)	2 (1.7)	0	**3 (2.5 %)**
Diagnostic lab	1	3	0	0	0	0	**0**
Total	**33**	**119**	**1**	**26**	**32**	**19**	**78 (65.5 %)**

The results obtained from panel testing, blinded rechecking and the onsite evaluation checklist is almost similar and consistent as overall proficiency result shows 75.6 % whose reading agreement was very good with kappa value of 0.87 (Table 4.1). Similarly, in blinded rechecking, the result showed (3.2 %) discordant readings with kappa value of 0.87. Thus, the reading agreement between the microcopy center and controller is almost perfect; hence result shown from proficiency testing and blinded rechecking is almost consistent with results from the onsite evaluation checklist with poor usage of 19 (57.6 %) posted smear preparation, staining procedure and grading chart, while 24 (72.7 %) examines at least 100 fields before reporting negative slides, where as 5 (15.2 %) and 31 (93.9 %) respectively examines 20-50 and 50-70 fields to report negative results. Together with these, the performance quality of the microscopy center was compromised with the availability and implementation of EQA protocol.

5. Discussions

The main objective of implementing EQA program is to establish inter-laboratory comparability, in agreement with a reference standard that can detect deficiencies through by determining the need for quality improvement.

5.1 Panel Testing

HFP and HFN results were considered to be major errors indicating misclassifications that fundamentally change the disease classification and management of a patient. The main thrust of an EQA program is to identify these errors. HFP result should be rare if the basic knowledge of laboratory professionals on the morphological appearance of AFB & a good microscope are met.

An isolated occurrence could often be considered to represent an administrative error such as erroneous recording or mislabeling of the slide. In the present study, 0.6 % of smears had HFP results in one of the 32 laboratories indicating major error and the remaining sites had zero incidence of HFP. An HFN, especially with 1+, could be due to quality of staining and unequal distribution of AFB in the smear. Other reasons include lack of technical knowledge in identifying AFB, poorly maintained microscopes, work overload, carelessness in reading, or not reading the slide at all. Low incidence of HFP (less than 2%) has also been reported from the study sites, similar study conducted in America by Pan American health organization shows FP is caused by specimen container, labeling and patient identification. Whereas FN results may also be caused by administrative or technical issues, specimen handling and registration or smear preparation, stain formulation, staining technique, microscope performance, and smear examination are some of the factors caused by technical aspects of the laboratory. (21)

All Proficiency tests slide sets should have the same number of low-positives, strong-positives and negatives to consistently measure performance. Error rates were higher in slides with low numbers of AFB (46 LFN in 22 sites, and the results might suggest that microscopy centers were unable to detect low AFB counts, probably because they didn't read all fields, or due to the high turnover rate therefore the outcome is a false negative result that is in agreement with other studies where the factor most strongly associated with PT performance was the number of low positive slides in the slide sets (26). This is significant error as patients with paucibacillary disease could give negative results in AFB microscopy and will not receive treatment, resulting in further community spread and failure in diagnosis of PTB. Similar study in Haiti shows occurrence of predominant FN errors that might be due to lack following proper EQA protocol. (27) Our finding is also similar to study in America by Pan American health organization shows FP is caused by specimen container, labeling and patient identification. Whereas FN results may also be caused by administrative or technical issues, specimen handling and registration or smear preparation, stain formulation, staining technique, microscope performance, and smear examination are some of the factors caused by technical aspects of the laboratory. (21)

In this study, majority of laboratories scored 14 (43.35 %) which is below acceptable performance of 80 %. Hospital laboratories were slightly better than higher clinic laboratories with (x^2= p value >0.05) showing no statistical association between hospital laboratories & higher clinic laboratories. The most likely explanation for this performance might be in working environment like better supply management & staff composition among health facilities. Similar study in Thimphu & Bhutan showed that AFB microscopy quality of 21.1 to 23.1 % laboratories was found to be unacceptable (23). This finding is also similar to study in Ethiopia, with problem of reading slides properly& unsatisfactory reporting (below acceptable score < 80 %) results, reporting both major & minor errors. (10)

In our study there were major errors in 3 (14.3 %) laboratories where as minor errors in 12 (57.1 %) laboratories. Errors were encountered on 23 (11.0%) of the smears. In contrast to our findings, a panel test done in Nepal, 1 (11.1 %) laboratory had quantitative (minor) error while there was no any major error encountered. (31)

In this study, 131 (40.9 %) of panel slides were incorrectly interpreted by laboratory personnel in the study sites. HFN observed in 7 (2.2 %) of smear, LFP in 4 (1.25 %) of smear, HFP in 1 (0.3 %) of smears, QE in 71 (22.2 %) of smears and LFN errors in 46 (14.4 %) of panel. Over all agreement was very good with kappa value of 0.87. Out of the total 131 (40.9 %) error committed by all health facilities, 2 (6.6 %), 90 (28.3 %), 8 (2.5 %) and 3 (0.9 %) were committed by hospitals, higher clinics, health centers and diagnostic laboratory respectively. However there were no statistically significant differences in errors types and health facilities (p value > 0.05). Unacceptable performance was obtained from one microscopy center with 2 major errors (1 HFP and 1 HFN) and 6 minor errors (2 LFN and 4 QE).A similar study done in Karachi hospital to assess sputum smear microscopy quality in private laboratories of TB control program shows that only 2 (28.6 %) private laboratories correctly classified all the specimens submitted, with 4 (57.1 %) laboratories correctly classifying less than half of submitted specimens which is almost similar to our study result. the possible cause of this result might be due to poor engagement, capacity building and rigorous monitoring of standards at private laboratories are of vital importance for the control of tuberculosis. (30)

By tracking back to the errors committed by the specific sites, the possible root causes were competency of professionals in identifying AFB, work overload, lack of appropriate updated trainings on AFB training, inability to filter Carbol fuchsin regularly, failure to check sputum quality due to pressure from physician and owners not to follow the spot-morning-spot protocol as physicians request only one sputum sample as well reliance on x-ray diagnosis alone. In addition, one diagnostic higher clinic scored 50 % performance which is far more from acceptable 80 % standard by committing major and minor error of 1 HFP and 2 LFN due to poor staining quality, staining problem and lack of detecting AFB. This finding is also consistent with study done in Ethiopia by Estifanos Biru shows false reading 3.2 % and 74 % disagreement with the regional laboratory readings staining quality due to poor staining quality. (35)

Out of 320 panel slides, 160 unstained and 160 stained: 6HFN, 0HFP, 25 LFN, 5 LFP and 37 QE and 3 HFN, 1 HFP, 20 LFN, 0LFP and 34 QE errors were occurred, respectively. Therefore, the possible causes could be due to failure to read adequate number of microscopy field, staining and reading problems. Quantification errors are also of minor importance, as they do not influence case management. This type of error can distinguish good laboratory personnel from very good laboratory personnel.

5.2 Blinded rechecking

Out of the 33 assessed laboratories, 14 (42.4 %) of them are on DOT program on which random blinded rechecking was conducted. Of these, 5 (15.2 %), 8 (24.2 %), and 1 (3.03 %) were hospitals, higher clinics and NGO health centers.

