ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF NURSING AND MIDWIFERY
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

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Performance of Tuberculosis Smear Microscopistson Fluorescent stained microscopy among Health facilities of Eastern Region of Oromia, Ethiopia

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A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES, SCHOOL OF NURSING AND MIDWIFERY, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT OF MASTER OF SCIENCE DEGREE IN CLINICAL LABORATORY SCIENCES (CLINICALLABORATORY MANAGEMENT AND QUALITY ASSURANCE SPECIALTY)

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This is to certify that the thesis prepared by **AmanNebiAyatoo**, entitled: To assess the performance of TB Smear Microscopists on Fluorescent stained microscopy among Health facilities of Eastern Region of Oromia, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical Laboratory Management and Quality Assurance Specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Lists of Tables

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Abbreviations

AFB Acid Fast Bacilli

AIDS	Acquired Immune Deficiency Syndrome
ANSV	Annual Negative Slide Volume
ART	Antiretroviral Therapy
ARTI	Acute Respiratory Tract Infection
DMC	District Tuberculosis Center
DOTS	Direct Observation Treatment Short-course
EQA	External Quality Assessment
FM	Fluorescent Microscopy
HFN	High False Negative
HFP	High False Positive
HIV	Human Immune Deficiency Virus
LED	Light-Emitting Diode
LFN	Low False Negative
LFP	Low False Positive
LPA	Line Probe Assay
LQAS	Lot Quality Assurance Sampling
MDR	Multi-Drug Resistant
MTB/RIF	Mycobacterium Tuberculosis/ Rifampicin
PLWHIV	People Living With HIV
PT	Proficiency Testing
QA	Quality Assurance
QC	Quality Control
QI	Quality Improvement
RBRC	Random Blinded Re-checking
RNTCP	Revised National Tuberculosis Control Program
STLS	Senior Tuberculosis Laboratory Supervisor
TB	Tuberculosis
WHO	World Health Organization
XDR	Extensive Drug Resistant

Abstract

Background: Tuberculosis (TB) is an infectious disease caused mainly by the bacillus Mycobacterium tuberculosis and it remains a major public health problem. Globally 10.0 people were ill and 1.3 million died of tuberculosis in 2017/18.In resource limited settings, tuberculosis diagnosis relies on sputum smear microscopy with many variables affecting the sensitivity and specificity.~~Tuberculosis is considered as a "global emergency" by the World Health Organization (WHO). Fluorescence microscopy (FM) can improve performance of microscopy and with the new light emitting diode (LED), technologies could be appropriate for peripheral settings.~~ The WHO defines External Quality Assessment (EQA) as a system for objectively checking a laboratory's performance using an external agency or facility. EQA participation is associated with improved laboratory performance over time and is a requirement for accreditation.

~~However, many professionals in Sub-Saharan countries including Ethiopia are unable to effectively implement quality improvement programs and many laboratories in Ethiopia remain without an accredited clinical laboratory.~~

Objective: To assess the Performance of TB Smear Microscopistson fluorescent stained microscopy among Health facilities of Eastern Region of Oromia, Ethiopia, 2018.

Methods: Afacility based cross sectional study design was conducted on 57 government Health facilitiesin Eastern Oromia Regional State from September to December 2018. ~~The study was included 57 governmental health facilities (42 health centers and 15 hospitals).~~ Panel slides were prepared and validatedat Adama Public Health Referral and Research Laboratory Centrecustomized checklist were used to conduct on site supervision and slides were collected for Random Blinded Re-checking by Lot Quality Assurance Sampling ~~Data were collected using the three external quality assessment methods.~~DataTechniques. Datawas~~were~~entered into EPI Info version 7 and transported to SPSS version 21.0 for analysis. The percentof agreements~~, differences~~ and different types of errors were calculated. The Sensitivity, Specificity, PPV, NPV of smear reading by TB smear M~~m~~icroscopist was calculated. The strength of an agreement among ~~between~~ participant readers and the reference readers were assessed using kappa statistics.

