ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Quality performance Evaluation of Laboratories on AFB smears Microscopy in Eastern Amhara Region, Ethiopia, 2011.

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A final thesis submitted to the School of graduate studies of Addis Ababa University in partial fulfillment to the requirements of the degree of Master of Science in Clinical Laboratory Sciences (Clinical and public health Microbiology track)

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June, 2011, Addis Ababa, Ethiopia

DECLARATION

I declare that this thesis (**Quality Performance Evaluation of Laboratories on AFB smear Microscopy in Eastern Amhara Region, Ethiopia, 2011)** is my own original work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used indicated reference and have been duly acknowledged.

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ACKNOWLEDGMENT

I gratefully acknowledge Addis Ababa University Graduate School and Department of Medical Laboratory Sciences that provided me this opportunity and financial support for thesis work.

I wish to express my deepest gratitude to my advisers Mr Kassu Desta, Getachew Belay and Mr Ibrahim Ali. I cannot have sufficient words to thank you for your dedicated and highly qualified support**,** friendliness, patience and understanding for their valuable advice from the beginning of research proposal to completion of thesis work. and I would also like to acknowledge Tilahun Muche and muluwork Getahun for assist this thesis.

I would also like to acknowledge for the laboratory personnel of participating laboratories of Eastern Amhara region. I would like to acknowledge all staff member of Dessie Regional Reference Laboratories

I would like to acknowledge all staff member of National reference TB Laboratory in Ethiopia, TB CAP and ITCH – Ethiopia for their assistance in the EQA of AFB microscopy evaluation. At the end my sincere appreciation goes to my beloved wife Amsal kuyaw , our baby Abel Mekete and my family for their crucial moral and financial support.

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V. OPERATIONAL DEFINITIONS

Blinded Rechecking: A process which involves collecting smears from the microscopy centre laboratory for blinded re-reading at regional reference laboratory and providing feedback to the microscopy center for corrective actions. Rechecking must always be blinded, whereby the controller does not know the results from the peripheral laboratory.

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

DOTS: Directly Observed Treatment, Short course chemotherapy. The internationally recommended strategy for TB control. The strategy includes (1) government commitment to TB control activities, (2) case detection by sputum smear microscopy, (3) directly observed treatment (DOT) with standardized short-course chemotherapy, (4) a regular, uninterrupted supply of anti-TB drugs, and (5) a standardized recording and reporting system.

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

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

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

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

Low False Negative (LFN): A scanty (1- 9 AFB / 100 fields) positive smear that is misread as negative. This type of minor error occurs occasionally even in laboratories that are performing well.

Low False Positive (LFP): A negative smear that is misread as a scanty (1-9 AFB / 100 fields) positive. This type of minor error occurs occasionally even in laboratories that are performing well.

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

Major error: This type of error is considered the most critical since it has the highest potential impact on patient management, and can result in an incorrect diagnosis or improper management of a patient. Major errors may indicate gross technical deficiencies, and include both High False Positive and High False Negative errors.

Microscopy Center (MC): A laboratory located at a health center or hospital, designated by EHNRI & TLCP as a facility which will provide smear microscopy services to an approximate population of 50,000.

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

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.

Quality Assurance (QA): System designed to continuously improve the reliability and efficiency of laboratory services. It includes internal quality control, external quality assessment and quality improvement.

Quality Control (QC): Also called Internal Quality Assurance, includes all means by which the TB smear microscopy laboratory controls operation, including microscope checks and checking new batches of staining solutions.

Quality Improvement (QI): A process by which the components of smear microscopy diagnostic services are analyzed with the aim of looking for ways to permanently remove obstacles to success. Data collection, data analysis, and creative problem solving are the key components of this process. It involves continuous monitoring, identifying defects, followed by remedial action including retraining when needed, to prevent recurrence of problems. QI depends on effective on-site evaluation visits.

Quality Officer (QO): A trained Lab supervisor entrusted with the responsibility of overseeing conduct of EQA activities as per guide line, including the responsibility of ensuring blinding of routines slides for RBRC, submission of summary reports, quarterly. Annual reports in time to all concerned, receipt, review of action taken reports from lower level labs, identification of problems, causes and corrective actions for lower level labs.

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

Region: The administrative level at which the TLCP is implemented.

Regional Health Bureau: Nodal centre for TB control activities in the respective region.

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

Scanty (Low Positive): Term used in this document to describe 1-9 acid-fast bacilli per 100 fields, which is the EHNRI/ TLCP standard for quantification. These results are reported to the physician as exact number of AFB seen.

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.

Smear negative pulmonary tuberculosis: are those patients who had clinical features of Tb and usually have abnormal chest x-ray feature and lack response to the broad spectrum drugs, at least two of sputum samples negative in AFB staining techniques.

Smear positive pulmonary tuberculosis: are those patients had clinical feature of TB and provide positive for AFB in microscopy

Tuberculosis and Leprosy Control Program (TLCP): A national program responsible for activities directed at controlling tuberculosis and leprosy through integrated efforts with the general health services for implementing the DOTS strategy.

Tuberculosis: is a bacterial diseases caused by *Mycobacterium* species.

Ziehl-Neelsen Stain (ZN): Acid-fast staining method using carbol fuchsin and methylene blue.

VI. LIST OF ABBREVIATION

AFB: Acid Fast Bacilli

ANSV: Annual Negative Slide Volume

DOTS: Directly 0bserved Treatment, Short course chemotherapy

DRRL: Dessie Regional Reference Laboratory

EHNRI: Ethiopian Health and Nutrition Research Institute

EQA: External Quality Assessment

HFN: High False Negative

HFP: High False Positive

IQC: Internal Quality Control

IRL: Intermediate Reference Laboratory

IUATLD: International Union against Tuberculosis and Lung Disease

LFN: Low False Negative

LFP: Low False Positive

LQAS: Lot Quality Assurance Sampling

LT: Laboratory Technologist/ Lab Technician

MC: Microscopy Center

NALC: N-acetyl-L cysteine

NRL: National Reference Laboratories

OSE: On-Site Evaluation

PDC: Peripheral Diagnostic Center

PT: Proficiency Testing

QA: Quality Assurance

QC: Quality Control

QE: Quantification Error

QI: Quality Improvement

RBRC: Random Blinded Re-checking

RLS: Regional Laboratory Supervisor

RNTCP: Revised National Tuberculosis Control Program me

RRL: Regional Reference Laboratory

SOP: Standard Operating Procedure

SPR: Slide Positivity Rate

TB : Tuberculosis

WHO: World Health Organization

Abstract

Background*:* External quality assessment *is* the most effective method for evaluation of AFB diagnostic methods for tuberculosis. Blinded rechecking of AFB slides using lot quality assurance sampling methods is currently practiced in many DOTS centers. In addition panel testing and onsite supervision are critical components of standard EQA program that can assess the overall performance of microscopic centers.

Objectives*:* This study aimed to evaluate the quality performance of laboratories in AFB smear microscopy and identifying TB slide positivity rate in peripheral diagnostic centers in Eastern Amhara Region.

Material and method*:* A cross-sectional study in 21 peripheral diagnostic centers in Eastern Amhara region, was conducted from October 2010 to April 2011.A total of 799staind smears were randomly collected for rechecking. Ten slides of panel testing smear were sent out to 21 microscopic centers to evaluate reading, staining and reporting performance of individuals. Check lists were used to assess quality issues of laboratories. Tuberculosis registration books were used to determine slide positivity rate. Data were captured, cleaned and analyzed using SPSS version 17. X^2 test, kappa values were used for comparison purpose. P value <0.05 was considerable to statistically significant.

Results: Proficiency panel test score of 100 %, 80-95 %, 60 % was performed by 9, 11, 1 laboratories, respectively.

Out of 799 randomly selected slides, 8.3 % and 8.6 % were reported to be positive for acid fast bacilli by the first and the second (regional) laboratory personnel, respectively. Overall, false reading was 1.6 % and the overall agreement was 98.4%. There is a slight difference among public and private microscopic centres in selected quality performance x^2 (p value < 0.05).

Annual PTB examinees were 6,212 in 2006 / 2007; 9537 in 2007 / 2008; 10499 in 2008 / 2009 and 13,086 in 2009 / 2010. while the positivity rate was 8.7, 9.9, 10.7 and 7.4 with respectively. The maximum positivity rate was between the ages of 15 and 34. Generally, positivity rate was 9.6 % among females and 8.4 % among males.

Conclusion and recommendation: The overall performance of majority of microscopic centers is acceptable. The overall slide positivity rate (SPR) was 9 % and showed an increasing trend from 2006/ 2007- 2009/ 2010 with slight decline at the end of 2010. Continuous quality performance assessment of tuberculosis microscopic centers should be considered as part of DOTS program for proper diagnosis and management of tuberculosis in the region. This should include panel testing, blinded rechecking and onsite supervision of laboratories along with strengthening of public private partnership for tuberculosis control in the region.

Key word: Panel testing; Onsite evaluation; random blinded rechecking; East Amhara,Ethiopia.

1. INTRODUCTION

1.1. Back ground

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), infects almost the third part of the world population and kills around two million people worldwide each year. About 80% of the global TB burden occurs in low-income countries, where pulmonary disease and its transmission are most serious public health problems. Among bacterial pathogens of human, Mycobacterium tuberculosis is best known for its slow growth rate and its acid-fast lipid-rich cell wall. Culture of mycobacterium is too slow for practical diagnosis, while their acid-fastness allows rapid detection in clinical specimens (1).

In many countries with a high prevalence of TB, direct sputum smear microscopy remains the most cost effective tool for diagnosing patients with infectious tuberculosis and for monitoring their progress on treatment (2). Sputum microscopy is an essential component of the DOTS strategy recommended by the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD). DOTS, relies on a network of laboratories to provide readily accessible and quality laboratory services to prevent the spread of infection in the community and prevent unnecessary treatment of cases who do not have TB (3).

Microscopy of sputum smears is a simple and inexpensive technique, quickly detecting those cases of pulmonary TB who are infectious; however, the main problem facing sputum smear microscopy is its low sensitivity which can seriously limit both the extent and quality of its application (4).

Acid fast microscopy can be highly specific test for mycobacterial disease but it should be done under careful supervision and that false positives could be reduced by initiation of a quality control program in the laboratory. Microscopy needs a good staining procedure that will be able to differentiate the bacilli from an artifact (5).

The results of sputum AFB microscopy are known to be influenced by various factors including the proficiency to read smears by microscopists. With the inherent limitations of sputum AFB microscopy, the highest sensitivity achievable by microscopists in reading

smears can be 95%. Several documents recommend the need for training the laboratory technicians to improve the quality of sputum acid fast bacilli (AFB) microscopy (6).

Quality assurance (QA) system for the sputum microscopy is aimed at minimizing the false positive and false negative results in the laboratory services. Failures to maintain the quality would result in loss of credibility and waste of precious resources, at individual level, and inaccurate data and poor performance of the programme, in terms of case detection, at the national level. According to the recommendations made by the national guideline, the quality control system for sputum smear microscopy for tuberculosis includes; internal quality control, quality improvement and external quality assessment (7).

The three components of external quality assessment (EQA) of acid-fast bacilli (AFB) smear microscopy are panel testing, blinded rechecking and on-site evaluation using a standard questionnaire. Panel testing can provide preliminary data on laboratory capabilities prior to a blinded rechecking programme. In practice, stained and unstained smear centrally prepared slides must be sent to peripheral laboratories to evaluate the ability of laboratory technicians to stain and/or read smears, although panel testing does not serve to assess routine laboratory performance (8).

Blinded rechecking is the most effective assessment method of external quality assessment (EQA) compare to panel testing and on-site evaluation for sputum smear microscopy. Blinded rechecking provides the insight of laboratory personnel capability and standard in carrying out day to day sputum microscopy. Blinded rechecking is time consuming and requires more number of competent human resources to conduct it regularly. However, it is the only assessment method that provides evidence about technical problem and individual's performance can be corrected and improved through this particular assessment method (9). The on-site evaluation identified potential problems underlying the substandard performance at deficient laboratories and helped with the formulation of corrective actions (10).

In 2002 external quality assessment (EQA) guidelines for acid-fast bacillus smear microscopy were implemented, and blinded rechecking of the slides was performed in Amahara regional state including the current study sites. Random blinded rechecking using the Lot Quality Assurance System (LQAS) method decreased the number of slides to be reviewed and assessment on smear quality determine by smear evaluation criteria such as smear size, thickness, staining quality, cleanness and evenness of smears to evaluate the

overall performance of individual laboratory personnel performing routine sputum microscopy that can identify gaps and improve the tuberculosis service at large. Hence this study aimed to evaluate level of agreement in sputum smear microscopic reading for AFB detection between peripheral diagnostic centers (PDCs) and the reference laboratory as well as to determine the trend of sputum smear positivity rate in Eastern part of Amhara Regional state of peripheral diagnostic centres in the previous four years (December 2006 to October 2010).

1.2. Statement of the problem

According to WHO 2010 TB report, there were 9.4 million incident cases, 14 million prevalent cases, 1.3 million deaths among HIV-negative people and 0.38 million deaths among HIV-positive people. Most cases were in the South-East Asia, Africa and Western Pacific regions (35%, 30% and 20%) respectively. An estimated 11– 13% of incident cases were HIV-positive; the African Region accounted for approximately 80% of these cases (11). It was estimated that in 2010, in Rwanda, through recent national survey, incidence of new sputum smear positive was estimated to be 162 per 100, 000 population compare to estimated incidence 300 per 100,000population in Ethiopia.

Tuberculosis control requires a functional laboratory set-up with quality diagnostic services and a trained diagnostician and a microscopist. However, the performance of such laboratories depends on continuous monitoring and quality improvement mechanisms put in place (12).

It is important to understand the limitation of smear microscopy in the detection of tuberculosis. The DOTS detection rate remains low, at 34 %, compared with WHO's target of 70% detection. The limited diagnostic capacity for TB in the country remains a challenge to improving case detection rate (13).

The reduced sensitivity is related to problems associated with the stringent requirements of the test and technicians used to handling large amounts of smears, can be expected to score high in the test. Because of high work load and fatigue, they often make more mistakes in their routine more compared to laboratories processing moderate number with regular positives (14). False results in diagnosis either lead to unnecessary treatment of the patient with potentially toxic drugs and puts precious resources of the programme to the drain, increasing the health care costs (false positive results); or deprive the potentially infectious TB patients from the benefit of treatment and cure (false negative results). Errors in reading follow-up of treatment smears can result in patients being placed on prolonged treatment or re-treatment, or in treatment discontinued prematurely (15). Therefore, quality assurance of AFB sputum smear microscopy is essential for any tuberculosis control programme.