Comparison of the results of the microscopy centers with the controller showed that they were similar in positive and negative findings 38 (13.4 %) and 236 (83.4 %) respectively. Considering the reading of the controller as a true value, there was 9 (3.2 %) discordant slides (2 FP and 7 FN) with99.3 % specificity and 84.4 % sensitivity. The PPV and NPV were 95 % & 97.1 %, respectively. There was no disagreement between microscopy center and controller having overall agreement of 97.5 % (X^2= p-value>0.05). Among the 9 discordant slides re-examined by the controller, 98.4 % were found to be in agreement with the microscopy center readings with kappa value of 0.87. Thus, the reading agreement between the microcopy center and controller is almost perfect. This shows that the result is similar with study in Nigeria showing with increased concordance rate of false positivity and false negativity rates of AFB microscopy results. (34)

From 283 random blindly selected slides, 14.1 % and 85.7 % were reported to be positive and negative for AFB by microscopy centers but 16.3 % and 80.6 % were reported as positive and negative by the controller respectively with (X^2= p-value <0.05) having a statistically significant difference between the microscopy center and the controller. This result is comparable with study in Kinshasa that shows 77 (10.4 %) discrepant results. And 67 (87 %) of these discrepant results were attributed to the peripheral laboratory. Other common problems involved here were poor microscope care, improper smear preparation, poor staining or reading techniques, incorrect data recording and slide storage, and lack of feedback from the NTP. (33)

The sensitivity and specificity of the microscopy centers whether they are or DOT or NOT for blinded rechecking ranges from 85-90 % & 90-100 % respectively. In general, the overall sensitivity, specificity, positive predictive value and negative predictive values of the blinded rechecking were 88.4 %, 99.3 %, 92.4 % & 98.9 % respectively. Moreover the overall kappa value found to be 0.87 and this shows us the reading agreement is almost perfect and ($X^{2\,=}$ p-value>0.05) showing no significant difference. Our result is almost similar with a study done in India whose sensitivity, specificity, PPV & NPV of reading smears were 94 %, 98 %, 99 % and 89 % respectively. The percentages of HFP & HFN were 0.06 % & 4 % respectively. (26)

The sensitivity, specificity, PPV, NPV and accuracy for microscopy centers by hospital, higher clinic, health center and diagnostic laboratory were 90.3 %, 99.3 %, 97 %, 98 %, and 97.6 %, respectively. All hospital microscopy centers had very good agreement with national having a kappa value of 0.93 which is almost perfect agreement.

Quality of smear was assessed during the random blinded re checking using visual assessment of the stained smears, likewise, we also assessed quality of smears during the visual re-examination of the sampled smears by the controller, on average it was found that 221 (78 %) had proper smear size, 188 (66.4 %) had proper thickness, 175 (61.8 %) had proper staining, 199 (73.3 %) had cleanness of smears and 161 (56.9 %) had evenness of smear.

Among the assessed slides an average of 203 (71.7 %), 220 (77.7 %), 190 (67.1 %), 161 (56.9 %), 185 (65.4 %) and 257 (90.8 %) slides have proper cleanness, size, thickness, evenness, staining and labeling qualities respectively. There was significant difference in slide quality among the 14 rechecked sites (X^2=p value <0.05).Which is almost similar to study done in America showing false negatives caused by specimen quality, patient identification, specimen labeling and transport condition; whereas false positive is caused by specimen container, specimen labeling and patient identification, where false negative results may also be caused by administrative or technical issues, specimen handling and registration or smear preparation, stain formulation, staining technique, microscope performance, and smear examination are some of the factors caused by technical aspects of the laboratory. Our finding of smears quality evaluation criteria; smear size, thickness, staining quality, cleanness &evenness were in agreement with study done in different countries of America. (21) This result is similar to study done in country by Mekete Mulat on AFB smear microscopy performance shows both private and public facilities had good slide quality which is more than 70 % for each quality components, except the private microscopic centers had poor quality in terms of smear evenness accounted only 28 % of the slides were even. There was no statistically significant difference between public and private health facilities except in the evenness of the smears which was better in the case of public health facilities. On the other hand, there were statistically significant differences for proper 28 smear size and evenness between hospitals and health centers where both measures were found to be better in health centers (p value < 0.05). (10)

Training of laboratory personnel on AFB and related topics would have an effect on reducing laboratory errors in AFB testing. This resulted in a marked improvement in the practical component (smear preparation, staining, and reading, our study finding also showed that training on AFB had better EQA performance similar to results of the Kinshasa showing shortages of materials such as distilled water, lens tissue, and disinfectant and the unavailability or the poor condition of the necessary equipment including wire loops, staining racks, a biohazard waste bin, and a microscope. Other common problems involved poor microscope care, improper smear preparation, poor staining or reading techniques, incorrect data recording and slide storage, and a lack of feedback from the NTP; in which 741 slides collected from the peripheral laboratories and reviewed by the NRL with 77 (10.4 %) discrepant results with 67 (87 %) of discrepant results were attributed to the peripheral laboratory and 10 (13 %) were attributed to the NRL (33)

5.3 On-site evaluation

We used standardized checklist adapted from the national EQA for sputum smear microscopy to assess the status of infrastructure, SOP, reagent and equipment, maintenance of microscope, biosafety and waste disposal, training related to AFB quality assessment, data and supply management to see associated problems of AFB smear microscopy performance of the selected private health facilities in Addis Ababa, Ethiopia.

In this study, among the selected 33 laboratories, 7 hospitals (21.2 %), 23 higher clinics (69.7 %), 2 health centers (6.06 %) and 1 diagnostic laboratory (3.03 %) were investigated. Of these, 14 (42.4 %) of them are on DOT program on which random blinded rechecking was conducted.

Based on the on-site evaluation, the facilities were assessed for infrastructure, availability and usages of internal quality control, staining reagents, safety and waste disposal practices, SOP's, training status, microscopy maintenance, data management and inventory management.

In our study, most health facilities had poor microscope maintenance, inventory management system and planning their supplies in advance 21 (63.6 %), 9 (27 %), 11 (33.3 %,) respectively. Our result is different from study in Gujarat India on proficiency panel testing and a reliable tool in EQA of sputum smear microscopy services that indicates high level of concordance in ZN smear laboratory whose readers' reported overall consistency level of more than 98 % in ZN grade agreement during the OSE visits. (32)

Only 5 (15.5 %) of participant laboratories were involved in panel testing which is critical for the overall AFB quality service due to lack of spare parts for microscope as only18 (54.5 %) of microscopy centers have spare parts and the rest may compromise the laboratory service delivery process mainly delay in tuberculosis diagnosis and treatment. Only 11 (33.3 %) have standard laboratory request and reporting form. Hence some laboratories are deficient which could result in poor data management for surveillance and other planning purpose. This result is similar to study done in California in which delays were associated with the type of laboratory in which testing was performed and delayed transport of specimens. (25)

In this study, only 2 (6.06 %) use control smears at least once a week by preparing positive and negative slides for reagent quality control. Nine (27.3 %) use standardized laboratory registration whereas 12 (36.4 %) report consistently with complete registration book. Concerning the supply chain managements of the PHFs, only 9(27.3 %) and 11 (33.3 %) have inventory system and plan their supplies respectively. Thirty (90.9 %) of laboratories obtain AFB reagents by direct purchase while the remaining 3 (9.09 %) obtain from AAHRB regional laboratory and distributed by pharmacist and laboratory professionals. No laboratory service interrupted due to supply problems and no facility prepares reagents in its laboratory. This result is similar to a study by Pan American health organization on smear microscopy quality assurance shows administrative issues like specimen container, transport condition, specimen quality, patient identification, specimen labeling, handling, patient identification and registration or smearing, stain formulation, staining technique, microscope performance, and smear examination are some of the factors that compromise quality laboratory service. (21)