Results: In this study, the overall achievements during onsite evaluation were 62.7% and the overall agreement in reading among smear ~~between~~ m~~M~~icroscopists and Reference readers were~~92.21%~~92.21 %(kappa=0.755),which was good ~~agreement.Overall~~agreement. Overall Sensitivity and Specificity of detecting TB-FM bacilli were 90.54% and 94.03% respectively. The overall errors for panel test were 185(9.3%) which indicates25(1.26%) major errors,

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160(8.04%) minor errors. The overall Sensitivity, Specificity, PPV and NPV for blinded rechecking were 93.755.1%, 99.608%, 96.151.67% and 99.3547% respectively.

Conclusions and Recommendation: The overall performance of panel testing and random blinded re-checking were satisfactory for this study but the overall facilities assessment results for on-site evaluation were (62.53%) this need more effort to improve the performance. ErrorsBesides, the total errors committed were 9.3% and majority of them were minor errors and the presence of this errorscommitted areare alarming for TB control program and due attention should be given for FM EQA method since the method is applied recently and not familiarized as that of ZN method. This study recommended that, much effort should be made by laboratory professionals need to up grade their educational level to maximize the level ofagreement.method. Adama Public Health Referral and Research Laboratory Center and re-checking laboratories should give corrective actions such as, consistent refreshment training and regular supportive supervisions as per the EQA guidelines and further researches should also be conducted.

Keywords: ExternalQualityAssessment,TuberculosisAssessment, Tuberculosis and ,FluorescenceMicroscopy.,

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~~1.~~Introduction

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1.1 Background

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Tuberculosis (TB) is an infectious disease caused mainly by the bacillus mycobacterium tuberculosis. It~~is~~ remains a major global health problem, responsible ill health. It typically affects the lungs (pulmonary TB) but can affect other parts of the body as well (extra pulmonary TB).The disease is spread via droplet infection when people with pulmonary TB expel the bacilli while coughing~~,sneezing,talking~~, sneezing, talking,~~etc~~etc[1].

Despite the availability of highly efficacious treatment for decades, TB remains a major global public health problem and, it is one of the leading cause of death from a single infectious agent,ranking above HIV/AIDS [3]. According to WHO 2018 report, there were 10 million new cases of TB ~~(Range, 9.0-11.1 million), equivalent to~~ 133 cases ~~(Range, 120-148)~~ per 100,000 ~~population~~populations; among ~~these~~these, TB causes 1.3 million deaths ~~(Range, 1.2-1.4 million)~~ among HIV negative people and there were an additional 300.000 deaths from TB ~~(Range, 266,000-335,000)~~ among HIV positive people [2].

According to the Ethiopia Ministry of Health Hospital statistics data; tuberculosis is one of the leading causes of morbidity, the fourth cause of hospital admission, and the second cause of hospital death in our country [3].

~~According to the~~ WHO Global TB report 2018 indicated that~~,~~ Ethiopia ranked 11th from30 high TB burden countries in the world and Third ~~Second~~in Africa with an estimated TB incidence (all forms) of 164~~172~~ new cases per 100,000 populations and the prevalence ~~105~~ new smear pulmonary positive cases among children age above 15 and adults were 105cases per 100,000 populations [4].

To eradicate TB, early detection and treatment of smear positive TB is a top priority. Smear microscopy is still the most crucial test for the diagnosis of pulmonary TB, especially in countries with limited resources. Accordingly, smear microscopy quality assurance has been emphasized in the WHO Directly Observed and Treatment Short Course (DOTS) strategy [5].

~~TB Laboratory can be diagnosed using different methods bacteriological such as (Direct Light Smear Microscopy, Light Emitting Diode (LED) Fluorescent Microscopy, TB Culture, Molecular Line Probe Assay (LPA),Gene X-pert MTB/RIF) and Interferon Gamma Release Assay[6].~~Currently tuberculosis bacteriologically can be diagnosedusing different methods such as Direct Light Smear Microscopy, Light Emitting Diode (LED) Fluorescent,TB-culture,Molecular Line Probe Assay (LPA),Gene X-pert(MTB/RIF) and Interferon Gamma Release Assay[6].

Sputum smear microscopy is an important component of Directly Observed Treatment Scheme (DOTS) Strategy. It is recommended for detection of infectious TB cases and monitoring of treatment progress in countries with limited resources[7].