1.2.1. Tuberculosis in Ethiopia

Ethiopia ranks seventh among the world's 22 high-burden countries with TB. According to WHO 2010 Global TB Report, the country had an estimated 44,398 TB cases in 2009, with an estimated incidence and prevalence rate of TB 300 and 470 per 100,000 populations, respectively. Similarly case detection was 50% for all forms of TB. Among all new TB cases 30% were smear positive, 35% smear negative, 34% extra pulmonary and ,<1% smear unknown cases (16).

Ethiopia's National TB and Leprosy Control Program began to implement Directly Observed Treatment, Short-course (DOTS) strategy for TB control in 1991. DOTS is a proven, cost effective strategy recommended by World Health Organization for countries with limited resources. While treatment is integrated into general health services and DOTS geographical coverage is 95 percent, due to the limited health infrastructure in the country, only approximately 60 to 70 percent of the population has access to DOTS services. The DOTS detection rate remains low, at 34 percent, compared with WHO's target of 70 percent detection. The limited diagnostic capacity for TB in the country remains a challenge to improving case detection rate (13).

According to the Ministry of health hospital statistics data, TB is one of the leading cause of morbidity and the fourth cause of hospital admission and the second cause of hospital death in Ethiopia (17).

Amhara Region has a total of 6 zonal hospitals and 9 district hospitals, 81 health centers, 508 health stations and 410 health posts. The health facility to population ratio is one hospital for 1,177,933 and one bed for 11,051 persons. Health service coverage in the region is still 51.8 % and a significant proportion of the population still lives beyond catchment areas of even peripheral health institutions (17).

1.3. Literature review

A study conducted in Tanzania in June 2006 showed that the proportion of well prepared smears was 86.2% and that of well stained smear was 81.2%. The overall agreement on reading was 89.2%; the overall sensitivity was 88.5% and specificity was 100%. High false negative (HFN) were the major errors found in this study and low false negative (LFN) and quantification error (QE) were the minor errors found and there were no false positive errors. Error found in this survey, HFN, LFN and QE suggests a systematic under-reading of smear in all surveyed health facilities probably due to a number of technical factors, quality of smear, poor stain, bad microscopy and inadequate training and other factors (2).

Study conducted in Malawi of 4,805 sputum specimens showed that documentation was complete in 95%; 93% reached the laboratory within 7 days of collection; 96 % of smears were well prepared and stained, and 97% concordance (96.4 % smear-positive and 97.6 % smear-negative) was demonstrated when 208 smears were re-examined by a second technician. The aggregate index of reliability was 86 %. The mean time spent on microscopic examination was 3.8 minutes, compared with the recommended time of 10 minutes. When all smears from 164 patients were assessed externally, 98.2% concordance (98.1 % smear positive and 98.2 % smear-negative) was demonstrated. False smear-negative and smear-positive rates were less than 2 % (18).

Another study conducted in Kinshasa in 2008, smear microscopy evaluation of laboratory technicians' knowledge and skills before and after refresher training and the distribution of new microscopes on the quality of smear microscopy at primary health care center laboratories through the implementation of the 2002 EQA guidelines for TB laboratories in resource-poor settings. It was found that the participants had a good understanding of the basic theoretical aspects of tuberculosis and sputum microscopy prior to participation in the refresher training course. Training resulted in a marked improvement in the practical component (smear preparation, staining, and reading), with median test scores increasing from 70 % pre training to 86 % post training (19).

A study conducted in Bhutan in 2008 8th and 9th proficiency testing results shown that in 8th round panel test, performance of four of 19 laboratories, who have reported back the results, were found unsatisfactory (below acceptable score < 90%), fourteen district labs reported six major errors (5 HFN & 1 HFP) and twenty one minor errors (17 LFN, 2 LFP & 2

QE). In the 9th round panel test, performance of six of 26 laboratories were found unsatisfactory (below acceptable score < 90%), thirteen district laboratories have reported five major errors (5 HFN) and twenty one minor errors (18 LFN and 3QE). Over all 21.05 % and 23.07 % of labs performance were found unacceptable in the 8th and 9th proficiency testing respectively. The overall findings from two round of panel testing showed not much difference in terms of performance improvement. Very few hospitals are consistent in their proficiency while some hospitals struggle to meet the acceptable standard level (20).

A study conducted in Ghana 2006, showed that the average marks for specimen quality, staining ability, smear cleanness, thickness, size and evenness increased from 64 %, 79 %, 69 %, 46 %, 67 % and 60 % in the last quarter of 2000 to 81%, 90 %, 86 %, 79 %, 80 % and 74 %, respectively, 24 months after the establishment of the QA system. Within the same period, the rate of false positives and negatives decreased from 14.8 % and 20.5 % to 0 % respectively, and agreements in positivity rate increased from 74 % to 95 %. The performance of the participating laboratories in keeping the laboratory registers were also improved (21).

Another study conducted in India, in 2005, determine the proficiency tests on 283 participants and comparison of results of smear read on day-1 and day-15 with the reference results. On day-1, the sensitivity, specificity, PPV and NPV of reading smears were 75 %, 88 %, 93 % and 63 % respectively. The agreement of smear results read on day-1 with the reference readings was poor (kappa value of 0.57) and on day-15, the sensitivity, specificity, PPV and NPV were 94 %, 98 %, 99 % and 89 % respectively. The agreement of smear results read on day-15 with the reference results was excellent (kappa value of 0.9). The number of errors committed by the trainees decreased gradually on subsequent occasions and the sensitivity of reading increased from 75 % to 94 %. In National Tuberculosis Programme, the managers expect that the microscopy technicians should have at least 85 % sensitivity (relative to controllers). The results of the study suggested that fresh trainees can achieve the expected level of 85 % sensitivity during the 15 days' training program me recommended by WHO and IUATLD (6).

In Thimphu, Bhutan in 2008 of 1st, 2nd, 3rd and 4th quarter results shown that all the labs failed to meet the minimum acceptable standard of smear quality , however; some laboratories have shown steady improvement in their performances. Few laboratories were

found reporting false positive and some still missing out AFB (false negative). Reporting of low false negative and quantification errors are expected especially if laboratory technicians are new but not high / low false positive and high false negative (9).

A study conducted in Nepal showed that quality assurance of Sputum Smear Microscopy in private laboratory was done for the first time in the region. Of the nine laboratories took part in the first round testing; only one laboratory had a QE. None of the laboratories have shown any major error (22).

A study conducted in India Overall, on-site evaluation was 44.15% errors in laboratory checklist-items were identified in ten IRLs during first year OSE (2005). Majority of errors occurred in EQA (92.6%), internal quality control (90%), staining reagents / equipment (56.6%), infrastructure (42%), and bio-safety practices (40%). Nine IRLs were performing clinical activities. Staffs were not adequate in IRLs. As a result of OSEs, IRLs discontinued the clinical activities (24).

Another study conducted in Taiwan found that the smearing and staining quality before and after training was 87 % to 99.6 % for proper staining, 48 % to 77 % for proper thickness and 63 % to 100 % for proper smear size (25).

A study conducted in Ethiopia, in nine zones of southern Ethiopia region between October 2000 and June 2002, showed that Out of 2209 slides, 54 % of which were positive and 46 % of were negative slides; the overall false reading was 3.2 % and the overall agreement was 96.8 %. Among the 95 discordant slides that were re-examined at the central reference laboratory, 74 % were found to be in agreement with the regional laboratory readings while 26 % were in agreement with the peripheral readings. Nine zones participated in the quality control activity in 2000, six zones in 2001 and three zones in 2002. A good agreement was recorded in the readings of AFB among the peripheral and reference laboratories. However, the false positive reading of 3.2% which exceeded the recommended cut-off point of 2%. The study recommended regular on-job training of the peripheral laboratory staff together with supportive supervision that would help improve their performance (23).

1.4. Significance of the study

The ultimate goal of quality assessment in health care program is to assess whether a program possesses the right things (input), is doing the right things (processes) and it leads to the right things (outcome) to happen. Hence, assessing the quality of sputum smear microscopy detection of AFB can have significance importance in evaluating tuberculosis control activities and in identifying area of improvement for better and effective tuberculosis control strategies.

WHO developed guideline for diagnosing patients with infectious tuberculosis and for monitoring their progress on treatment based on external quality assessment of direct sputum smear microscopy.

In order to achieve the required quality in laboratory diagnosis, a continuous system of quality assessment (QA) needs to be established. Therefore, this study were conducted to assess the quality of sputum smear microscopy detection of tuberculosis by proficiency testing, blinded slide rechecking, on-site evaluation and slide positivity rate in Eastern part of Amhara region to identify any gap in providing direct microscopy examination of tuberculosis services and information to decision makers and program managers that could improve tuberculosis 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 and international levels.

2.0. Objective of the study

2.1. General objectives of the study

- The general objective of this study is to evaluate the quality performance of laboratories in AFB smear microscopy and identifying TB slide positivity rate in peripheral diagnostic centers in Eastern Amhara Region.

Specific objectives of the study

- To identify major gaps of microscopic centers in performing sputum smear microscopy examination in Eastern Amhara Region.
- To determine level of agreement in sputum smear microscopic reading for AFB detection between the peripheral microscopic centers and the reference laboratory.
- To determine the trend of sputum smear positivity rate in selected microscopic centers.

Hypothesis: We hypothesized that, the overall performance of tuberculosis microscopic centers in Eastern Amhara region is above 80 %.

3.0. Materials and methods

3.1. Study design and period

A cross – sectional study was conducted on selected laboratories of Eastern part of Amhara Region, from October to April 2011. The performances of the peripheral diagnostic centers was assessed using panel testing (proficiency testing), random blinded rechecking, on-site evaluation and retrospectively data were collected from the study sites for determination of trend in smear positivity rate of tuberculosis.

3.2. Study area

Samples were collected in Selected peripheral diagnostic centers found in Eastern part of Amhara Region includes South Wollo zone, Oromia zone, North Wollo zone, Wagemera zone and North shoa zone. Based on the figures from the central statistical agency in 2005,

South Wollo has an estimated total population of 2,942,886, of which 1,446,752 were males and 1,496,134 were females, with an estimated area of 16,956.06 square kilometers, with population density of 173.56 people per square kilometer.

Oromia zone has an estimated total population of 639,107, of which 319,521 were males and 319,586 were females. With an estimated area of 4,434.53 square kilometers, this zone has an estimated population density of 144.12 people per square kilometer.

Again North Wollo zone has also an estimated total population of 1,731,849, of which 864,907 were males and 866,942 were females. With an estimated area of 16,400.98 Square kilometers, North wollo has an estimated population density of 105.59 people per square kilometer.

Wagemra zone has an estimated total population of 375,600, of which 187,915 were males and 187,685 were females. With an estimated area of 8,329.70 square kilometers, this zone has an estimated population density of 45.09 people per square kilometer.

Similarly, North Shoa zone has also an estimated total population of 2,159,301, of which 1,080,266 were males and 1,079,035 were females; 255,275 or 11.8% of its population are

urban dwellers. With an estimated area of 16,070.23 square kilometers, North Shoa has an estimated population density of 134.37 people per square kilometer (27).

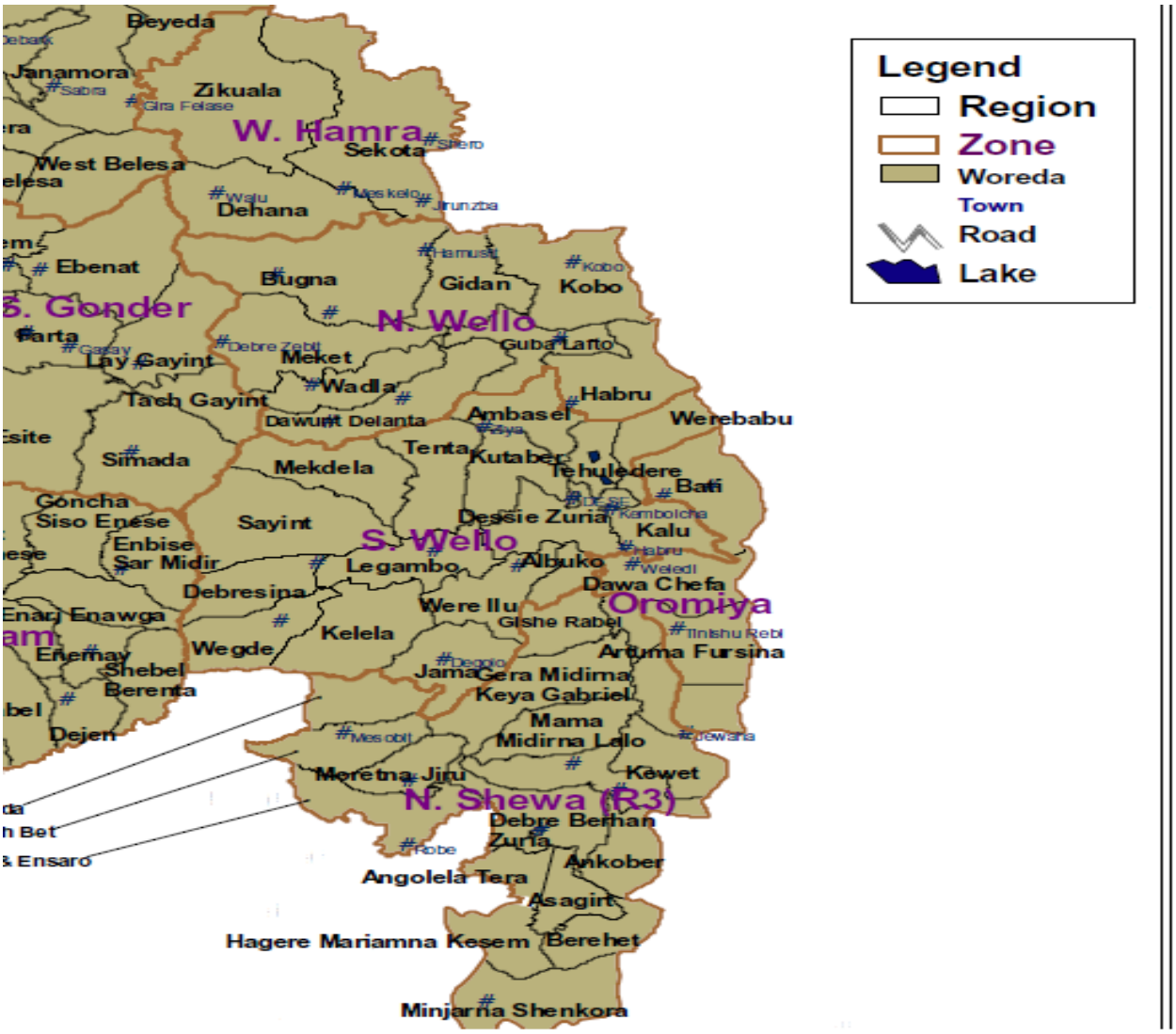


Figure 1: Map of Eastern Amhara Region, Ethiopia, February 2011

3.3 . Source Population

The study population is all AFB diagnostic centers in Eastern Amhara Region.