In this study, only 6 (18.2%) have separate area of AFB laboratory for specimen receipt and smear preparation. And 15 (45.5%) have well ventilated windows while 29 (87.8 %) have regular water supply but most institutions 31 (93.95) and 30 (90.9 %) have permanent electric power with backup generator respectively. Likewise,19 (57.6 %) of laboratory have posted smear preparation, staining procedure and grading chart, but only 9 (27.3 %) have arranged slides in slide box as per lab register and slide number. Twenty four (72.7 %) examines at least 100 fields before reporting negative slides, where as 5 (15.2 %) and 31 (93.9 %) respectively examines 20-50 and 50-70 fields to report negative results. This result is similar to study in Indonesia that shows 9 to 53 % of tuberculosis cases and 4-18 % of sputum smear positive tuberculosis cases in hospitals were not served with standardized diagnosis. (24)

All laboratory personnel in the microscopy centers were responsible for sputum collection, along with quality of the sample; smears were dried and fixed prior to staining. However, 22 (66.7 %) collects sputum in spot morning spot system for AFB microscopy by checking sputum quality. Although all the 33 study sites use new slides for sputum smears, only 23 (69.7 %) of them label these slides with lab code, serial number and sequence identifier. Only one laboratory 1 (3.0 %) filters carbol fuchsin before staining and 10 (30.3 %) filters monthly but 22 (66.7 %) of them do not filter at all.

Twenty eight (84.8 %) use functional binocular microscope where regular maintenance of microscope given for 21(63.6 %) while 23 (69.9 %) have knowledge of repairing simple microscope problems and only 11(33.3 %) document their daily preventive maintenance which is cleaning their microscope. Seventy five eight percent of the facilities have standard waste container with lid while 32 (96.9 %) disinfect their working area. But only 11 (33.3 %) of them have adequate space for sputum processing. EQA protocol is available and followed in 6 (18.2%) and slides are collected by RRL for EQA from 7 (21.2%). This is also similar to study by WHO in resource-poor countries in which many smear microscopy laboratories are single roomed and understaffed with poorly maintained microscopes, and some of these laboratories lack consistent sources of electricity and clean water. There are few opportunities for the training of staff and little staff capacity to handle high volume workloads. (13)

5.4 Staffing

There were a total of 119 laboratory personnel in 33 private health facilities. Among these, 50 (42%) were female and the remaining were male laboratory professionals. 1 (0.8 %) MSc, 43 (36.1 %) BSc, 49 (41.2 %) diploma and the remaining 26 (21.8 %) were level IV laboratory technicians. Out of these, 18 (15.1 %) of BSc level took additional training for AFB smear microscopy services. On the other hand, 19 (16 %) diploma and the remaining 7 (5.9 %) were level IV medical laboratory technicians trained on AFB smear microscopy TB.

The age group in years 20-25, 26-30, 31-35, 36-40 & > 41 years and professional levels were 30(25.2 %), 52 (43.7 %), 23 (19.3 %), 11 (9.2 %), & 3 (2.2 %) and 10 (8.4 %), 20 (16.8 %, 15 (12 %), 41 (34.5 %), 31 (26.1 %) and 2(1.7 %) were expert, senior and junior medical laboratory technologist, and junior, senior, chief medical laboratory technicians respectively.

Thirty one (26.1 %) were working in hospitals, 79 (66.4 %) in higher clinics, 6 (5.04 %) in health center and 3(2.5 %) working in diagnostic laboratory. The work experience is dominated by 1-5 years with 71 (59.7 %), 30 (25.2 %) were 6-10 years, 4 (3.4%) of them were 16-20 while the remaining 11 (9.2 %) were having greater than 21 years of experience. Twenty five (21 %) were laboratory heads, 11 (9.2 %) quality officer and the remaining 83 (69.7 %) of respondents were laboratory experts. Facility based TB related training was given for 17.6 % working in hospital, 45.4 % in higher clinics, 2.5 % in health center and no personnel trained from the diagnostic laboratory.

6. Strength and Limitation of the study

6.1 strength of the study

- We assessed the performance quality of AFB smear microscopy of private health facilities using proficiency testing, random blinded rechecking and OSE.
- Used innovative onsite blinded rechecking method that saves time, transportation cost and slides for other concerned bodies
- Generated data on private healt0h facilities for other future studies
- Provided feedback for the MCs at spot

6.2 Limitation of the study

- Annual slide positivity rate was not calculated due to lack of stored data of previous performances at all MCs
- We were unable to include all health facilities in the region for better evaluation of the microscopy centers.
- The blinded rechecking we did was only for one quarter which would be better if four quarters to elucidate the correct picture of the microscopy centers.

7. Conclusion and recommendation

7.1. Conclusion

Proficiency test score of 100 %, 80-95 % and 50-75 % was performed by 2, 15 and 15laboratories, respectively, since microscopy centers scored a panel test score of 75.5 % which is below acceptable 80 %. Out of 283 randomly selected slides, the overall, false reading for blinded rechecking was 3.9% with overall agreement of 97.5 % and sensitivity of 88.4 % and specificity of 99.3 %. On-site evaluation indicated poor supply and usages of infrastructure, SOPs, reagent quality, equipment maintenance, data management and training issues. Results of the on-site evaluation revealed problems in terms of infrastructure, standard operating procedure, reagent quality, equipment maintenance, data management and training issues.

The results obtained from panel testing, blinded rechecking and the onsite evaluation checklist is almost similar and consistent as overall proficiency result shows 75.6 % whose reading agreement was very good with kappa value of 0. 87; similarly, in blinded rechecking, the result showed 3.2 % discordant readings with kappa value of 0.87. Thus, the reading agreement between the microcopy center and controller is almost perfect; hence result shown from proficiency testing and blinded rechecking is almost consistent with results from the onsite evaluation checklist with poor usage of 19 (57.6 %) posted smear preparation, staining procedure and grading chart, while 24 (72.7 %) examines at least 100 fields before reporting negative slides, where as 5 (15.2 %) and 31 (93.9 %) respectively examines 20-50 and 50-70 fields to report negative results. Together with these, the performance quality of the microscopy center was compromised with the availability and implementation of EQA protocol.

7.2. Recommendation:

Based on the finding, the following recommendations are forwarded:

- Continuous assessment of tuberculosis microscopy centers should be considered as part of DOTS program for proper diagnosis and management of tuberculosis in the city by including panel testing, blinded rechecking and onsite supervision of laboratories.
- The regional health and research laboratory should implement external quality assessment schemes for all health facilities.
- Public private partnership has to be strengthened in the city in particular for proper diagnosis and management of tuberculosis.
- The region should develop appropriate communication channel and maintain a significant means of monitoring and evaluation system for all microscopy centers.