Sputum Smear Microscopy has been the corner stone of TB diagnosis and disease monitoring in Revised National Tuberculosis Control Program ~~me~~ (RNTCP).To achieve and maintain the national objectives of 85% treatment success rate among new Pulmonary Sputum Positive TB cases, and to achieve and maintain detection of at least 70% of such cases, quality assurance of Sputum Smear Microscopy is most immediate need[8].Currently, Ethiopia reports treatment success and case detection rates of 83 and 62 % of all forms of TB, respectively[9].

In countries with a high burden of TB, direct sputum smear microscopy remains the most cost effective tool for diagnosing patients with infectious tuberculosis and monitoring their progress on treatment. Quality assured laboratory services for sputum smear microscopy is crucial to deliver accurate, reliable and timely Acid Fast Bacilli (AFB) test results[10].

Accurate and reliable laboratory diagnosis will be provided if an efficient, excellent and quality assured system is maintained in the laboratory. Testing system must be monitored to ensure the quality of the overall process, to detect and reduce errors, and to improve laboratory performance across all the testing sites. External Quality Assurance (EQA) is a system designed to monitor the performance of laboratories in different methods by external body [11].

Microscopy errors are likely to result in failure to detect persons with infectious TB who will then continue to spread infection in the community or unnecessary treatment for' non-cases'.

Errors in reading follow- up smears can result in patients being placed on prolonged treatment, retreatment and in treatment discontinued prematurely [12].

EQA programsareneededto ~~be~~ensur~~e~~ing that smears are performed and stained properly, results are interpreted correctly and all microscopy centers achieve an acceptable level of performance. Effective EQA programs require dedicated and qualified staff for rechecking of smears. The implementation of EQA for microscopy has the advantage, not only of strengthening laboratory networks, but of improving diagnostic quality [13].

External quality assessment comprises proficiency-testing, blind rechecking of samples slides and on-site evaluation. Blind rechecking is the most widely practical and performed four times a year and sample slides are collected blindly by trained person or by TB supervisor using Lot quality assurance sampling (LQAS) method based on the Ethiopian national TB EQA guideline [10]. Proficiency Test (PT) is a process of sending stained and/or unstained smears from the ~~EQA~~External Quality Assessment(EQA) provider to participant laboratories to evaluate their proficiencyand each laboratory results are compared with those of other laboratories in the group or with pre-assigned value and the feedback reported to the participating laboratories [14].

On-site evaluation is one of the three components of External Quality assessment (EQA) methods other than panel and blinded recheckingthat conducted ~~at least once a month~~biannually by Senior Tuberculosis Laboratory Supervisor (STLS) at the District Microscopy Centre (DMC). It is the best method to obtain a realistic picture of the conditions and practices in the laboratory that was~~revealed~~revealedcomprehensive assessment of laboratory safety procedures, condition of equipment, adequacy of supplies as well as technical components of AFB smear microscopy like preparation, staining and reading of ~~smears[~~smears [15].

Light Emitting Diode (LEDs) provide a cheap and reliable light source with a more robust and long lifespan (>50,000 hours); additionally, no darkroom is required for their operation; LED microscopy has been shown to have equivalent specificity and improved sensitivity over conventional ZN microscopy [16].

Fluorescence microscopy (FM) using aura mine staining has been shown to have 10% higher Sensitivity and decreased the time required for sputum examination as compared to routine light microscopy using Ziehl-Neelsen (Z-N) staining, without compromising specificity in detecting

AFB in sputum smear microscopy [17]. Light-Emitting Diode Fluorescence Microscopy (LED-FM) for sputum smear examination is recommended by the WHO for detection of acid-fast bacilli in high TB burden countries [18].

There were selected TB rechecking laboratories in the country mandated by the regional health bureaus that act as controllers to re-read the blindly collected slides from smear microscopy centers and selected diagnostic health facilities mandated to diagnosis Fluorescent stained smear microscopy by the Regional Health ~~bureaus. As discussed above, TB was one of the leading causes of death among top 10 diseases in Ethiopia as well as in the world. Therefore, Fluorescent microscopy one of the recently used methods for TBdiagnosis among laboratories in high patient load to identify TB within a short time. Even though there was no locally conducted research on this method and to assess the current problems that were existed during the utilization of the method. Hence~~Bureaus. Hence, the main purpose of this research was to assess the performance of TB Smear Microscopists on Fluorescent stained microscopy among Health facilities of Eastern Region of Oromia, Ethiopia, 2018.