3.4. Study population

The study population for random blinded rechecking was all sputum smears examined in selected peripheral diagnostic centers, appropriately stored stained slides and documented

which was stained by Ziehl Neelson method. For panel testing 21 laboratories that are given a set of ten slides smears which was centrally prepared and validated.

3.4.1. Site selection

Among 114 Tb diagnostic laboratories in Eastern part of Amhara Region, 21 were selected based on the smear microscopy workload of 2010. The selected laboratories included: 12 of the12 hospital laboratories (9 public and 3 private) and 9 of the 102 health center laboratories. The median annual workload at the selected sites was 1839 sputum smears (range, 427 to 4557 sputum smears) for AFB smear microscopy examination.

3.5. Inclusion and Exclusion criteria

3.5.1. Inclusion criteria

All hospitals and health centers laboratories were participating in the national external quality assessment (EQA) program in Eastern part of Amhara Regional state providing sputum smear microscopy services were included.

3.5.2. Exclusion criteria

The exclusion criteria used are peripheral diagnostic centers, which do not provide sputum smear microscopy services in regular bases.

3.6. Study variable

3.6.1. Dependant variable for EQA and SPR

Results of panel testing, random blinded rechecking, on-site evaluation and slide positivity rate (SPR).

3.6.2. Independent variable for EQA

Smear quality, stained and un stained smear size, thickness, quality of staining, cleanness and evenness, availability and use of laboratory reagents / equipments, bio safety and waste

disposal, on-site cross rechecking standard and international guidelines on external quality assessment (EQA) of AFB smear microscopy services.

3.6.3. Independent variable for SPR: positivity rate by Age and sex group.

3.7. Data collection and sampling technique

3.7.1. Panel testing

3.7.1.1. Preparation of AFB smear for panel testing: Panel testing smears were prepared using N-acetyl-L-cysteine (NALC) methods described by international procedures are summarised in (Figure 2).

Panel testing smear preparation procedure

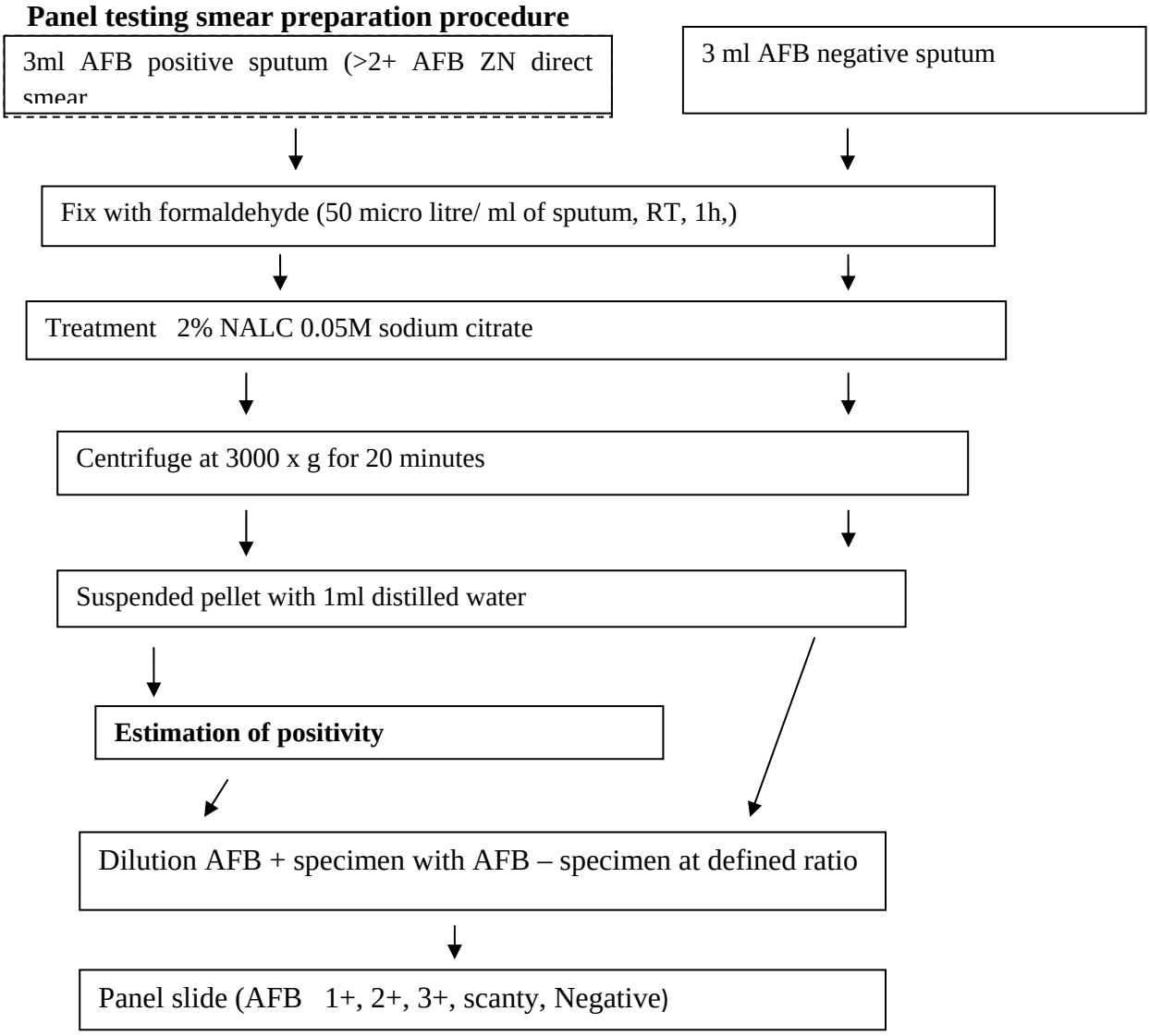


Figure 2: Smear preparation procedure using the NALC methods for panel test slides AFB = acid-fast bacilli; ZN= Ziehl-Neelsen; RT = room temperature; NaOH = sodium hydroxide; NALC = N-acetyl-L-cysteine

3.7.1.2. Sample size for panel testing

A set of 10 slides was considered as acceptable number based on previous experience and guidelines (12). This set of slides consists of five stained and five pre-fixed unstained smears. For this study sites, a total of 210 validated panel testing slides including : negative (105) and positives (105) of different grade;3+ →21 , 2+ →21 , 1+ →21and scanty →42 respectively.

3.7.1.3. 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 were 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 technicians to properly grade positive slides. Multiple 3+ smears was not include (Annex-7).

3.7.1.4. System for sending slides to the laboratories

Validated panels were distributed to participant microscopy centres with out breakage or degradation of the slides. The time allotted for reading the 10 slides were 50 to 70 minutes according to national guideline. To classify smear as negative, International guideline recommend examination of 200 / HPF (100x objective). This 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 was reported by using national and WHO-IUATLD guidelines as stated in (Annex7) The supervisors were collected the smear results in a sheet.

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

Table 1: 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	**Correct**	**LFN**	**HFN**	**HFN**	**HFN**
1-9AFB/100F	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

3.7.1.5. 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) result in a score of 0 where as minor error (LFP, LFN and QE) result in a scores of 5 points. Passing score was 80 points. Generally the Overall agreement rate (OAR) were considered excellent > 95%, Satisfactory >90% and poor performance < 80%.While Interpretation of results was done using International Union Against Tuberculosis and Lung Disease (26).

3.7.1.6. Feedback

Feedback was given to the participating laboratories by regional laboratory supervisor and a result of panel testing was discussed with the personnel's in majority of sites and will be completed soon.

3.7.2. Blinded rechecking

All the routinely examined slides (from July 2010 to September 2010) were brought to Dessie Regional Reference Laboratory (DRRL) for AFB smear rechecking.

3.7.2.1. Sample size Determination for blinded rechecking

The sample size was based on the following considerations: a sensitivity of 80% (i.e., the percentage of detection of positive smears including low positives by the laboratory technician (LT) relative to the controller), a specificity of 100% (meaning that there should not be any false-positive error tolerated), an acceptance number of zero, a slide positivity rate (SPR) and annual negative slide volume, based on these parameters, the quarter sample size needed for the blinded rechecking was 799 slides from 19 study sites, smears were systematically selected after the slide number was recorded in the register using national guideline stated in (Table 2). Two microscopic centers were not involved in the blinded rechecking due to natural accidents that destroy the stored slides.

Table 2: Recommended annual sample size (80% sensitivity, 100% specificity and '0' acceptance number .

Number negative slides of in the MC in a year	**Slide positivity rate (SPR %)**				
	2.5 - 4.9	**5.02 - 7.49**	**7.5 - 9.9**	**10 - 14.9**	**>15**
301 - 500	243 (62)	154 (40)	114 (30)	89 (23)	62 (16)
501 - 1000	318 (81)	180 (45)	128 (33)	96 (25)	66 17)
>1000	456 (114)	216 (54)	144 (37)	104 (27)	69 18)

***Annual sample size of both positive and negative slides (quarterly sample size in parenthesis)**

3.7.2.2. Slide Collection and selection

Slides were collected by well trained laboratory technologist in order to ensure that a random sample obtained. To avoid bias, the technicians in the peripheral laboratory were never performing the sampling. In order to eliminate selection bias, slides were randomly selected using the TB register log book by an independent supervisor from list of specimens processed during the third quarter of 2010. This ensures that the technicians kept all slides regardless of result but slides were not selected from the slide box.

3.7.2.3. Slide transportation system

Randomly collected slides were kept in boxes that did not allow the slides to touch each other, and slides were in closed boxes away from direct sunlight and the results of selected slides were entered by sample collector in sealed, and handed over to supervisor, who was immediately transfer the LQAS slide box and sealed cover to regional quality officer.

3.7.2.4. Slide evaluation and Interpretation

Smear was evaluated for, appropriate smear size and thickness, and quality of staining, cleanness and evenness by senior laboratory technologist who acted as the controller as given in (Table 21) The results of the finding was reported and interpreted according to national and WHO-IUATLD standard guidelines as stated in (Table 20).

3.7.2.5. Discordant slides

Discrepant results were resolved by second controller. 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. Interpretation of the results was recorded according to the recommendation by WHO and national tuberculosis external quality assessment (EQA) guideline (12, 26).

3.7.2.6. Feedback

Feedback was given to the participating laboratories by regional laboratory supervisor and blinded rechecking were discussed with the personnel's in majority of sites and will be completed soon.

3.7.3. On-site evaluation

On-site evaluation of each laboratory was performed using the national comprehensive on-site evaluation checklist. The checklist was consisted of closed and open ended questions, observation and a limited on-site slide rechecking (annex-1).

3.7.4. Slide positivity rate

Proportion of positive smears was estimated from the total suspected cases using the laboratory TB register log books. Four years data were collected from the selected peripheral microscopic centers (Annex-6).

3.8. Data quality assurance

To assure data quality, quality control for panel testing, smear preparation of sputum smear, microscopy AFB detection were accomplished starting from sample collection, microscopy, smearing , staining and reagent quality according to IUATLD / WHO guidelines / national guideline.

Each new batch of Ziehl-Neelson reagents were checked by known unstained positive and negative control smears during on-site evaluation. Completeness, accuracy and consistency of selected random blinded rechecking smears, panel smear reading, standard checklist preparation and retrospective data recording were done according to recommended methods (12).

3.9. Data management

Cross-checking and data cleaning was done and missing information was obtained by going back to the questionnaire. All laboratory and clinical data were recorded on a logbook during the study period and the data were also stored in a CD and USB device as a backup. The logbook and CD were stored in lockable shelves and only the investigator had an access to the files. Dependent and Independent variable to SPSS version 17.

3.10 .Data analysis

Data processing and statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) software (Windows version 17.0). The percentages of different types of errors were calculated for smears microscopy center. The sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) of smear reading by microscopic center laboratory technician was calculated. Chi square test is used to see any association with different variable. The agreement in reading between the peripheral

diagnostic centers and the reference laboratory readings was done using kappa statistics and p value less than 0.05 was considered to be statistically significant.

3.11. Ethical Consideration

The research proposal was evaluated by the research and ethics committee of School of Medical Laboratory Science and reviewed and cleared by Institution of Review Board (IRB) before the start of fieldwork.

Addis Ababa University Faculty of Medical, School of Medical Laboratory Science wrote official letter of co-operation to those administrative zone health departments and EHNRI for the purpose of panel testing slide preparation and batch validation. Each administrative zone health department also wrote co-operation letters to those selected administrative woredas health office and those woreda administration offices sent a letter of co–operation to those selected health facilities. During data collection there were a high degree of confidentiality and informed consent also obtained from each study sites, no name and other identifier on the questionnaire. At the end of the study, feedbacks were given from panel and blinded rechecking test results for each study site.

4.0. RESULT

4.1. Participants microscopic centres

Among the twenty-one peripheral diagnostic centers included in the study, one was a regional referral hospital, two were zonal hospitals, six were district hospitals, three were private general hospitals and the remaining nine were health centers. All heads of the peripheral diagnostic centers were willing to participate in the study.

4.2. Panel Testing

For the assessment of panel testing a total of 210 AFB smears of various grades of slides were used. The response rate of participant laboratories was 100 % and the results were given back within one day of panel distribution. Among these laboratories, 9 (42.9 %) could correctly detect negative and positive slides. About 2 laboratories (9.5 %) have misread negative slides as 1+ to 3+ positive. One laboratory (3.8 %) has misread 1+ to 3+ positive as negative; 8 laboratories (38.1%) have misread scanty as negative; one laboratory (3.8 %) has misread negative slides as scanty and 6 laboratories (28.6 %) had problems in grading the positive smears, quantification errors (Table 3).