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9. ANNEXES

Annex-I: Questionnaire: socio demographic characteristics of laboratory professionals working in private health facilities in Addis Ababa, 2013(n=119)

S/No.	Variables	Tick (√)
1	Gender	
	Female	
	Male	
2	Age group in years	
	20-25	
	26-30	
	31-35	
	36-40	
	>41	
3	Professional level	
	Chief Medical Laboratory Technologist	
	Expert Medical Laboratory Technologist	
	Senior Medical Laboratory Technologist	
	Junior Medical Laboratory Technologist	
	Junior Medical Laboratory Technician	
	Senior Medical Laboratory Technician	
	Chief Medical Laboratory Technician	
4	Educational level	
	MSc	
	BSc	
	Diploma	
	Level IV	
5	Working Organization	
	Hospital	
	Higher clinic	
6	Work Experience in years	
	1-5	

	6-10	
	16-20	
	>21	
7	Position in this organization	
	Laboratory head	
	Quality officer	
	Section/department head	
	Laboratory expert	

Annex II: AFB onsite evaluation checklist for MCs during direct observation

I. General Information		
1	Name of laboratory (MC): use code	
2	Zone / Woreda	
3	Region	
4	Head of MC	
5	Date of visit	
6	Name of the visiting laboratory supervisor (s)	

II. Human resource & development: Number of staff in the laboratory				
Staff category of laboratory by level of education		Number	Frequency training on TB	Not trained on TB
1	Laboratory scientist (MSc)			
2	Laboratory technologist (BSc)			
3	Laboratory technician (Dip)			

III. Infrastructure			
Descriptions		Adequate /Acceptable	
		Yes	No
1	Separate area for AFB laboratory work		
2	Separate tables for specimen receipt/smear preparation/ microscopy		
3	Well ventilated laboratory (window)		
4	Do you have regular water supply?		
5	Is there permanent electric power?		

6	Do you have a generator as a back up?		
IV. Availability of Standard Operating Procedure:			
1	Is there a posted smear preparation & staining procedure?		
2	Is there a posted grading chart?		
3	Are all slides available in the slide box as per lab register?		
4	Are slides arranged as per their slide numbers?		

V. Material:							
Material descriptions		Adequate supply		Inadequate supply		Conditions (properly functional)	
		Yes	No	Yes	No	Yes	No
1	Frosted slide						
2	Slide box						
3	Sputum container approved by NTP						
4	Pencil used for frosted slides						
5	Wire loops or sticks						
6	Funnel						
7	Filter paper						
8	Staining rack						
9	Sprit lamp/Bunsen burner						
10	Lens tissue						
11	Red pen for recording positive result						
12	Water supply						

VI. Reagents							
Reagent Descriptions		Adequate supply		Inadequate supply		Conditions (properly functional)	
		Yes	No	Yes	No	Yes	No
1	Carbol fuchsin						
2	Methylene blue						
3	3% acid alcohol						
4	Oil immersion						
VII. Items							

Items Descriptions		Adequate /Acceptable	
		Yes	No
1	Functional Binocular light Microscopes		
2	Regular maintenance of microscope		
3	Do you know how to repair simple microscope problem? (E.g. changing a bulb of the microscope)		
4	Do you have spare-parts at the lab? E.g. bulbs		
5	Do you document preventive maintenance for the microscope?		
5.1	If, Yes, how frequently?		
5.2	What is your major preventive maintenance?		
VIII. Biosafety and waste disposal			
1	Standard waste containers with lid		
2	Do you disinfect your working area?		
3	Do you autoclave any contaminated materials before disposal?		
4	Do you have incinerator?		
5	Do you dispose contaminated materials by incinerator?		
7	Is the working area cleaned & well organized?		
8	Do you wear personal protective equipment during work?		
9	Is there adequate space for sputum processing?		
10	Is there availability of safety supplies (lab coat, glove etc.)?		
11	Is there a good sewerage system, staining, washing area?		
IX. Trainings related to TB			
1	Trained on NTCP/ EQA		
2	Is there training need?		
3	If yes, please specify what training		
4	Has new staff received proper training as required by the NTP?		
5	Never trained		
X. Quality Assurance			
1	EQA Protocol available and followed		
2	Do you do use internal quality control for reagents?		
3	Do you use control smears at least once a week to check quality of stains?		

4	Do you prepare positive & negative slides for reagent quality control? If yes, observe			
5	Did you collect and send slides for EQA to regional laboratory in the last two years? If yes, please state when, how many times			
6	Does your laboratory participate in panel testing?			
7	Did you receive EQA feedback from regional laboratory? If No, please state			
8	Does your laboratory Participate in blinded re- checking?			
9	Are slides collected quarterly?			
10	If the answer is yes, for the previous Quality, EQA feed back?			
11	Do you receive the RBRC results of previous quarter/quarterly?			
12	Have results of rechecking or panel testing been acceptable?			
XI. Data management				
1	Is a standard laboratory register book in use, If not, why?			
2	Is there a standard laboratory Request form?			
3	Is the registration book completed with the necessary information?			
4	Do you record when you sent slides for EQA? If yes observe			
5	Are there standard reporting formats?			
6	Is there consistent reporting system?			
XII. Inventory and Supply management				
1	Do you have an inventory list of supplies and stains?			
2	How often do you receive supplies like stains and others?	A. Monthly		
		B. Every 6 months		
		C. Once in a year		
3	Do you plan your supply requirements in advance?			
4	Do you have difficulties in receiving your supplies? If yes, why?			
5	Do you prepare stains and reagents by yourself in your lab?			
XIII. Basic AFB microscopy procedure to observe the skill of laboratory professionals				
1	Is the lab technician responsible for collecting sputum sample? If no, who is responsible?			
2	Do you collect sputum for AFB microscopy Spot, Morning, and Spot? If No, explain			

3	Is the quality of sputum checked? Yes/No			
4	When the patient produces saliva, do you repeat sputum sample? If No, explain			
5	Are new slides used for sputum smears?			
6	Are slides cleaned prior to use?			
7	Are slides labeled with lab code, serial number & sequence identifier?			
8	Are smears completely air dried prior to fixing?			
9	Are slides heat fixed by passing 3-5 times through flame?			
10	How often is carbol fuchsin filtered?	A. Always before use		
		B. Monthly		
		C. Weekly		
		D. Never		
11	Is there a wooden stick is to prepare each smear? If No, what you use to smear the sputum?			
12	How many fields are examined to report a negative smear?	A. 20-50		
		B. 70-100		
		C. 50 – 70		
13	Do you clean microscope lenses with tissue paper? If No, explain:			
14	Where do you get prepared AFB reagents?	A. Regional laboratory		
		B. Zone/ Woreda		
		C. Direct purchase		
		D. Other (specify)?		
15	Who is responsible to distribute reagents and supplies to the institution?	A. Lab technologist		
		B. Pharmacist		
		C. Zonal TB / Lab expert		
		D. AAHR Regional lab		
16	Are there prepared reagents supplied by standard container? If no, list the container			
17	Is there a service interruption to laboratory work due to shortages of supplies and equipment? If yes, explain			

Annex III: Evaluation and interpretation of errors between controllers and microscopist

Result of PDC-LT	Regional reference laboratory Result (controller)				
	Negative	1- 9AFB/100F	1+	2+	3+
Negative					
1-9AFB/100					
1+					
2+					
3+					

Annex IV: Performance level of microscopy centers in PHFs in Addis Ababa (33, 2104)

Lab Code	HFN	HFP	LFN	LFP	QE	Total error	Total scores
LAB-01							
LAB-02							
LAB-03							
LAB-04							
LAB-05							
LAB-06							
LAB-07							
LAB-08							
LAB-09							
LAB-10							
LAB-11							
LAB-12							
LAB-13							
LAB-14							
LAB-15							
LAB-16							
LAB-17							
LAB-18							
LAB-19							
LAB-20							