One of the 21 laboratories reported back unsatisfactory (below acceptable score < 80%) results and the remaining twenty had acceptable performance (passing score). However, three peripheral diagnostic centers have reported both major and minor errors (3 HFP, 1 HFN, 4LFN and 3 QE) and eight sites had reported minor errors (6 LFN, 1LFP and 5 QE).

One peripheral diagnostic center had unsatisfactory scored (score of 60) with major and minor errors [2 HFP ; 1 LFN ; and 3 QE] (Table 3). In addition, one diagnostic health center scored 80 that had major and minor error of 1HFP and 2 LFN.

Out of 210 panel testing slides distributed , 11 % (23 / 210) panel slides were reported wrongly that contain major errors of 1.9 % (1 HFN ; 3 HFP) and minor errors of 9 % (10 LFN ; 1LFP, and 8 QE) (Table 3). The total score of microscopic center ranges 60 to 100 points. Nine centers had scored 100, six had scored 95, and one center scored 60 points (Table 3).

Table 3: Performance level of microscopic centers in Eastern Amhara Regional state.

LAB. CODE No.	HFN	HFP	LFN	LFP	QE	Total error	Total score
LAB-01	0	0	0	0	0	0	100
LAB-02	0	0	0	0	0	0	100
LAB- 03	0	1	2	0	0	3	80
LAB- 04	0	0	1	0	1	2	90
LAB- 05	1	0	1	0	0	2	85
LAB- 06	0	0	0	0	1	1	95
LAB- 07	0	0	0	0	0	0	100
LAB- 08	0	0	0	0	0	0	100
LAB- 09	0	0	0	0	0	0	100
LAB- 10	0	0	1	0	0	1	95
LAB- 11	0	0	0	0	1	1	95
LAB- 12	0	2	1	0	3	6	60
LAB- 13	0	0	1	0	0	1	95
LAB- 14	0	0	0	0	1	1	95
LAB- 15	0	0	0	0	0	0	100
LAB- 16	0	0	0	0	0	0	100
LAB- 17	0	0	1	0	1	2	90
LAB- 18	0	0	0	1	0	1	95
LAB- 19	0	0	0	0	0	0	100
LAB- 20	0	0	0	0	0	0	100
LAB- 21	0	0	2	0	0	2	90
Total	1	3	10	1	8	23	

The highest incidence of errors observed from public microscopic centers in Eastern Amhara region for major, minor and quantification errors (Table 4).

Table 4: performance of different microscopic centers for Panel testing, 2010.

Type of error	Categories of Health facilities	Error types	# of error	Percent (%)
Major error	**Public**	HFN	0	0
		HFP	3	13.0
	Private	HFN	1	4.3
		HFP	0	0
Minor error	**Public**	LFN	8	34.8
		LFP	1	4.3
		QE	6	26.1
	Private	LFN	2	8.7
		LFP	0	0
		QE	2	8.7
Total			23	100 %

About 23 panel slides (11 %) were incorrectly interpreted by laboratory personnel in the centers. HFN error observed in 0.5 % of smear , LFP in 0.5 % of smear, HFP in 1.4 % of smears, QE in 3.8 % of smears and LFN errors in 4.8 % of panels. Over all agreement was very good with kappa value of (0. 85) (Table 5).

It was found that a total of 8.6 % and 2.4 % of errors were committed by the public and private health facilities respectively. Out of the total 11.0 % error committed by all health facilities, 7.2 % and 3.8 % were committed by hospitals and health centers respectively (Table 5). However there was no statistically significant differences in errors types and health facilities (p value > 0.05).

Table 5: Performance of peripheral diagnostic centers for panel slides in Eastern Amhara region.

Institutions types		Major errors		Minor errors			Total error / 210 slides
		HFN No (%)	HFP No (%)	LFN No (%)	LFP No (%)	QE No (%)	
Heath Sector	Public	0 (0.0%)	3(1.4%)	8 (3.8%)	1(0.5%)	6 (2.9%)	18 (8.6%)
	Private	1(0.5%)	0 (0.0%)	2 (1.0%)	0 (0.0%)	2 (1.0%)	5 (2.4%)
Facility Level	Hospital	1(0.5%)	2(1.0%)	5(2.4%)	1(0.5%)	6 (2.9%)	15(7.1%)
	Health Centres	0 (0.0%)	1(0.5%)	5 (2.4%)	0 (0.0%)	2 (1.0%)	8 (3.8%)
Total		1(0.5%)	3(1.4%)	10(4.8%)	1(0.5%)	8(3.8%)	23(11%)

Out of 210 panel slides distributed to the selected sites 50 % (105) were unstained and 50 % (105) were stained panel slides. About 60 .9 % (14 / 23) of errors were reported from unstained panel smears while errors rate from stained panel smears accounted for 39% (9 / 23). There was no statistically difference in the frequency of errors and panel slides types x^2 (P value > 0.05) (Table 6).

Table 6: Comparison of stained and unstained panel testing smear in Eastern Amhara region, Ethiopia, April 2011.

Types of panel slides	Major error		Minor error			Total error
	HFN	HFP	LFN	LFP	QE	
Stained panel slide	0	3	3	0	3	9
Un stained panel slide	1	0	7	1	5	14
Total	1	3	10	1	8	23

The overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of panel reading by microscopic centers were 89 % , 96% , 96% , and 90 % respectively stated in (Table 7).

Table 7: Performance of panel testing that compares PDCs to NRL, Eastern Amhara region, Ethiopia, April 2011.

Peripheral diagnostic center (Test value)		**Regional reference laboratory (True value)**		**Test total**
		positive	Negative	
Test value	Positive	94	4	98
	Negative	11	101	112
True value total		105	105	210

***K = kappa value**

The sensitivity and specificity of the microscopic centers for panel test by zone ranged 85-90 % and 90 – 100 % respectively. All zonal microscopic centers had very good agreement with national with kappa value of 0.85 (Table 8).

Table 8: Sensitivity, specificity and predictive values of the peripheral diagnostic centers for panel slides by zones, Eastern Amhara region, 2010

Microscopic centers by Zone	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Kappa value**
S/ Wollo (n, 90)	88.9	97.8	97.6	89.8	0.8
Oromia (n , 20)	90.0	100	100	90.9	0.9
N / Wollo (n, 40)	90.0	90.0	90.0	90.9	0.8
Wagmra (n , 20)	90.0	100	100	90.9	0.9
N / Shoa (n , 40)	85.	95	94.4	90.5	0.8
Total (n, 210)	89.5	96.2	95.9	90.2	0.85

4.3. Random blinded rechecking

Out of 9,288 quarterly examined routine slides, 799 stained smears were blindly selected from the peripheral diagnostic centers of five zones of the Eastern Amhara region during a period of 3rd quarter. Majority of the peripheral diagnostic centers (PDCs) (60 %) were having the Annual Negative Slide Volume (ANSV) and Slide Positivity Rate (SPR) within the accepted range (15 % -15% for SPR and >300 for ANSV)

The PDCs with low SPR (> 5 %) & low ANSV (< 300) contributed to 31 (3.9 %) of the total PDCs, the PDCs with low SPR (<5 %) & ANSV (301 - 500) accounted 27 (3.3%) for the total PDCs ; the PDCs with low SPR (< 5 %) & ANSV (501 - 1000) accounted to 250 (31.3 %) of the total PDCs; PDCs with ANSV of >1000 but SPR of < 5 % accounted to 42 (5.3%) of the total PDCs. and PDCs with ANSV of 300 – 500 but SPR of >15% accounted to 17(2.1%) of the total PDCs (Table 9).

Table 9: **EQA -Annual negative slide volume (ANSV) and Slide positivity rate (SPR) at PDC level for the year 2010 in Eastern Amhara Region, Ethiopia.**

Annual Negative Slide Volume (ANSV)	**Number of Peripheral diagnostic centers (PDCs)**			**Total PDCs**
	Slide positivity rate (SPR)			
	< 5 %	**5 % to 15 %**	**>15 %**	
≤ 300	0%	31 (3.9%)	0%	31 (3.9%)
301- 500	27 (3.3%)	0 %	17 (2.1%)	44 (5.5%)
501- 1000	250 (31.3%)	63 (7.9%)	0%	313 (39.2%)
>1000	42 (5.3%)	369 (46.2%)	0%	411 (51.4%)
Total PDCs	**319 (39.9%)**	**463 (57.9%)**	**17 (2.1%)**	**799 (100%)**

4.3.1. Smear quality in blinded rechecking

Quality of smears were assessed during the visual re-examination of the sampled smears by the regional laboratories, on average it was found that 570 (71.3%) had proper smear size,

585 (73.2 %) had proper thickness, 618 (77.3 %) had proper staining, 743 (93.0 %) had cleanness of smears and 453 (56.7 %) had evenness of smear (Table 10). There was no difference in slide quality among the five zones X^2 (p value > 0.05).

Table10: Smear quality of microscopic centers for third quarter of 2010 evaluated by Regional Reference Laboratory, Eastern Amhara Region, Ethiopia.

Zone	# slides	Regional reference laboratory result(RRL)				
		Proper smear size No (%)	proper smear thickness No (%)	Quality of staining No (%)	Cleanness No (%)	Evenness of slides No (%)
South Wollo	250	173(69.2%)	168 (67.2%)	176 (70.4%)	220 (88.0%)	93 (37.2%)
Oromia Zone	30	20 (66.7%)	15 (50.0%)	25 (83.3%)	25 (83.3%)	15 (50.0%)
North Wollo	338	250 (74.0%)	265 (78.4%)	289 (85.5%)	322 (95.3%)	239 (70.5%)
Wagmra zone	33	30 (90.9%)	27 (81.8%)	27 (81.8%)	33 (100%)	29 (87.8%)
North shoa	148	97 (65.5%)	110 (74.3%)	101 (68.2%)	143 (96.6%)	77 (52.0%)
Total	799	570 (71.3%)	585(73.2%)	618(77.3%)	743(93.0%)	453(56.6%)

We have also analyzed the slide smear quality among public and private and hospitals and health centers microscopic centers (Table 11). 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

smear size and evenness between hospitals and health centers where both measures were found to be better in health centers (p value < 0.05).

Table 11: Quality of smear by center types evaluated by regional reference laboratory in Eastern Amhara Region, Ethiopia in 3rd quarter 2010.

Diagnostic centers		**# slides examined**	**Regional reference laboratory result(RRL)**				
			Proper smear size **No (%)**	Proper smear thickness No (%)	**Quality of staining** **No (%)**	**Cleanness** **No (%)**	**Evenness** **No (%)**
Health Sector	Public	735	523 (71.2%)	540 (73.5%)	563 (76.6%)	684(93.1%)	435(59.2%)
	Private	64	47 (73.4%)	45 (70.3%)	55 (85.9%)	59(92.2%)	18 (28.1%
	X^2 (p value)		0.15 (p>0.05)	0.29(p>0.05)	0.29(p>0.05)	0.07(p>0.05)	23.1(p<0.05)
Facility Level	Hospital	383	259 (67.6%)	277(72.3%)	295 (77.0%)	363(94.8%)	200(52.2%)
	Health centre	416	311(74.8%)	308 (74.0%)	323(77.6%)	380(91.3%)	253(60.8%)
	X^2 (p value)		4.96(p<0.05)	0.29(p>0.05)	0.04(p>0.05)	3.6(p>0.05)	6.0(p<0.05)

From 799 random blindly selected slides , 8.3 % and 91.7 % were reported to be positive and negative for acid fast bacilli by the first laboratory personnel (at peripheral results) and 8.6 % and 91.4 % were reported as positive and negative by the second laboratory personnel (regional result) respectively.

Comparison of the results derived from the first laboratory personnel with those of the second technician showed that they were similar in 61 (7.6 %) cases for positive findings and in 725 (90.7 %) cases for negative findings. Considering the reading of the Regional reference laboratory (RRL) as a true value, there was 13 (1.6 %) discordant slides (five false positive and eight false negative) that result in specificity of 99.3 % and sensitivity of 88.4 %. The PPV and NPV were 92.4 % and 98.9 %, respectively. There was no disagreement between regional AFB EQA quality officer (second controller) and central reference laboratories. Overall, false reading was 1.6 % and the overall agreement was 98.4 %. Among

the 13 discordant slides that was re-examined at the quality officer of regional reference laboratory, 98.4 % were found to be in agreement with the PDCs laboratory readings (with kappa value of 0.89).

Table 12: Blinded rechecking results between PDC s and RRL in Eastern Amhara region

S. No	Lab.code No.	No.of slides	Peripheral result		Regional result		No. of discordant slide	Frequency of false result	
			positive	Negative	positive	Negative		False positive	False negative
1	LAB-01	26	2	24	2	24	0	0	0
2	LAB-02	47	1	46	1	46	0	0	0
3	LAB- 03	27	0	27	0	27	0	0	0
4	LAB-04	24	1	23	1	23	0	0	0
5	LAB-05	23	3	20	3	20	0	0	0
6	LAB-06	17	5	12	5	12	0	0	0
7	LAB-07	32	2	30	4	28	2	0	2
8	LAB-08	31	1	30	2	29	1	0	1
9	LAB-09	23	3	20	1	22	2	2	0
10	LAB-10	None	NA	NA	NA	NA	NA	NA	NA
11	LAB-11	30	3	27	2	28	1	1	0
12	LAB-12	46	5	41	5	41	0	0	0
13	LAB-13	92	3	89	5	87	2	0	2
14	LAB-14	42	3	39	5	37	2	0	2
15	LAB-15	158	4	154	4	154	0	0	0
16	LAB-16	33	6	27	6	27	0	0	0
17	LAB-17	none	NA	NA	NA	NA	NA	NA	NA
18	LAB-18	33	5	28	6	27	1	0	1
19	LAB-19	39	4	35	4	35	0	0	0
20	LAB-20	25	3	22	3	22	0	0	0
21	LAB-21	51	12	39	10	41	2	2	0
	Total	799	66	733	69	730	13	5	8

The sensitivity, specificity, positive predictive value, negative predictive value and kappa value were 88.4%, 99.3%, 92.4% and 98.9%, k = 0.89 respectively as shown in (Table13). Thus the reading agreement between the peripheral and regional laboratories is almost perfect.

Table13: Sensitivity, specificity and predictive values of sputum microscopy at the peripheral diagnostic centers for blinded rechecking, Eastern Amhara Region.

Peripheral diagnostic center (Test value)		**Regional reference laboratory (True value)**		**Total**
		Positive	**Negative**	
Test value	positive	61	5	66
	negative	8	725	733
True value total		**69**	**730**	**799**

The sensitivity and specificity of the diagnostic centers by zonal microscopic centers (n, 19) 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 0.89 (Table 14).

Table 14: Sensitivity, specificity and predictive values of zonal microscopic centers for blinded rechecking sputum smear, Eastern Amhara Region, Ethiopia, 2010.

Microscopic centers by Zone	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Kappa value
S/ Wollo (n, 250)	84.2	99.1	88.8	97.7	0.85
Oromia (n, 30)	100	96.4	66.7	100	0.80
N / Wollo (n, 338)	78.9	100	100	98.7	0.87
Wagmra (n , 33)	100	100	100	100	1.0
N/ Shoa (n, 148)	95.6	98.4	91.6	99.1	0.9
Total (n, 799)	88.4	99.3	92.4	98.9	0.89

4.4. On-site evaluation

During the assessment on-site evaluation was accompanying the panel testing and blind rechecking of the evaluation of the diagnostic centers. Therefore, 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 TB quality assurance, data management and supply management.

In this study 9 public hospitals (42.9 %), 9 public health centers (42.9 %) and 3 private hospital (14.3 %) laboratories were investigated. 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 (Table 16). Most of the health facilities had poor microscope maintenance (53.5%) and inventory management (25.0 %) system (Table 15). Detail on-site assessment is depicted in (Table 16).

Table 15: Number and percent of average score in each sub head in twenty one PDCs of OSE, Eastern Amhara region, Ethiopia.

sub-head	# checklist items	average score (yes x # checklist items)	Number of laboratory	%
Infrastructure	6	14.2	21	67.5
External Quality Assessment	13	14.8	21	70.4
Internal quality control	3	16.7	21	79.3
Staining reagents/ equipment	13	14.7	21	75.5
Standard operating procedure	4	16.5	21	78.6
TB training status	5	17	21	80.9
Maintenance of microscopy	4	11.3	21	53.5
Bio safety & waste disposal	11	14.8	21	70.5
Data management	6	18.7	21	89.0
Inventory &supply management	4	5.3	21	25.0
Average	**69**	**14.4**	**21**	**69.2**

Table 16: Detail assessment for on-site evaluation with standardize checklist in peripheral diagnostic centers of Eastern Amhara regional state, Ethiopia.

Variables		
1. Infrastructure	**Frequency for yes**	**Percentage (%)**
Separate area for TB laboratory work	10	47.6
Separate tables for specimen receipt / smear preparation / microscopy	12	57.1
Well ventilated laboratory (window)	14	66.7
Do you have regular water supply ?	17	81.0
Is there power interruption ?	18	85.7
Do you have a generator as a back up?	14	66.7
2. Available Standard Operating Procedure(SOP)		
Is there a posted smear preparation & staining procedure?	19	90.5
Is there a posted grading chart	14	66.7
All slides are available in the slide box as per Lab register	16	76.2
Slides are arranged as per their slide numbers	17	81.0
3. Reagents and equipments		
Do you collect sputum for AFB microscopy spot, morning, spot ?	21	100
Is the quality of sputum checked?	18	85.7
Are new slides used for sputum smears ?	21	100
Are slides labeled with lab. code, serial number and sequence identifier?	21	100
carbol fuchsin filtered always before use	5	23.8
carbol fuchsin filtered weekly	6	28.6
carbol fuchsin filtered monthly	3	14.3
No filter carbol fuchsine	7	33.3
Is a wooden stick is used to prepare each smear?	20	95.2
Carbol fuchsin adequate available	21	100

3% acid alcohol available	21	100
Methyl blue adequate available	21	100
Adequate Oil immersion	21	100
4. Maintenance of microscope		
Functional binocular light Microscopes	21	100
Maintenance of microscopes	7	33.3
Do you know how to repair simple microscope problem? Eg. Change bulb of microscope	10	47.6
Do you have spare-parts at the lab? (E.g. bulbs,	7	33.3
5. Biosafety and waste disposal		
Standard waste containers with lid	16	76.2
Do you disinfection your working area ?	20	95.2
Do you autoclave contaminated materials before disposable or re-use?	6	28.6
Do you have incinerator?	19	90.5
Do you dispose contaminated materials by incinerator?	18	85.7
Does the laboratory have adequate ventilation?	13	61.9
Does working area cleaned & well organized?	14	66.7
Do you wear personal protective equipments during work?	15	71.4
Are there adequate spaces for sputum processing?	10	47.6
Is there Availability of safety supplies (lab coat, glove etc.) ?	16	76.2
Is there a good sausage system, staining, washing area ?	16	76.2
6. Training status		
Training in NTCP/ EQA	20	95.2
Is there training need?	19	90.5
Has there been any change in staff since last supervisory visit?	14	66.7

Has new staff received proper training as required by the National TB program (NTP)?	11	52.4
Have results of rechecking or panel testing been acceptable?	21	100
7. External quality assessment		
EQA Protocol available and followed	19	90.5
Do you do internal quality control for reagents?	18	85.7
Did you collect and send slides for EQA to regional laboratory in the last two years?	16	76.2
If yes ,please state when,& how many times(3x)	21	100
Does your lab. Participate in panel testing?	12	57.1
Did you receive EQA feedback from regional laboratory?	14	66.7
Did the MC participate in EQA?	21	100
Does your lab. participate in blinded re-checking?	20	95.2
If the answer is yes for the previous quality, EQA feed back	17	81.0
Did the centre receive the results RBRC of previous quarter/quarterly?	13	63.2
Technical staff stated that they examined 100 or even more field per slide (positive or negative)	16	76.2
The Peripheral diagnostic centers examined 50 - 70 field per slide.	2	9.5
Peripheral diagnostic centers of TB technicians examined 20 -50 field per slid	3	14.3
8. **Internal quality control**		
Are you using Control smears at least once a week to check quality of stains?	15	71.4
Do you have prepared positive & negative slides for reagent quality control?	17	81.0
Do you do internal quality control for reagents?	18	85.7
9. Data management		

Is a standard laboratory register book in use?	20	95.2
Is a standard lab. request form use?	17	81.0
Is the registration book complete with the necessary information?	19	90.5
Do you record when you sent slides for EQA?	20	95.2
Are there standard reporting forms?	17	81.0
Is there consistent reporting?	19	90.5
10. Inventory and Supply management		
Do you have an inventory list of supplies and stains?	7	33.3
Do you plan your supply requirements in advance?	12	57.1
Do you have difficulties receiving your supplies?	1	4.76
Do you prepare stains and reagents by yourself in the lab?	1	4.76

All laboratory personnel in the microscopic centers in the region were responsible for sputum collection, along with quality of the sample; smears were dried and fixed prior to staining. However, about 19 (94.4%) of microscopic centers uses new slides, 17 centers (80 %) cleans slides prior to smear preparation, about 16 centers (76 %) clean their microscope using lens paper, 18 centers (86 %) uses readymade reagents and only 3 centers uses standard container for AFB reagents. About 4 centers (14 %) reported that there is frequent interruption of services due to shortage of consumables.

Staffing

There were a total of 99 laboratory personnel in 21 peripheral diagnostic centers. About 47 of them (47.5 %) were BSc laboratory technologists and the remaining 52 (52.5%) were diploma laboratory technicians. Out of this 37 (37.4%) laboratory personnel at BSc level took additional training for TB while 10 (10.1%) BSC level laboratory personnel did not take additional training for TB. On the other hand 42 (42.4%) diploma level personnel took TB related training and the remaining 10 (10.1%) were untrained for TB. (Table17).

Table 17: Distribution of laboratory professionals with TB related training in Eastern Amhara Region in 2010.

Health sectors	# of HF	# of lab. staff	Trained for TB.		Untrained for TB.	
			BScs n, (%)	**Diploma n,%**	**BScs n, %**	**Diploma n,%**
Public Hospital	09	65	29 (44.6)	19 (29.2)	10 (15.4)	7(26.2)
Public Health centre	09	24	7(29.2)	14 (58.3)	0	3(12.5)
Private Hospital	03	10	1(10)	9 (90)	0	0
Total	21	99	37 (37.4)	42 (42.4)	10 (10.1)	10 (10.1)

Work Load

Our study showed that 4.8 % of microscopic centers **(**n=1) were receiving less than 100 specimens per quarter, 38.1% of PDCs (n=8) were receiving between 100 to 300 specimens per quarter, 19.0 % of PDCs (n=4) were receiving between 301 to 500 specimen per quarter, 28.6 % of PDCs (n=6) were receiving between 501 to 800 specimen per quarter and 9.5 % of them (n=2) were receiving more than 1000 specimens per quarter.

Furthermore, we noticed that five peripheral diagnostic centers (23.8 %) were filtering carbol fuchsin solution before using it, six peripheral diagnostic centers (28.6%) were filtering carbol fuchsin per week, three peripheral diagnostic centers (14.3%) were filtering per month and seven peripheral diagnostic centers (33.3%) were not filtering carbol fuchsin at all.

In 16 laboratories (76.2%), the technical staff stated that they examine 100 or even more field per slide (before they declare negative acid fast bacilli), 2 peripheral diagnostic centers (9.5 %) of TB technicians examine 20 -50 field per slide, 3 peripheral diagnostic centers (14.3 %) examine 50 -70 field per slide. Additionally, all peripheral diagnostic centers had no biological safety cabinet (BSC), labeled expired date on AFB reagent and in most of peripheral TB diagnostic centers 14 (66.7%) had a well ventilated room.

Table18: Laboratory work load by health institution types / data on slide volume of 3rd quarter 2010

Health facilities	# of PDCs	Total no. of Sample examined	# of smear positive	# of smears negative	SPR
Public Hospitals	9	6141	556	5585	9.0
Public Health centers	9	2256	134	2122	5.9
Private Hospitals	3	891	75	816	8.4
Total	**21**	**9288**	**765**	**8523**	**8.2**

4.5. Slide positivity Rate from document review

A total of 41,325 clients tested for pulmonary TB in four years time in the 21 peripheral diagnostic centers, sex and age were recorded for 98.03 % (40,511) of them. About 9 % (3,728) of the total examinee were smear positive; 55.8 % (22,591) of the total clients and 52.6% (1,902) of the smear positive clients were males. The maximum positivity rate was between the ages of 15 and 34. Generally, positivity rate was 9.6 % among females and 8.4 % among males (fig 3).

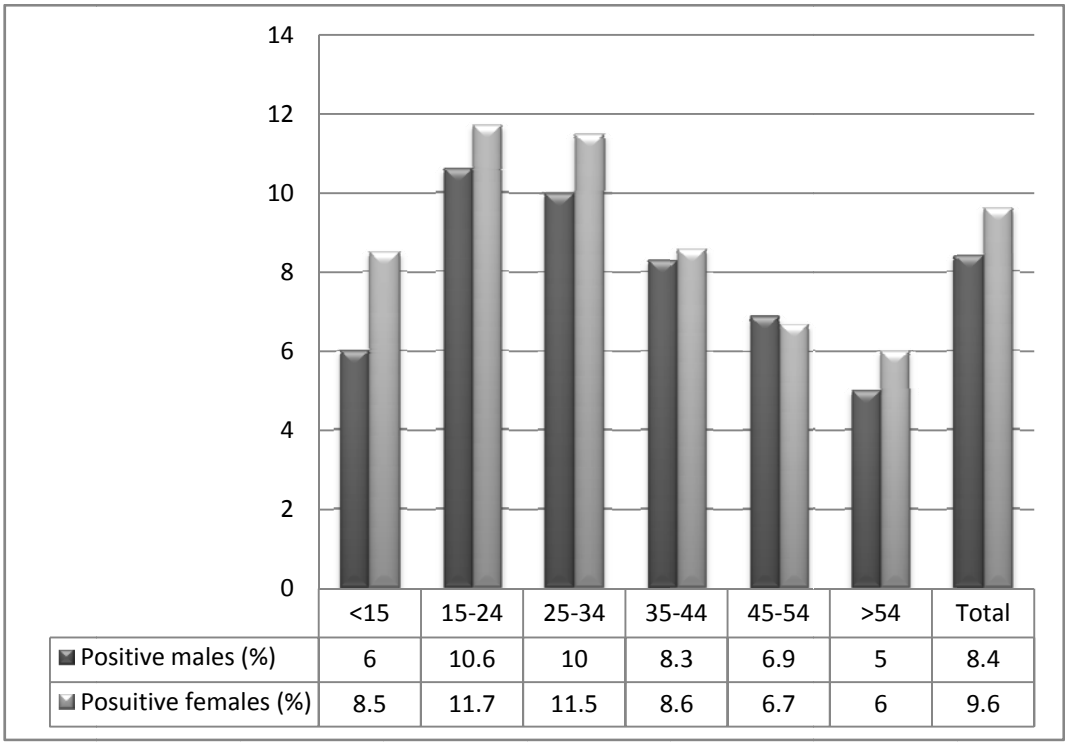
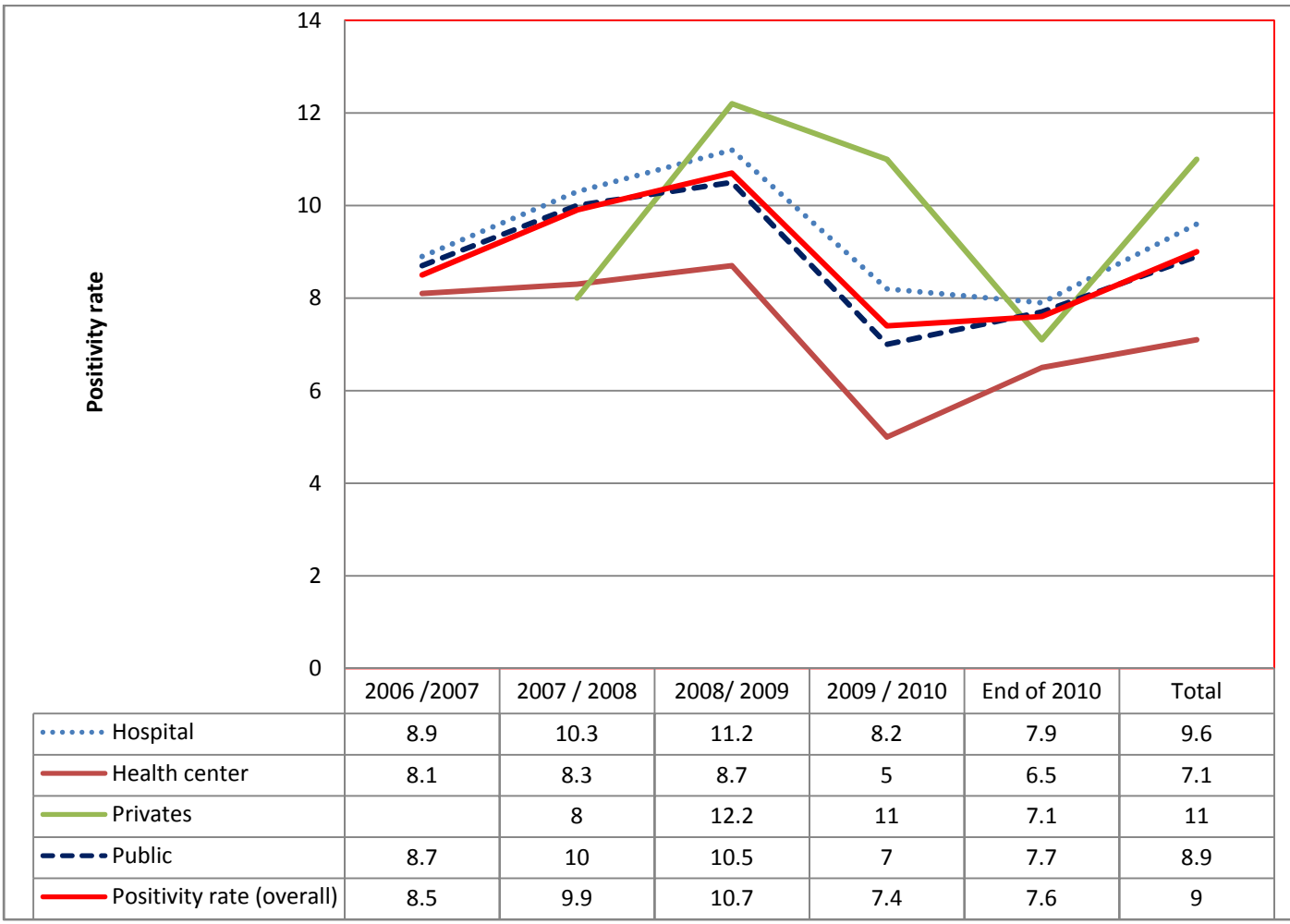