LAB-21							
LAB-22							
LAB-23							
LAB-24							
LAB-25							
LAB-26							
LAB-27							
LAB-28							
LAB-29							
LAB-30							
LAB-31							
LAB-32							
LAB-33							
Total							

Annex V: Results of the study

Type of error	Categories of Health facilities	Error types	No of error	Percent (%)
Major error	Hospitals	HFN		
		HFP		
	Higher clinics	HFN		
		HFP		
	Health centers	HFN		
		HFP		
Minor error	Hospitals	LFN		
		LFP		
		QE		
	Higher clinics	LFN		
		LFP		
		QE		
	Health centers	LFN		
		LFP		

		QE		
Total				

Annex VI: Types of errors encountered

Health institution	Major errors		Minor error			Total
	HFN No (%)	HFP No (%)	LFN No (%)	LFP No (%)	QE No (%)	
Hospitals						
Higher clinics						
Health centers						
Total						

Annex VII: Comparison of stained and unstained panel-testing smear

Types of panel slides	Major error		Minor error			Total error
	HFN	HFP	LFN	LFP	QE	
Stained panel slide						
Un stained panel slide						
Total						

Annex-VIII: Performance of panel testing that compares PHFs to NRL

PHFs (Test value)		National reference laboratory (True value)		Test total
Test value	Positive	Positive	Negative	
	Negative			
True value total				

Annex-IX: Sensitivity, specificity & predictive values of the microscopy for panel slides

Microscopic centers by facilities	Sensitivity	Specificity	PPV	NPV	Kappa value
LAB-01					
LAB-02					
LAB-03					
LAB-04					
LAB-05					
LAB-06					
LAB-07					
LAB-08					
LAB-09					
LAB-10					
LAB-11					
LAB-12					
LAB-13					
LAB-14					
LAB-15					
LAB-16					
LAB-17					
LAB-18					
LAB-19					
LAB-20					
LAB-21					
LAB-22					
LAB-23					
LAB-24					
LAB-25					
LAB-26					
LAB-27					
LAB-28					
LAB-29					

LAB-30					
LAB-31					
LAB-32					
LAB-33					
Total					

Annex-X: Values for blinded rechecking at diagnostic centers and controller

Diagnostic center			Controller value (True value)		Total
			Negative	Positive	
Test value	Positive				
	Negative				
True value total					

Annex XI: Sensitivity, specificity and predictive values for blinded rechecking

Microscopic centers	Sensitivity	Specificity	PPV	NPV	Kappa value
Hospital					
Health centers					
Higher clinics					

Annex XII: Distribution of laboratory professionals with TB related training

Facilities	No of HFs	Trained for TB				Untrained for TB			
		PhD n, %	MSc n, %	BSc n, %	Diploma n, %	PhD n, %	MSc n, %	BSc n, %	Diploma n, %
Hospitals									
Higher clinics									
Health Centre									
Total									

Annex XIII: Laboratory workload on slide volume during the onsite evaluation

Health facilities	No. of lab centers	Total no of sample examined	No. of smear positive	No. of smears negative
Hospitals				
Higher clinics				
Health Centre				
Diagnostic laboratory				
Total				

Annex IXV: External quality assessment program of panel testing for AFB Microscopy

Reporting Format for Proficiency Test Result

S. No	Lab code	1	2	3	4	5	6	7	8	9	10	Sig nature
		stained	Unstained	Stained	Unstained	Stained	Unstained	Stained	unstained	Stained	unstained	
1	LAB 01											

Total score: __________ Percentage: _______ Performance acceptable / unacceptable

Classification	Description	Interpretation	Score
Correct resp.		No error	10 point
LFP, LFN	Low false positive & low false negative respectively	Minor error	5 point
QE	Quantification error	Minor error	5 point
HFP, HFN	High false positive &high false negative respectively	Major error	0 point

Annex-XV: External quality assessment microscopy center smear result sheet for random blinded rechecking

Slide #	Lab code	Slide quality**						MC result	RBR result	Agree	Disagree	NRL result
		Clearness	Size	Thickness	Evenness	Staining	Labeling					

Annex XVI: Manufacturing procedure for panel test smear

This procedure is a self-explanatory laboratory method for producing multiple test slides from AFB positive and negative samples. Laboratory staff should read and understand both the procedure and the testing protocols before developing test slides. This procedure has been reproduced /validated in NRL. National TB laboratory has continued difficulties with clumping of AFB that prevents slide-to-slide consistency; the use of N-acetyl-L cysteine (NALC) may improve the quality of the slides. The NRL should demonstrate proficiency in producing samples with a minimum of 25-30slides that are consistent for negative and low numbers of AFB before proceeding to developing test slide sets

Materials Required

Processing was performed in a Biological Safety Cabinet level II

- 50 ml plastic screw cap tubes
- 40% Formaldehyde
- 4% NaOH
- 2% of N-acetyl-L-cysteine
- 2.9% sodium citrate.2H2O
- 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)

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

AFB Smear Panel Preparation protocol

Determining how many AFB/field in starting material (Pooled sputum)

- Put on personal protective equipment (PPE)
- Wipe don work area with 1% Sodium hypochlorite
- Receive and pool (about 10ml) of 3+ smear positive sputum from diagnostics
- Arrange reagents, sputum, pipettes, vortex, slides and discard container in a biosafety cabinet if available (Perform all steps in a BSC)
- Add 1 drop of 40% formalin per ml of sputum
- Vortex vigorously for 30 seconds, invert, vortex vigorously for 30 seconds
- Incubate at room temperature for 1 hour
- Vortex the pooled 3+ positive sputum for 2 minutes (use a timer)
- Label and prepare 2 direct smear from the pool
- Dry, heat fix, and stain using Ziehl-Neelsen staining method

Pooled Positive Starting Material (Sputum):

$$\mathbf{averagepooled}\left(\frac{\mathbf{AFB}}{\mathbf{field}}\right) = \frac{\mathbf{slide1}\left(\frac{\mathbf{AFB}}{\mathbf{field}}\right) + \mathbf{slide2}\left(\frac{\mathbf{AFB}}{\mathbf{field}}\right)}{\mathbf{2}}$$

Guide for achieving highly concentrated stock ($\mathbf{80 - 100}\,\frac{\mathbf{AFB}}{\mathbf{field}}$):

$\geq 50\,\frac{\mathbf{AFB}}{\mathbf{field}}$ on direct smear = prepare 1 tube following procedure

$50 - 20\,\frac{\mathbf{AFB}}{\mathbf{field}}$ on direct smear = prepare 2 tubes on the following procedure

$<20\,\frac{\mathbf{AFB}}{\mathbf{field}}$ on direct smear = prepare 3 tubes on the following procedure

Preparation of positive stock suspension (for each tube):

- Using the guide above determine how many tubes are needed to produce a concentrated stock
- Working in a biosafety cabinet, vortex the pooled positive starting material for 1 minute