Figure 3: **positivity rate by sex and age group**

Of 41, 325 clients tested for pulmonary TB (PTB), the overall positivity rate was 9 % (3,728) from December 2006 to October 2010. It was 11% and 8.9% among private and public health faculties, respectively. When it was categorized by level of health facilities, the positivity rate was 9.6 % among hospitals and 7.1% among health centers. The positivity rate among the sites ranged from 2.1 % to 15.1 %. The number of total smears examined by the health facilities ranged from 457 to 7,745 with an average of 2,013.7.

The annual client number tested for PTB using acid fast bacilli methods was 6,212 in 2006 / 2007 ; 9537 in 2007 / 2008 ; 10499 in 2008 / 2009 and 13,086 in 2009 / 2010. The positivity rate was 8.7, 9.9, 10.7 and 7.4, respectively (fig 4).



	2006 /2007	2007 / 2008	2008/ 2009	2009 / 2010	End of 2010	Total
Hospital	8.9	10.3	11.2	8.2	7.9	9.6
Health center	8.1	8.3	8.7	5	6.5	7.1
Privates		8	12.2	11	7.1	11
Public	8.7	10	10.5	7	7.7	8.9
Positivity rate (overall)	8.5	9.9	10.7	7.4	7.6	9

Figure 4: **positivity rate by different levels and types of health facilities and trend over years.**

Even though the number of clients examined for AFB increased from 2006 to 2010, the positivity rate decreased to 7.4 % (968 / 13,086). There was a statistically significant difference between hospital and health centers over the four years period. On the other hand statistically significant difference was observed in slide positivity rate between public and private health institution for the year 2009 / 2010 (P value < 0.05).

5. Discussions

The main objective of an EQA program me is to establish inter-laboratory comparability, in agreement with a reference standards that can detect deficiencies through this system will determine the need for quality improvement.

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 programme is to identify these errors. An HFP result should be rare if the basic requirements technician's knowledge about the morphological appearance of acid-fast bacilli (AFB) and 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, 1.4 % of smears had HFP results in two sites of the 21 laboratories indicating there is major error of results and the remaining sites had zero incidence of HFP. An HFN, especially with 1 grade, could be due to quality of staining and unequal distribution of AFB in the smear. Other reasons include technician's lack of 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 other regions, such as Vietnam and Algeria (28, 29), while a study in Senegal (30) reported a false-positive rate of 11 % to 12 %. An HFN result, on the other hand, can be expected occasionally, necessitating the use of a critical value for HFN.

The result of this study 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 (29, 31). All PT 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 (10 LFN in 8 sites), and the results might suggested that microscopists are unable to detect low AFB counts , probably because they do not read all fields , or due to the high turnover rate therefore the outcome is a false negative result. This kind of error is important, as patients with paucibacillary disease could give negative results in AFB

microscopy and will therefore not receive treatment, resulting in further community spread and failure in diagnosis of pulmonary TB (31).

In the present study, majority of peripheral diagnostic centers were scored acceptable performance. However, of which 1(4.8 %) of our laboratories were found to be below the acceptable performance (80 %). On the other hand, a study conducted on 19 to 26 laboratories in Thimphu and Bhutan showed that the AFB microscopy quality of 21.1 to 23.1% laboratories was found to be unacceptable (9). So the diagnostic qualities of our study laboratories were found to be better than other studies. Besides that, public and health centre laboratories were slightly better than private and hospital laboratories, respectively as there is a statically significant difference x^2 (p value < 0.05). The most likely explanation for this difference in performance might be difference in working environment among public and private health facilities.

Proficiency testing in Mexico revealed that 46 % (196) of laboratory personnel scored below 80 % in contrast to our study (3). The large sample size of personnel they used may contribute the difference and effect of training / supervision could have impact in the overall PT performance.

Among 9 private TB diagnostic laboratories in Nepal, one (11.1 %) laboratory had quantitative (minor) error while there was no any major error encountered. However, in our study there were major errors in three (14.3%) of the laboratories and minor errors in twelve (57.1%) of the laboratories. Errors were encountered on twenty three (11.0%) of the smears. Only one (4.8%) of the laboratory scored unacceptable (<80%) result. Unexpectedly, hospital laboratories were lesser than health center laboratories in overall performance. On average, private laboratories were found to be lesser than public health laboratories for different error types. The majority of error was false negative [52.4%] (11), especially on smears with low density (scanty) of mycobacterium (22).

Unacceptable performance was obtained from one peripheral diagnostic center with major (2HFP) and minor errors (1LFN and 3QE). By tracking back to the errors committed by the specific sites, the possible root causes were competency of technician in identifying AFB. In addition, one diagnostic health center scored 80 that had major and minor error of 1HFP and 2 LFN due to poor staining

quality, staining problem and lack of detection AFB by the technician which needs to be improved.

Out of 210 panel slides, 105 unstained and 105 stained: 1HFN, 0HFP, 7LFN, 1LFP and 5QE and 0HFN, 3HFP, 3LFN, 0LFP and 3QE 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 a good laboratory personnel from a very good laboratory personnel.

Blinded rechecking

Comparison of the results derived from testing sites with the reference laboratory at regional level showed that there were similar results in 61(7.6%) of cases for positive smears and in 725(90.7%) of cases for negative smears. This study showed an excellent agreement between slides coming from peripheral diagnostic centres, and the proportion of false readings was insignificant. The study showed that the overall false reading was 1.6 % and the overall agreement was 98.4% which were better than other studies with false reading rate of 3.2% and an agreement of 96.8% (23).

In Iran reported from 930 slides, re-reading for positive slides ranges 5.8- 7.0 % and 93-94.2 % for negative slides. This finding is almost comparable with our findings (33).

Our finding of smear quality evaluation criteria; smear size, thickness, staining quality, cleanness & evenness were in agreement with study done in different countries of Ghana and Taiwan (21, 25). Even though the smearing and staining quality was not adequately good, reading ability was found to be better, as it was seen by the sensitivity of 88.4% and specificity of 99.3%. Similarly, a study conducted in Ghana showed that staining quality, smear cleanness, thickness, size and evenness was found to be 79 %, 69 %, 46 %, 67 % and 60 % with improvements to 90 %, 86%, 79 %, 80 % and 74 % after the establishment of the QA system, respectively (21). Another study conducted in Taiwan found that the smearing and staining quality before and after training was 87 % to 99.6 % proper staining, 48 % to 77 % proper thickness and 63 % to 100 % proper smear size (25). Similar smear quality has been reported in Beijing (34).

Evenness of smears in private health facilities was very low (28.1 %) compared to public facilities in the study sites (59.2 %) p value < 0.05. The big difference in terms of smear evenness could be explained that private facilities, especially laboratory personnel may have additional burden other than tuberculosis microscopy, high turnover of staffs which needs further investigation.

High false negative (HFN) were the major errors found in this study and low false negative (LFN) and quantification error (QE) were the minor errors found and there were no false positive errors. In this study, the overall false reading was 1.6 %, the overall agreement was 98.4 % the sensitivity of (88.4) and specificity of 99.3 %, respectively. Similarly, a study conducted in Tanzania in 2006 showed that the proportion of well prepared smears was 86.2 % and that of well stained smear was 81.2 %. The overall agreement on reading was 89.2 %; the overall sensitivity was 88.5 % and specificity was 100 %.

A study conducted in Thimphu (2008) of 1st, 2nd, 3rd and 4th quarter results shown that some laboratories have shown steady improvement in their performances. Few laboratories were found reporting false positive and some still missing out AFB (false negatives) (9). Reporting of low false negative and quantification errors are expected especially if laboratory personnel are new but not for high / low false positive and high false negatives.

Training of laboratory personnel on AFB and related topics would have an effect on reducing laboratory errors in AFB testing. In Kinshasa (2008), training resulted in a marked improvement in the practical component (smear preparation, staining, and reading), with median test scores increasing from 70 % pre training to 86 % post training (19). Our study finding also showed that training on AFB had better EQA performance similar to results of the Kinshasa (19).

On-site evaluation

Evaluation of laboratories through on-site evaluation showed that the overall achievement of the laboratories was 69.2% and the lowest score was recorded on maintenance of microscope (53.5 %) and highest score were recorded on data management (89.0 %). In contrast to this, a study conducted in India in 2006 shows that 44.15% errors in laboratory checklist-items were identified in ten Intermediate reference laboratories (IRLs). Majority of errors occurred in

EQA (92.6%), internal quality control (90%), staining reagents / equipment (56.6%), infrastructure (42%), and bio-safety practices (40 %) (24).

About 43 % of participant laboratories were not involved in panel testing which is critical for the overall tuberculosis quality service. All health centers should be participated and the quality problems observed could be improved. Lack of spare parts for microscope in 14(66.7%) microscopic centers require an urgent remedial action as microscope without spare parts like bulbs will compromise the laboratory service delivery process mainly delay in tuberculosis diagnosis and treatment. Although more than 81 % (17/ 21) microscopic centers have standard laboratory request form and reporting form, still some laboratories are deficient which could result in poor data management for surveillance and other planning purpose.

This may strengthen recommendation recently given by African Field Epidemiology Network were good infrastructure, equipment and laboratory personnel are key for control of infectious diseases in Africa (35).

Majority of participant laboratories were using commercially prepared reagent for acid fast staining. Nothing is stated about the quality of this stain. This finding is also observed in Iranian study were 68 .4 % of laboratories used commercially prepared staining reagent with poor quality (33). Still 33.3 % of laboratories were not filtering the carbol fuchsin reagent prior to staining. This will lead to false positive result. It is well known that none of the laboratories have biological safety cabinet that could have impact on safety of laboratory personnel. This is not a problem of the study areas rather all centers in the country except research centers lack this important safety equipment.

Slide positivity rate (SPR)

Data from 21 laboratories on sex, age with positivity rate and results of sputum smear examination were electronically captured from the registers and a uniform data recording TB log book was used by all selected peripheral diagnostic centers. The overall positivity rate was 9 % (3,728) in December 2006 to October 2010.

Slide positivity rate among public and private centers was 8.9 % and 11 % respectively. This may reflect there is difference in terms of tuberculosis patient visit as noted by the no of TB suspects from the observed data.

It has been noted that public private partnership could contribute for case detection of TB and Tb control program at large. This has to be further strengthened. (36).

SPR is high among age group between 15-34 years. This might be such age group is very active, mobile and acquire tuberculosis from index cases.

The annual clients tested for PTB is steadily increasing from 6,212 in 2006 / 2007 to 13,086 in 2009 / 2010. The same is true for positivity rate that increased from 8.7 % to 10.7 % and reduced slightly to 7.4 % for year 2010/ 2011 (fig 4). It is known that Ethiopia has high incidence of tuberculosis which is not exceptional in the study site. Slight decrease in SPR for year 2010 /2011 might be due to small sample size we obtain for this calendar year, which was not completed.

It is impossible for more than one reader to examine the same 100 fields and report the exact number of AFB, taking into consideration the unequal distribution of bacilli in a smear with less than 10 AFB. The danger in such a procedure is that any error by the first reader will be reflected in the readings of the subsequent readers (32). Further, the technicians participating in a proficiency testing programme have unlimited time for the slide examination and they are aware of being tested. This method therefore does not allow a true assessment to be made of the quality of slide reading under routine conditions; it can only measure the ability of the technician to read slides correctly. Thirdly, the present system does not address the quality of smear preparation and staining. This approach, however, requires a more sophisticated preparation by the reference laboratory using more homogenous sputum specimens with the desired numbers of AFB per slide, which are difficult to prepare.

Acid-fast microscopy is not difficult, but it is tedious if large numbers of smears have to be examined. Thus, there is the danger of loss of technical accuracy if a large number of proficiency panel slides are read on a single day. Hence, there is a need to have the smallest sample size and to specify the maximum number of slides to be examined each day. The technical sensitivity and specificity at lower-level laboratories is also influenced by the quality and condition of the microscopes available (32).

Finally, acid-fast microscopy is a technique that has inherent errors, like any other technique, even when performed by the most experienced technician. Thus, the results of one laboratory simply cannot be assumed to be more correct than another. This calls for referee reading of all discrepant results by a third reader under blind conditions. Any errors in the reading of the referee have to be accepted as inherent limitations of the method. Thus, there is no possible practical alternative for establishing a gold standard. The present investigations helped in the identification of a few good laboratories and separate them from those that are poor performers. In the case of centers with poor performance, a letter was sent out pointing out the deficiencies observed.

This study indicates that it is possible for a national reference laboratory to prepare consistent Proficiency testing (PT) slide sets for many microscopists. PT also allowed the effect of training on the performance of microscopists to be evaluated.

6. Limitations of the study

We are unable to include all health facilities in the region for better evaluation of the microscopic centers.

The blinded rechecking we did is only for one quarter which was better for four quarters to elucidate the correct picture of the microscopic centers.

The participant microscopic centers do not have the same exposure for external quality program as observed in our study, which may create little bias on the overall performance of the centers.

7. Conclusion and recommendation

7.1. Conclusion

- The overall performance of microscopic centers both in public and private centers is acceptable except for a single laboratory which scored below 80 % according to the national and international guidelines for panel testing.
- All types of errors has been observed in the participants centers that indicate improvement in the laboratory service.
- The overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of panel reading by microscopic centers were 89 %, 96 %, 96 %, and 90 % respectively.
- The overall false reading for blinded rechecking by participant laboratories was 1.6 % and the overall agreement was 98.4 %, with sensitivity of 88.4 %, specificity of 99.3 %.
- Majority of the Peripheral diagnostic centers (PDCs) (60%) were having the Annual negative slide volume (ANSV) and a slide positivity rate (SPR) within the accepted range.
- On-site evaluation of participant microscopic centers revealed a number of problems in terms of infrastructure, standard operating procedure, reagent quality, equipment maintenance, data management and training issues.
- The overall slide positivity rate (SPR) was 9 % which shows an increasing trend from 2006/ 2007- 2009/ 2010 with slight decline at the end of 2010.

7.2. Recommendation:

Based on the finding, the following recommendations are forwarded:

- Continuous assessment of tuberculosis microscopic centers should be considered as part of DOTS program for proper diagnosis and management of tuberculosis in the region. This should include panel testing, blinded rechecking and onsite supervision of laboratories.
- The region should implement external quality assessment schemes for all health facilities in the region including public and private sectors which can improve the overall performance of the laboratories.
- The overall agreement of microscopic centers for blinded rechecking should be maintained and mechanism has to be implemented for proper communication among national, regional and peripheral microscopic centers.
- Public private partnership has to be strengthened in the country at large and in the region in particular for proper diagnosis and management of tuberculosis.
- The region should supply adequate consumables, spare parts for microscope, safety tools, for all microscopic centers through appropriate channel and communication.
- Microscopic centers should be dedicated for proper handling, recording and storing of tuberculosis slides which is critical for external quality assessment purpose and as part of their duties.
- Microscopic centers must take quality measures including pre-analytical, analytical and post analytical quality control to improve the overall performance of their microscopic centers together with their region, national laboratories and other governmental and nongovernmental organization.

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Annex-1: Questioner

Questionnaire identification number: ----------

Hospital / Health center: ----------

Checked by: Supervisor Name: ---------- Signature: ----------

Date: ----------

Name of interviewee: ---------- signature ---------- date ----------

Date of interview: ----------/ _____ / _____

Date Month Year

TB On site evaluation check list for supervision of MCs by supervisors

Part-1: General Information

1.1	Name of laboratory (MC):	
1.2	Zone / Woreda:	
1.3	Region:	
1.4	Head of MC:	
1.5	Date of visit:	
1.6	Name of the visiting laboratory supervisor (s):	

Part-2: Human resource & development:

Number of staff in the laboratory

	Category of laboratory staff	**number**	**How many trained on TB**	**Un trained on TB**
2.1	Laboratory technologist (BSc)			
2.2	Laboratory technician (Dip)			
2.3	Junior technician (certificate)			

Part-3: Laboratory Work load / Data on slide volume for the last quarter

S.No.	**Types of slide include diag. & follow up**	**Last 3rd months**	**Last 2nd months**	**Last 1st month**
3.1	Total No. of sputum sample(slide examined)			
3.2	Number of smear positive slides			
3.3	Number of smear negative slides			
3.4	Sputum smear positive rate			

Current visit particulars

S. No	**Item**	**Adequate / Acceptable [1]**	**Problems Identified**
Part-4	**Infrastructure:**		
4.1	Separate area for TB laboratory work	Yes / No	
4.2	Separate tables for specimen receipt / smear preparation / microscopy	Yes / No	
4.3	Well ventilated laboratory (window)	Yes / No	
4.4	Do you have regular water supply?	Yes / No	
4.5	Is there power interruption?	Yes / No	
	Do you have a generator as a back up?	Yes / No	
Part-5	**Available Standard Operating Procedure:**		
5.1	Is there a posted smear preparation & staining procedure?	Yes / No	
5.2	Is there a posted grading chart	Yes / No	

S. No	Item	Adequate / Acceptable [1]	Problems Identified
5.3	All slides are available in the slide box as per Lab register	Yes / No	
5.4	Slides are arranged as per their slide numbers	Yes / No	

Part -6	Material	Available	Good condition	Adequate supply	Problem identified
6.1	Slide	Yes / No	Yes / No	Yes / No	
6.2	Frosted slide	Yes / No	Yes / No	Yes / No	
6.3	Slide box	Yes / No	Yes / No	Yes / No	
6.4	Sputum containers approved by NTP	Yes / No	Yes / No	Yes / No	
6.5	Pencil used frosted slides	Yes / No	Yes / No	Yes /No	
6.6	Wire loops or sticks	Yes / No	Yes / No	Yes / No	
6.7	Funnel	Yes / No	Yes / No	Yes / No	
6.8	Filter paper	Yes / No	Yes / No	Yes / No	
6.9	Staining rack	Yes / No	Yes / No	Yes / No	
6.10	Sprit lamp/Bunsen burner	Yes / No	Yes / No	Yes / No	
6.11	sprit for sprit lamp or gas for burner	Yes / No	Yes / No	Yes /No	
6.12	Lens tissue	Yes / No	Yes / No	Yes / No	
6.13	Red pen Recording for positive result	Yes / No	Yes / No	Yes /No	
6.14	Water supply	Yes / No	yes / No	Yes / No	

	Reagent	Available	With expired date	Adequate supply
6.15	Carbol fuchsine	Yes / No	Yes / No	Yes / No
6.16	Methylene blue	Yes / No	Yes / No	Yes / No
6.17	3% acid alcohol	Yes / No	Yes / No	Yes / No
6.18	Oil immersion	Yes / No	Yes / No	Yes /No

	Item	Adequate/acceptable	**Problem identified**
6.19	Functional Binocular light Microscopes	Yes / No	
6.20	maintenance of microscopes	Yes / No	
6.21	Do you know how to repair simple microscope problem? (E.g. changing a bulb of the microscope)	Yes / No	
6.22	Do you have spare-parts at the lab? (E.g. bulbs,	Yes / No	
6.23	Do you documented preventive maintenance for the microscope?	Yes / No	
	If, Yes, how frequently_____		
	what are your major preventive maintenance_____		

Part-7	**Bio safety & waste disposal:**	Adequate/acceptable	**Problem identified**
7.1	Standard waste containers with lid	Yes / No	
7.2	Do you disinfection your working area ?	Yes / No	
7.3	Do you autoclave contaminated materials before disposable or re-use?	Yes / No	
7.4	Do you have incinerator?	Yes / No	
7.5	Do you dispose contaminated materials by incinerator?	Yes /No	
7.6	Does the laboratory have adequate ventilation?	Yes / No	
7.7	Does working area cleaned & well organized?	Yes / No	
7.8	Do you wear personal protective equipments During work?	Yes / No	
7.9	Is there adequate spaces for sputum processing?	Yes / No	
7.10	Is there Availability of safety supplies (lab coat, glove etc.) ?	Yes / No	
7.11	Is there a good sausage system, staining, washing area ?	Yes / No	
Part-8	**Training related to TB**		
8.1	Training in NTCP/ EQA	Yes / No	
8.2	Is there training need?	Yes / No	

	If yes , please specify what training _____ _____		
8.3	Has there been any change in staff since last supervisory visit?	Yes / No	
8.4	Has new staff received proper training as required by the National TB program (NTP)?	Yes / No	
8.5	Have results of rechecking or panel testing been acceptable?	Yes / No	
8.6	If no, any problems identified and is there any plan for additional training / refresher course?	Yes / No	
Part-9	**Quality assurance**		
9.1	EQA Protocol available and followed	Yes / No	
9.2	Do you do internal quality control for reagents?	Yes / No	
9.3	Are you use Control smears at least once a week to check quality of stains?	Yes / No	
9.4	Do you have prepared positive & negative slides for reagent quality control? If yes observe	Yes / No	
9.5	Did you collect and send slides for EQA to regional laboratory in the last two years?	Yes / No	
	If yes ,please state when,& how many times _____ _____		

9.6	Does your lab. Participate in panel testing?	Yes / No	
9.7	Did you receive EQA feedback from regional laboratory?	Yes / No	
	If No, please state _____		
9.8	Did the MC participate in EQA?	Yes / No	
9.9	Does your lab. Participate in blinded re-checking ?	Yes / No	
9.10	Are slides collected quarterly?	Yes / No	
9.11	If the answer is yes for the previous Quality , EQA feed back	Yes / No	
9.12	Did the centre receive the results RBRC of previous quarter/quarterly?	Yes /No	

Part10	**Data management**		
10.1	Is a standard laboratory register book in use?	**Yes / No**	
	If no why_____ ----------		
10.2	Is a standard lab. Request form use ?	**Yes / No**	
10.3	Is the registration book complete with the necessary information?	**Yes / No**	
10.4	Do you record when you sent slides for EQA ? If yes observe	Yes / No	
10.5	Are there standard reporting forms ?	Yes / No	

10.6	Is there consistent reporting?	Yes / No	
Part-11	**Inventory and Supply management**		
11.1	Do you have an inventory list of supplies and stains?	Yes / No	
	If yes, when did you last update these lists? ---------- ----------		
11.2	How often do you receive supplies like stains and others? One month, every 6month , once a year,	**Yes / No**	
11.3	Do you plan your supply requirements in advance?	Yes / No	
11.4	Do you have difficulties receiving your supplies?	Yes / No	
	If yes why? ----------		
11.5	Do you prepare stains and reagents by yourself in the lab?	Yes / No	
	If yes, do have difficulties doing so? ---------- ---------- _____		

Part -12 Some basic AFB microscope procedure to observe the skill of lab tech.

12.1. Is the lab technician responsible for collecting sputum? Yes ☐ No ☐

If no who is responsible----------

12.2. Do you collect sputum for AFB microscopy spot, morning, spot ? Yes ☐ No ☐

If No explain ----------

12.3. Is the quality of sputum checked? Yes ☐ No ☐

12.4. When the patient produces saliva, is repeat sputum collected? Yes ☐ No ☐

If No explain----------

12.5. Are new slides used for sputum smears? Yes ☐ No ☐

12.6. Are slides cleaned prior to use? Yes ☐ No ☐

12.7. Are slides labelled with lab code, serial number & sequence identifier ? yes / No

12.8. Are smears completely air dried prior to fixing ? yes / No

12.9. Are slides heat fixed by passing 3- 5 times through flame ? yes / No

12.10. How often is Carbol Fuchsine filtered?

- Always before use ☐ - Monthly ☐
- Weekly ☐

12.11. Is a wooden stick is used to prepare each smear? Yes ☐ No ☐

If No what you use to smear the sputum?----------

12.12. How many fields are examined to report a negative smear?

- 20- 50 ☐ - 70- 100 ☐
- 50 – 70 ☐

12.13. Do you clean microscope lenses with tissue paper? Yes ☐ No ☐

If No explain----------

12.14. From where do you get prepared AFB reagents?

- Regional laboratory -Zone/ woreda
- Hospital - Other

12.15. Who is responsible to distribute reagents and supplies from Regional lab. zone/ woreda or hospital?

- Lab technicians - Pharmacist
- Zonal TB / Lab expert - Regional lab

12.16. Are there prepared reagents supplied by standard container ?

If yes list the type of container ----------

If no list the container in use ----------

12.17. Is there service interruptions to laboratory work due to shortages of supplies and Equipment? Yes ☐ No ☐

If yes, please explain _____

13. On- site evaluation Summary

List any major problems identified during on site visit

a. Operational problems

_____

_____

_____

_____

_____

b. Technical problem

_____

_____

_____

_____

c. Action required

_____

_____

_____

Name of Supervisor ---------- Signature----------Date----------

Lab head of MC ----------Signature ----------date----------

Annex-2: External quality assessment program of panel testing for AFB microscopy

Participant laboratory ----------

Date arrived: ----------

Date returned: ----------

Name of analyst: ----------

Slide code No.	Type	Result
AM1	Stained	
AM2	Stained	
AM3	Stained	
AM4	Stained	
AM5	Stained	
AM6	Un Stained	
AM7	Un Stained	
AM8	Un Stained	
AM9	Un Stained	
AM10	Un Stained	

Comment: ----------

Annex- 3: Panel testing recording and feedback form

Test slide set # --10

Date sent: ----------Date result received: ----------

Peripheral laboratory: ----------

Name of technician reading test smears: ---------- Date of result reporting :----------

Slide ID.	Type of slide	result	Expected result	Error type	point
AM1	Stained				
AM2	Stained				
AM3	Stained				
AM4	Stained				
AM5	Stained				
AM6	Un stained				
AM7	Un stained				
AM8	Un stained				
AM9	Un stained				
AM10	Un stained				

Total score: _____ Percentage: _____ Performance acceptable / unacceptable

	Result as deemed by EQA unit				
Negative	**Negative**	**1 -9 AFB/100F**	**1+**	**2 +**	**3+**
1 -9 AFB/100F					
1+					
2+					
3+					

Classification	Description	Interpretation	Score
Correct resp.		No error	10 point
LFP, LFN	Low false positive & low false negative are 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-4: External quality assessment microscopic centre smear result sheet for random blinded rechecking

H/C / Hospital ---------- region: ---------- zone: ----------

Quarter / year : ---------- date of compiled: ----------

PDC serial No.	PDC smear result	RRL smear result	remark

Sample sends by:

Name ----------

Date ----------

Sample collector

Name ----------

Date ----------

Annex-5:Blinded rechecking of sputum smear examination for AFB

Peripheral laboratory ---------- first controller :----------

Local technicians: ---------- laboratory :----------

Date sampled: ---------- second controller: ----------

Period in lab. register checked: ---------- laboratory: ----------

Table1: The six criteria of smear evaluation

Slide No.	PDC result	1st controller	2nd controller	Specimen Quality		Size		thickness		staining		cleanness		evenness	
				good	poor	Good	Poor	normal	Poor	Good	Poor	Good	Poor	evenness	Unevenness

Peripheral diagnostic centres						Regional reference laboratory				
Total reported results on the samples Positive ---- scanty ---- negative ---------						Total reported results on the samples Positive ---- scanty ---- negative ------				
Summary of errors identified						Summary of errors identified				
HFN	HFP	LFN	LFP	QE		HFN	HFP	LFN	LFP	QE

HFN = High false negative; HFP = High false positive HFN = High false negative High false positive

LFN =Low false negative; LFP = Low false positive LFN =Low false negative; LFP = Low false positiQE QE= Quantification error QE = Quantification error

Conclusion: ----------

Recommendation: ----------

Annex- 6: Data from the registered TB log book of sputum smear examined

Hospital/ Health center ----------

Data collector ----------signature ---------- date----------

Lab. head of MC----------signature ----------date----------

S. No.	Month /year	Age	Sex	Case type	Results of AFB			Final result
					Spot- 1	morning	Spot-2	

Annex- 7: 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 National reference TB laboratories. 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. National reference laboratory should demonstrate proficiency in producing samples with a minimum of 25-30 slides that are consistent for negative and low numbers of AFB before proceeding to developing test slide sets (26).

i. Materials Required

- Processing should be performed in a Biological Safety Cabinet.
- 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)

Amount: 5 ml

Color: white to green

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

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

ii. Procedure using (N-acetyl-L-cysteine (NALC)

1. **Collection of sputum specimens**:

Sputum specimens with numerous AFB should be collected from the patients and be stored for not more than 2 days after collection in order to prevent destruction of sputum cells. Fresh AFB negative sputa also must be selected from the routine specimens.