- Pipette 3ml of the positive starting material into a 50ml Falcon tube labeled "Positive Stock" for each tube
- Add 1ml of 4% NaOH (or 2ml if sputum is very viscous) to each tube
- Vortex vigorously for 1 minute
- Add 20ml of distilled water
- Add 5ml of Sputasol (Tween 20), or 7.5ml if sputum is very viscous, and mix by inverting 3-5 times (do not vortex)
- Incubate in a 55-60^{o}C water bath for 30 minutes, mix by inversion once or twice during the incubation
- Add distilled water up to 40 ml
- Mix by inverting 3-5 times
- Centrifuge at 3000xg for 20 minutes (rpm =$\sqrt{\frac{\mathbf{RCF}}{\mathbf{1.12Rmax}}}$ $\mathbf{xxmm}$)
- Decant the supernatant into 2% sodium hypochlorite disinfectant
- Re-suspend the sediment from tube 1 in 1ml of Phosphate Buffered Saline PH 6.8 (If available) or distilled water
- Vortex 1 minute
- If more than one tube is being prepared, add all the suspension from tube 1 to the next sediment. Vortex for 1 minute and repeat for tube 3 if necessary
- Prepare 6 slides of the re-suspended sediment using a 10µl loop
- Allow to air dry, heat fix, and stain using the ZN method
- Store prepared stock at 2-8^{o}C if dilutions will not be prepared the same day

Preparation of negative stock suspension:

- Place 3 ml of smear negative sputum (verified by direct smear) in a labeled 50 ml Falcon tube
- Add 3 drops of 40% formaldehyde
- Vortex vigorously for 30 seconds, invert the tube, vortex again for 30 seconds
- Incubate for 1 hour at room temperature
- Add 1 ml of 4% NaOH, and mix gently by inversion 3-5 times
- Add 20ml of distilled water, and mix well by inverting several times
- Incubate in 55-60^{o}C water bath for 10 min, mixing by inversion 3-5 times during and after period

- Store at 2-8^{0}C if dilutions are not prepared the same day

Table XVII: AFB results from positive stock suspension:

Have six different readers and read each of the six slides to determine the average number of AFB per field (ideally, between 60 to 80 per field)

The 6 different readers	Slide 1 (AFB/field)	Slide 2 (AFB/field)	Slide 3 (AFB/field)	Slide 4 (AFB/field)	Slide 5 (AFB/field)	Slide 6 (AFB/field)	Average
Reader 1:							
Reader 2:							
Reader 3:							
Reader 4:							
Reader 5:							
Reader 6:							
Average							

$$\textbf{the sum of the averages from all readers (A)} = \frac{?}{6} = \textbf{70 AFB, averageAFB/field}$$

Formulas for preparing the 3+ dilutions:

The target concentration for the 3+ dilution is 50 AFB/field where:

A = Concentration of the positive stock solution (average AFB/field determined above)

B = the amount of positive stock solution added to the 3+ dilution tube (total volume = 1ml)

C = The amount of negative stock solution to the 3+ dilution tube (total volume = 1 ml)

All dilutions are prepared in a total volume of 1 ml

$$B = \frac{\text{targetconcX1ml}}{\text{concofpositivestock}} = \frac{50\frac{AFB}{field}X1ml}{A}\quad C = 1ml - B$$

Table XVIII: Target dilution concentrations for smear panel preparation:

Reporting Scale	Expected concentration of AFB to observe	Target concentration of AFB in each dilution	Formula
3+	>10 AFB/field	50 AFB/field or 5000 AFB/100 fields	$\frac{50\frac{\text{AFB}}{\text{field}}\text{X1ml}}{\text{A}} = \text{B}$
2+	1-9 AFB/field	10 AFB/ field or 1000 AFB/100 fields	$\frac{10\frac{\text{AFB}}{\text{field}}\text{X1ml}}{50\frac{\text{AFB}}{\text{field}}} = 0.2\text{ml}$
1+	10-99 AFB/field	2 AFB/field or 200 AFB/100 fields	$\frac{2\frac{\text{AFB}}{\text{field}}\text{X1ml}}{10\frac{\text{AFB}}{\text{field}}} = 0.2\text{ml}$
Actual	1-9 AFB/field	0.4 AFB/field or 40 AFB/100 fields	$\frac{0.4\frac{\text{AFB}}{\text{field}}\text{X1ml}}{2\frac{\text{AFB}}{\text{field}}} = 0.2\text{ml}$

Preparation of dilutions from positive stock solution:

- Label four 2ml screw plastic tubes (Cryovials) with: "3+, 2+, 1+, and Actual" and place in a rack
- Place the positive and negative stock suspensions that were prepared earlier in a rack in the work place
- Calculate the amount of positive stock to be added to the 3+dilution by using the formulas listed above. (B = Amount of the positive stock to be added to 3+ dilution tube =? ml)
- Calculate the amount of the negative stock: $(\text{C} = 1\text{ml} - \text{B} = ?\ \text{ml})$
- Vortex the negative stock for 20 seconds
- Add amount C? ml of the negative stock to the 3+ vial
- Add 0.8ml of the negative stock to each of the 2+, 1+, and the Actual vials
- Vortex the positive stock for 2 minutes
- Add amount B? ml of the positive stock to the 3+ vial
- Cap and vortex the 3+ vial for 2 minutes
- Add 0.2ml from the 3+ vial to the 2+ vial

- Cap and vortex the 2+ vial for 2 minutes
- Add 0.2ml from the 2+ vial to the 1+ vial
- Cap and vortex the 1+ vial for 2 minutes
- Add 0.2ml from the 1+ vial to the Actual vial
- Cap and vortex for 2 minutes
- Prepare 6 smears from each dilution using a 10µl loop, vortex each dilution 30 seconds directly before smear preparation
- Dry, heat fix and stain using the ZN method
- Have 6 reader examine each slide and document results in the following tables

Annex –IXI Panel validation procedure, 3+ Dilutions:

The 6 different readers	Slide 1 (AFB/field)	Slide 2 (AFB/field)	Slide 3 (AFB/field)	Slide 4 (AFB/field)	Slide 5 (AFB/field)	Slide 6 (AFB/field)	Average
Reader 1:							
Reader 2:							
Reader 3:							
Reader 4:							
Reader 5:							
Reader 6:							

Annex-XX: 2+ Dilutions:

The 6 different readers	Slide 1 (AFB/field)	Slide 2 (AFB/field)	Slide 3 (AFB/field)	Slide 4 (AFB/field)	Slide 5 (AFB/field)	Slide 6 (AFB/field)	Average
Reader 1:							
Reader 2:							
Reader 3:							
Reader 4:							
Reader 5:							
Reader 6:							

Annex-XXI: 1+ Dilution

The 6 different readers	Slide 1 (AFB/field)	Slide 2 (AFB/field)	Slide 3 (AFB/field)	Slide 4 (AFB/field)	Slide 5 (AFB/field)	Slide 6 (AFB/field)	Average
Reader 1:							
Reader 2:							
Reader 3:							
Reader 4:							
Reader 5:							
Reader 6:							

Annex-XXII: Actual Dilutions:

The 6 different readers	Slide 1 (AFB/field)	Slide 2 (AFB/field)	Slide 3 (AFB/field)	Slide 4 (AFB/field)	Slide 5 (AFB/field)	Slide 6 (AFB/field)	Average
Reader 1:							
Reader 2:							
Reader 3:							
Reader 4:							
Reader 5:							
Reader 6:							

Annex-XXIII: Statistical Validation of Panel:

Use the standard deviation (SD) calculation form to statistically validate the panel

Step 1: Record a set of 6 readings from one reader in the reading column (R). Record the dilution and the Reader at the top of the from

Step 2: Calculate and record the mean (the average of the 6 readings) in the “Mean of the Readings (M)” box

Step 3: Using the spaces provided in the third (M) and forth column (R – M), subtract the mean from each individual reading and record the answer

Step 4: Square each of the 6 values in the "(R – M)" column and record the answer to the right in the "Square of R – M" column

Step 5: Calculate the sum of the squares of R – M and record it in the space provided

Step 6: Divide the sum of the squares R – M (answer from Step 5) by n – 1 (n = number of values in the data set in this case n= 6 and 6 – 1 = 5)

Step 7: Calculate the square root of the answer from Step 6 ($(\mathbf{sumofsquaresofR-M})\ /\ (\mathbf{n-1})$). This number is the standard deviation (SD) for this data set, record it in the space provided.