2. **Preparation of mucolytic solution**:

2% of N-acetyl-L-cysteine is mixed with an equal amount of 2.9% sodium citrate.2H2O right before use.

3. **Liquefaction of sputum specimen**

AFB positive and negative sputum samples are mixed with an equal amount of mucolytic solution separately and shake gently to liquefy specimens.

4. **Dilution of AFB positive sputum homogenate**:

The liquefied AFB-positive sputum is diluted with varying proportions of AFB-negative specimen

5. **AFB counts of sputum dilutions**

One drop of each sputum dilution is spread on a slide with a smear size of 2 cm^2 and dry and sterilize in a hot oven for one hour without scorching. 10 smears are prepared with each sputum dilution and stained with Ziehl-Neelsen staining method and count AFB per 1, 10 or 100 microscopic fields.

Sputum dilutions whose average AFB counts fall into “1-9/100 fields”, “10 - 99/100 fields (1+)”, “1-10 per microscopic field (2+)” or “more than 10 per microscopic field (3+)” are selected and used to prepare as many smear slides as possible. Negative slides must also be prepared.

AFB counts should be rechecked with randomly selected 10 to 15 slides again after completion of smear preparation in order to confirm AFB counts of every batch of slides (26)

iii: Ziehl neelsen 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 decolourisation by weak acid. Those bacteria that resist decolourisation by acid are called Acid Fast Bacilli (AFB). This property differentiates AFB from other bacteria, cells and mucus which 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

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

Result: The AFB was stained bright pink.

Procedural notes: It is important for reading and interpretation of results that appearance of the smears was 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.

iv. 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 will help 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 length 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 technicians. For high positives (2+ or 3+) the technicians may estimate the number AFB/100 fields by Selecting a sufficient number of representative fields. For low positives (exact count AFB/ 100 fields and 1+) and AFB negatives slides the technicians should read a minimum of 200 fields per slide and record the average number AFB/100 fields (26).

Table19: **The Set of smear composition of panel testing**

1 Slide grade =3 + 1 slide grade = 2+ 1 slide grade = 1+ 2 slide graded scanty 5 negative slides	1 Slide grade =3 + 1 slide grade = 2+ 2slide grade = 1+ 3slides graded scanty 3negative slides	1slide graded= 2-3+ 2slides graded=1+ 3slidegrade scanty 4 negative	1slide graded=2-3+ 2slides graded=1+ 4slidegrade scanty 3negative

*scanty: slides graded in 1-9AFB/ 100F

v: System for sending slides to the laboratories

The success of Panel testing will relying on the ability to deliver slides to the peripheral laboratories with minimal breakage or degradation of the slides. If examinees receive packages of broken and faded smears, they will be poorly motivated to perform well, and confidence in EQA methods will decline. Each country will need to determine the best mechanism for delivering slides based on the services and resources available.

vi. Reporting criteria

Reporting criteria was done using National and WHO/IUATLD guidelines shows in (Table20).

Table 20: 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+

Annex-8: 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 will be selected using the laboratory register by one of the study personnel during the quarterly visits.

1. sample size determination

Ideally, the collected smears should constitute a statistically representative and random sample based on both test volume in the laboratory being evaluated, and the expected performance parameters as defined by EHNRI.

2. Slide evaluation

Re-examined the sampled smears visually to assess the quality of smear preparation as size, thickness, staining, cleanness and evenness using the national guideline (Table 21).

Table 21 The Criteria of blinded rechecking smear evaluation

#	**Criteria**	**Definition**
	Smear size	This is evaluated macroscopically. A smear size of approximately 1x2 and 2x3 cm was considered acceptable
	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
	Staining quality	This is evaluated by microscopic observation. A good staining technique ensures that the smear is neither under-stained nor over-stained
	cleanness	This is evaluated by microscopic observation. A smear was considered clean if stained smear was free from stain deposits, dirt, debris and crystals produced by overheating during staining
	evenness	Slide is evaluated by macroscopic observation. A smear of acceptable evenness should not be too thick or not too thin on the slide

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 or xylol 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-9: Standards for reagents and equipments

1) Standards for Reagents

a. Specifications

A. Basic fuchsine

The chemical name: Pararosaniline hydrochloride

- The chemical structure: $C_{19}H_{18}N_3Cl$
- 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 = 10 gms, required amount = 10/0.85 = 11.76 gms. (12)

B. Carbolic acid:

The chemical name: Phenol

- The chemical structure: C_6H_5OH
- Molecular Wt: 94.11
- Melting point: $40^{o}C \pm 2$
- Purity: 99.5%
- Please note: The critical concentration of Phenol in Carbol fuchsin is 5%.
- Phenol is highly corrosive, handle with extreme care.

C. Methylated spirit

- Chemical name: Ethanol denatured + 5% Isopropyl alcohol + 5% Methanol
- Molecular structure: C_2H_5OH
- Molecular wt: 46.07
- Purity: 90%

D. Acid Alcohol:

- 96% Ethanol
- 37% Hydrochloric acid

E. Methylene blue:

- The chemical name: Methylthionine chloride
- The chemical structure: $C_{16}H_{18}ClN_3S$.
- 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 amount by dye content. For example: Dye content = 82%, actual amount 1 gms, required amount = 1/0.82 = 1.22 gms.)

F. 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 colourless, odourless, transparent, free from fluorescence in day light with relative density of 0.827 to 0.890, viscosity of 110 to 230 mPa s., specific gravity of 0.76-0.78 at 15.5 ^{o}C.

G. Shelf life of prepared reagents:

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

H. Identification:

- All reagents should have a 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: 1.3 mm
- Edges: Polished
- Sealed in a moisture absorbing dessicant pack

ii. Balance:

Type: Electronic or Analytical balance

a. **Electronic balance**:

- 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 – 120 gms, (two digit decimal)
- Resolution: 0.01 gm

b. **Analytical balance**:

- ✓ Enclosed in a glass box with shutters, dimensions of the box in cms: 46 x 34 x 20
- ✓ Oscillator type of balance, with levelling screws, two aluminium pans, plumb line for adjusting horizontal level
- ✓ Weighing capacity: 1 mg to 200 gms, with fractional weight and regular weight in boxes including rider and forceps to handle weights (12)

iii. Binocular microscopes

Binocular microscope, for use with electric light via power line or alternative via battery pack. Battery pack can be used as un interruptible power supply and should be provided with the microscope.

Observation tube: binocular, 30^0 inclination (viewing angle) and 360^0 rotation

Stage: rectangular,built in mechanical stage with vernier scale (mimimum: 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 weares

Bright field illumination in transmitted light mode: white light LED, minimum 3w

Fluorescence illumination in reflected light mode: blue light LED, minimum 3w

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- 10: Information sheet and Verbal Consent form (English version)

Onsite evaluation: To assess quality performance evaluation of laboratories on AFB smear Microscopy in Eastern Amhara region, Ethiopia, **2010.**

General information sheet about the study

Name of the hospital / microscopic center ----------

Questionnaire identification number----------

My name is Mekete Mulat and currently I am master student of clinical laboratory science in the track clinical and public health microbiology program launched in Addis Ababa university faculty of medicine school of medical laboratory science. now I am working a research undertaken by Addis Ababa University, School of Medical Laboratory student research project and the aim of the study is to assess quality performance evaluation of sputum smear microscopy detection of tuberculosis with panel testing, blinded slide re-checking and on-site evaluation by WHO EQA guidelines to determine the gape of technical error & performance of the technician on selected health facilities in Eastern Amhara region and which will be improve the diagnosis outcome. It will be 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 to participate. However, your participation is important to full fill the study purpose.

For any information you can contact

1. Faculty of Medicine Addis Ababa University
   Office of Associate Dean, Postgraduate Programs and Research

   P.O. Box 9086. Addis Ababa, Ethiopia

   Tel. 251-11-55128765

E-mail:aaumfirb@yahoo.com

2. Dessie Health Science College

Tel. 0331-11-19-81, Dessie, Ethiopia

3. The address of the principal investigator is: Mekete Mulat, BSC

Department of Medical laboratory sciences, Clinical and public Microbiology track

Faculty of Medicine, Addis Ababa University

Addis Ababa, Ethiopia.

Tel: +251-091-0519308
E-mail:mekamsal@gmail.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

Annex-11: <u>የመረጃ መስጪያና የፈቃደኝነት መጠየቂያ ቅጽ</u> (Amharic Version)

ስለ ጥናቱ አጠቃላይ መረጃ

1. የጤና ተቋሙ ስም ----------

2. የመጠይቁ መለያ ----------

3. የተጠናቀቀ (የተሟላ መጠይቅ) ----------

ስሜ ማከተ ማላት እባላለሁ፡፡ **በአሁኑ ሰዓት በአዲስ አበባ ዩኒቨርሲቲ በላቦራቶሪ ሳይንስ ትምህርት ክፍል ውስጥ በተከፈተው በክሊኒካል ላቦራቶሪ ሳይንስ የማስተርስ ፕሮግራም ውስጥ በክሊኒካል ና ፐብሊክ ማይክሮባዮሎጂ ትራክ ክፍል ትምህርቴን በመከታተል ላይ እገኛለሁ፡፡ አሁን ወደ እርስዎ የመጣሁ**በት አላማ የቲቢ በሽታን ከአክታ ናሙና በሚይክሮስኮፕ ምርመራንና ለመምርመራው የሚያገለግሉ መሳሪያና ኬሞካሎችን የጥራት አሰሳ ማድረግ ሲሆን በተጨማሪ በአለም ጤና ድርጅት መመሪያ መሳረት የተዘጋጁ እስላይዶችን ለጥናቱ ለተመረጡ ጤና ድርጅቶች በማሰራጨት የባለሞያዎችን የመመርመር ችሎታ ለመመዝንና እስላይዶች ደግሞ በመመርመር የጤና ድርጅቱንም ሆነ የባለሞያወን የጥራት ምዘና በማድረግ ወደፊት ለማሻሻል የሚያስችል ዘዴ ለመፍጠር ነው

ሁሉም በዚህ ማጠይቅ ወስጥ የምትሞልሳቸው መረጃዎች በማባ ማስጥር ሆነው ይያዛሉ ጥቅም ላይ የሚወሉትም ለጥናት ብቻ ይሆናል ቃለ ማጠይቁ በፈቃደኝነት ላይ የተመሳረተ ሲሆን እረሰዋም በጥናቱ ወስጥ የመሳተፍ ያለመሳተፍ መበተዎ የተጠበቀ ነው፡፡

ነገር ግን እርስዎ በጥናቱ ተሳትፈው የሚሰጠን መረጃ የጥናቱን አላማ ለማሳካትና ያለወን የቲቢ ሚይክሮስኮፒክ ናሙና ምርመራ ጥራት ማስ ለበሽተኞች አለስፈላጊ መድሐኒት እንድጠቀሙ ከማድረግ የጥናት አሰሳው ለወጥ ለማምጣት ከፍተኛ ጠቀሜታ አለው፡፡

አድራሻ ማወቅ ካስፈለገ

1. **ሕክምና ፋካልቲ፣ አዲስ አበባ ዩኒቨርሲቲ ድህረ ምረቃ ፕሮግራምና ምርምር የተባባሪ ዲን ቢሮ የመ.ሳ.ቁ. 9086 አዲስ አበባ ስልክ 251- 11-551-28-765**

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2.ደሴ ጤና ሳይንስ ኮሌጅ

ስልክ፡ 0331111981

3. **ዋና ተመራማሪው** መከተ ሙላት **ሜዲካል ላቦራቶሪ ሳይንስ ዲፓርትመንት፣ ክሊኒካል እና ፐብሊክ ማይክሮባዮሎጂ ትራክ፣ ህክምና ፋካልቲ፣ አዲስ አበባ ዩኒቨርሲቲ፣ ስልክ 091-051-93-08፣ ኢሜል** mekamsal@gmail.com

ከመጠይቁ በፊት የተጠያቂውን ስምምነት ማረጋገጫ ቅፅ

በጥናቱ ለመሳተፍ ፈቃደና ነዎት

1. አዎ ( ) 2. አይ / አልስማማም ( )

መልስዎ አዎ ከሆነ፣ ለጠያቂው መመለስዎትን ይቀጥሉ፡፡ በዚህ ጠቃሚ ጥናት ስለረዱን እናመሰግንዎታለን፡፡