Step 8: Insert the SD into the following formula to determine if the panel is acceptable.

$$(\text{Mean} - 2\text{SD}) = ???$$

$$\text{If the value} > 0\ then\ the\ panel\ is\ acceptable.$$

$$\text{If the value} < 0\ then\ the\ panel\ is\ rejected.$$

Determine the SD for each reader's 6 slides for each set of dilutions. There should be 24 SD calculations

Once all the SDs have been calculated and it has been determined if each will be accepted or rejected, examine all the results for each dilution. There will be 6 accept or reject results for each dilution. The entire dilution is acceptable if at least 5 reader's results are acceptable (1 reader's results can be rejected and the dilution still is acceptable). When 2 or more readers' results are rejected then the dilution is considered rejected.

Calculation of SD and Statistical Validation of the AFB Smear Microscopy EQA Panel

$$\text{Acceptable} \quad (\text{if } M - 2\text{SD is} > 0)$$

$\text{Rejectable} \quad (\text{if } M - 2\text{SD is} < 0)$

<u>Remark:</u> the panel is acceptable according to the above statistical calculation validation

Annex-XXIV: Ziehl Nelson staining for AFB

Principle:

Mycobacterium tuberculosis is known as AFB because it resists decolorisation by acid. This acid fastness is due to the presence of mycolic acid in the cell wall. In this method, the primary stain (Carbol fuchsin) is heated, which facilitates the stain to penetrate the waxy covering of mycobacteria and resist decolorisation by weak acid. Those bacteria that resist decolorisation by acid are called Acid Fast Bacilli (AFB). This property differentiates AFB from other bacteria, cells and mucus that get decolorized by the action of weak acid. The counter stain (Methylene blue) is used to stain other materials and gives a contrast background for easy visibility of the acid-fast bacilli.

Method

1. Heat fixed sputum smears were placed on the staining rack.
2. Flood the smear with Carbol fuchsin.
3. Heat the slides from underside with a spirit lamp until vapor just began to rise. Leave the stain for about 5 minutes.
4. Rinse the stain with clean water and drain off excess water on the slide.
5. Decolorize the smear by covering the whole slide with 3% acid for about 3-4 minutes or until the smear is sufficiently decolorized. Decolorisation is repeated, if necessary.
6. Rinse well with clean water.
7. Cover the smear with methylene blue for 1 minute. Gently rinse the slide with clean water.
8. Wipe the back of the slide clean and place upright on the slide rack to air dry.
9. Examine the smear under oil immersion objective for the presence or AFB.

Result: The AFB is stained bright pink

Procedural notes: It is important for reading and interpretation of results with appearance of the smears of 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. It would be also useful when making 1+ suspension to consider making two different concentrations: 50 AFB/100 fields for 1+ smear preparation and 15 AFB/ fields for further dilution to “exact” count smear.

Annex-XXV: Prepare and Validate Batches of Slides

Using diluted stock preparations; prepare slide batches (50-100 slides per batch was recommended). Note: If laboratories are proficient in developing consistent slides, then developing many slides from fewer samples helps to save time. 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. Number of slides made: The laboratory should record how many slides are made from each sample to determine how many slides are available for test slide sets. National reference recommends that laboratories prepare 50-100 slides so that sufficient slides are available to put duplicate samples (one stained and one unstained) in test slide sets. Date slides made: The date that the test slides are produced. The lengths of time that slides are stored for two weeks without affecting performance before distribute our study sites.

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 technologists. For high positives (2+ or 3+) the technologists 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 technologists should read a minimum of 200 fields per slide and record the average number AFB/100 fields

Reporting criteria

Reporting criteria was done using National and WHO/IUATLD guidelines

Annex-XXVII: Grading of AFB smears by Z-N microscopy WHO /IUATLD criteria

No. of AFB	Reporting
No AFB /200F	Negative
1 -9 AFB /100F	Scanty
10 -99 AFB/ 100F	1+
1 – 10 AFB / F	2+
More than 10 AFB / F	3+

Anne XXVIII: Procedure for Blinded slide rechecking

Check the contents of the slide box to ensure that all slides are present and that laboratory numbers match those on the blinded re-checking result sheet. This is based on the 'Lot Quality Assurance System statistical sampling method and minimal sensitivity will be set at 80%. The slides were selected using the laboratory register by PI from the last quarter.

1. Sample size determination

Ideally, the collected smears should constitute a statistically representative and random sample based on test volume.

2. Slide evaluation

Re-examined the sampled smears visually to assess the quality of smear preparation as size, thickness, staining, cleanness, evenness and labeling using the national guideline (Annex 29).

Annex XXIX: The Criteria of blinded rechecking smear evaluation

S. No	Criteria	Definition
1	Smear size	This is evaluated macroscopically. A smear size of approximately 1x2 and 2x3 cm was considered acceptable
2	Smear thickness	This is evaluated by both microscopic and macroscopic observations. A smear is considered to be of acceptable thickness if the entire depth of the smear layer can be focused sharply in each field
3	Staining quality	This is evaluated by microscopically. A good staining technique ensures that the smear is neither under-stained nor over-stained
4	Cleanness	This is evaluated by microscopically. A smear was considered clean if stained smear is free from stain deposits, dirt, debris and crystals produced by overheating during staining
5	Evenness	Slide is evaluated by macroscopically. A smear of acceptable evenness should not be too thick or not too thin on the slide
6	Labeling	Observed microscopically and it should contain slide number and name of number of the health institution

3. Slide Storage

The laboratory must store slides in a way that allows retrieval of every slide identified for the rechecking sample. Therefore, it is best to save all slides, storing them in the slide boxes in the same order as they are listed in the laboratory register. In order to maintain consistency with the laboratory register, two blank spaces should be left behind the first slide from a suspect patient so that the second and third slides can be added after they are read. Slides must be labeled in a manner consistent with the laboratory register to ensure that the correct slide is matched to the result. The result of the smear examination must not appear on the slide. Prior to placing slides in the storage boxes, slides may be cleaned with tissue paper or inverting the examined slides on soft tissue paper but xylene should not be used for removal of oil. Store slides in boxes that allow the immersion oil to drip off, and the slides are not touching each other (e.g., do not stack or press slides together). Filled slide boxes should be stored closed and as far removed from heat and humidity as possible until they are sampled for re-reading. Slides should not be dried and stored under direct UV light. The sampling and re-reading of slides should be done as soon as possible, because long-term storage under tropical climatic conditions will cause fading of the ZN staining.

Annex XXX: Standards for reagents and equipment

1) Standards for Reagents

a. Specifications

A. Basic fuchsine

The chemical name: Pararosaniline hydrochloride

The chemical structure: C19H18N3Cl

Molecular Wt: 323.8

Color: Metallic green

Dye content: Should be available on the container. Approximately 85% - 88% (to calculate the required amount of basic fuchsine; divide the actual amount by dye content. For example: Dye content = 85%, actual amount = 10gms, required amount = 10/0.85 = 11.76gms.

B. Carbolic acid:

The chemical name: Phenol

The chemical structure: C6H5OH

Molecular Wt: 94.11

Melting point: 40^{o} C$\pm$2

Purity: 99.5%

Note: The critical concentration of phenol in carbol fuchsin is 5%.

Phenol is highly corrosive, handle with extreme care.

C. Acid Alcohol: 96% Ethanol and 37% Hydrochloric acid

D. Methylene blue:

The chemical name: methylthionine chloride

The chemical structure: C16H18ClN3S

Molecular Wt: 319.9

Dye content: Should be available on the container.

Approximately 82% (to calculate the required amount of Methylene blue; divide the actual amountby dye content. For example: Dye content = 82%, actual amount 1gms, required amount = 1/0.82 = 1.22gms.)

E. Immersion oil: Immersion oil supplied by the manufacturer of microscope with refractive index closer to that of Glass or 1.515; liquid paraffin (heavy), refractive index of 1.48, a colorless, odorless, transparent, free from fluorescence in day light with relative density of 0.827 to0.890, viscosity of 110 to 230 mPa s., specific gravity of 0.76-0.78 at 15.5^{o}C.

F. Shelf life of prepared reagents:

Carbol fuchsine, acid alcohol, methylene blue reagents may be kept for a maximum period of 4 months.

G. Identification:

All reagents should be label with name of the reagent, name of the TU, name of MC, the date of preparation and the expiry date.

The containers of Carbol fuchsin, Acid alcohol, Methylene blue reagents should in addition have the name of the person preparing the reagent.

Freshly prepared reagents should not be mixed with old stock.

2. Equipment:

i. Slides:

- Size: 76 mm x 26 mm,
- Thickness: 1.3mm
- Edges: Polished

- Sealed in a moisture absorbing desiccant pack

ii. Balance:

Type: Electronic or Analytical balance

a. Electronic balance: a general purpose table top laboratory balance, 220-230V, stainless steel platform, keypad auto calibration function, auto off, prolonged battery life, overload and under load, low battery LCD indicator.

Range: Wide range, 0.01 – 120gms, (two digits decimal)

Resolution: 0.01gm

b. Analytical balance: Enclosed in a glass box with shutters dimensions of 46 x 34 x 20 in cm. Oscillator type of balance, with leveling screws, two aluminum pans, plumb line for adjusting horizontal level

Weighing capacity: 1 mg to 200gms, with fractional weight and regular weight in boxes including rider and forceps to handle weights

iii. Binocular microscopes

Binocular microscope, for use with electric light via power line

Observation tube: binocular, 300 inclination (viewing angle) and 3600 rotations

Stage: rectangular, built in mechanical stage with venire scale (minimum: 140mm x135mm)

Condenser: Abbe type condenser (0.9/1.25) with iris diaphragm

Objective: 10x, 20x, 40x, 100x, oil immersion; color corrected infinity optics

Eye pieces: wide field, 10x/18, Fov 18 mm, adjustable, can be used by spectacle wears

Power supply: wide range in put 100-240V, 500-600HZ

Microscope has to full the standard: ISO9001

Accessories: Additional mirror for daylight usage, photo tube

Annex-XXXI: Information sheet and Verbal Consent form (English and Amharic version)

General information:

I the undersigned name assure and no complain if the indicated title is performed in our institution for the sake of the sector's quality improvement and consistent laboratory quality assurance in terms of TB laboratory.

Information sheet and Verbal Consent form (English version) Onsite evaluation: To assess quality performance evaluation of laboratories on AFB smears in private health facilities in Addis Ababa, Ethiopia, 2014

General information sheet about the study

Name of the hospital / microscopic center ---
Questionnaire identification number---

My name is Lemi Mosissa and currently I am student of clinical laboratory science in the track clinical laboratory management and quality assurance program launched in Addis Ababa University College of health sciences school of medical laboratory science. Now, I am working a research thesis project and the aim of the study is to assess quality performance evaluation of sputum smear microscopy detection of tuberculosis with panel testing and on-site evaluation by WHO EQA guidelines to determine the gap of technical error & performance of the technician on selected health facilities in private health institution in Addis Ababa, Ethiopia and which will inspire the improvement of the diagnostic outcome. It will also important for government to make decision.

All the information, which you are being asked to provide in this questionnaire will be kept strictly confidential; and will be used only for study purposes, the interview is voluntary and you have the right to participate or not. However, your participation is important to full fill the study purpose.

For any information you can contact

1**. Addis Ababa University College of health sciences school of clinical laboratory sciences**

Tel. 251-11-2753470, school of clinical laboratory science

2. Advisors:

- ✓ Kassu Desta (BSc, MSc, PhD fellow), Tel: 0911107099, E-mail: kassudesta2020@gmail.com
- ✓ Tedla Mindaye (BSc, MSc), Tel: - 0911634324, E-mail: tedlamin@yahoo.comand
- ✓ Abebaw Kebede (BSc, MSc), Tel: - 0911634324, E-mail Abebaw@live.com

3. The address of the principal investigator is: Lemi Mosissa, BSc Department of Medical laboratory sciences, Clinical laboratory management and quality assurance track

Tel: +251-0911-18-23-59 E-mail:lammii1515@gmail.com/lemimosissa@yahoo.com

I hope that you will be frank and honest in providing answers to the following questions: -

Do you agree to answer the following questions to the best of your ability?

Yes () No () If you answer yes, please continue responding to the interviewer

Thank you in helping with this important study.

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በዚህ መጠይቅ ውስጥም ሆነ በሌላ አጠቃላይ የጥናቱ ውጤቶች በሚስጢር ተይዘው ለጥናቱ ብቻ አገልግሎት ላይ የሚውሉ ይሆናል፡፡ በቃለ መጠይቁም ሆነ በጥናቱ ላይ ለመሳተፍ በርስዎ ፈቃደኝነት ላይ የተመሰረተ ይሆናል፡፡ ነገር ግን የርስዎና የተቋምዎ በጥናቱ መሳተፍ የሚያስገኘው መረጃ የጥናቱን ዓላማ ለማሳካትና የቲቢ ማይክሮስኮፒን ምርመራ ጥራት ከፍለ ማድረግ ለበሽተኞችም አስፈላጊና ተገቢ መድሓኒት በወቅቱ እንዲጠቀሙ ከማድረግ አኳያ የጥናት አሰሳው ያለው ሚና ከፍተኛ ነው፡፡

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   ከመጠይቁ በፊት የተጠያቂውን ስምምነት ማረጋገጫ ቅፅ፡-

   1. በጥናቱ ለመሳተፍ ፈቃደኛ ነዎት? ሀ. አዎ ለ. አይደለም

   በዚህ ጠቃሚ ጥናት ስለረዱኝ አመሰግናለሁ!

Declaration

I the undersigned, declare that this is my original work and has not been presented for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

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Signature: ______________________

Approval of advisors

Name of the advisors:

Kassu Desta (MSc, Assistance professor, PhD fellow)

Signature ___________________ Date: _____________

Place ______________________

Tedla Mindaye (MSc, PhD fellow)

Signature ___________________ Date: _____________

Place ______________________

Abebaw Kebede (MSc)

Signature ___________________ Date: _____________

Place ______________________