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1.2. Statement of the problem

As TB is a global threat these days, detection errors have serious ~~consequences.~~consequences. Though the methods were not similar,s~~S~~ome studies conducted in Ethiopia concerning EQA indicated that, even if the overall performances are acceptable, huge amount of minor ~~errors~~errors (17%) and Major errors (3.7%)were noted.Additionally,gaps were identifiedduring onsite evaluation~~on rechecking facilities; there was promising performance of those facilities~~

~~selected for rechecking services during decentralization [~~ the quality ofAcid Fast Bacilli (AFB) staining reagents was sub-standard in Public-Private Mix laboratories[13, 19].

In Ethiopia, the TB incidence rate was increasing from time to time, for example in 2013/14 Ethiopian Ministry of Health report there were 116,633 TB cases of all form with in a TB case notification rate of 133 per 100,000 population [13]. However, after five years that was in 2017/18 the rate increased by 0.91% and reached 117,705 within a TB case notification rate of 172 per 100,000 populations. MDR-TB cases increased from time to time and miss-diagnosis of TB patients as ~~TB negative~~No AFB (Acid Fast Bacilli)were reasons contributing to high TB incidence rate [3].

The optimum performance in diagnostic process is derived from skillful practice of a series of procedures that begins with sampling and carries through smearing, staining and grading. If there is a weak link in any of the procedures, then it is difficult to attain the desired performance. Identifying the problem area certainly makes the corrective action on easier tasks and ultimately, customizes training according to the needs of the diagnostic system. Even if ~~though~~, the method was not similar many studies conducted in Ethiopia ~~i~~ showed~~ndicated~~ that, a number of underperformance ~~in~~ in the quality of tuberculosis laboratories~~infrastructure~~, standard operating procedure, reagent utilization, equipment maintenance, data management, training issue, internal quality control and external quality control practices were the major gaps identified behind the underperformance of laboratory professionals [20].

Another study also revealed that the overall performance of the laboratories for smear microscopy reading and smear quality was poor. Moreover, irregularity of IQC measures, poor smearing techniques, poor smear thickness, poor evenness and size of smear were significantly associated with false negative ~~results. Moreover, laboratories with poor staining procedures had more likely to have false negative. At the same time, poor smear thickness resulted in having high chance of false negative. Those laboratories with no IQC measures had significantly higher false negative results. On~~results. On the other hand, absence of IQC measures andno previous participation in EQA programhad also statistically significant association with false positive results [21].

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Different researchers conducted different local studies concerning ZiehlNelson(ZN)~~N~~,this was the second study conducted concerning FM method next to Bahirdar Public Health Referral and Research LaboratoryCentrein line with Heal-TB using Random Blinded Rechecking as a pilot test before staining & after re-staining~~but there was only one study conducted using random blinded re-checking in Bahirder Public Health Referral andResearch Laboratory Center~~ [22],~~and this study was the second study conducted concerning FM method.~~ In light of the above facts, so far there was no narrated document on FM staining method that compressed three EQA methods regional as well as in our country. Therefore, this study tried to assess the performance of TB microscopist on fluorescent stained smear microscopy using three EQA methods.

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~~**In light of the above facts, so far there was no narrated document on FM staining techniques that compressed concerning on-site evaluation, panel testing and random blinded re-checking regional as well as in our country so, this study tried to assessthe performance of TBmicroscopist on fluorescent stained smear microscopy using Random Blinded re-checking, to assess the different gaps observed using on-site supervision regarding AFB Microscopy and to assess the performance of smear microscopies through paneltesting.**~~

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1.3. Significance of the study

The finding of this study~~results of this study will~~ provides significant and tangible information for different stakeholders about the performance of TB smear microscopists in the ~~laboratory so~~

~~that improvement projects can be planned and corrective measures can be taken.~~ laboratory. The~~In addition, recommendations that indicate the necessity of~~ implementation of continuous External Quality assessments aids ~~are given~~ to make the testing service and EQA program sustainable and reliable. Assessing the performance of Microscopists in TB detection can have significance importance for tuberculosis control activities and help to identify area of improvement for better and effective tuberculosis control program forORHB and FMOH

~~Therefore, assessing the performance of Microscopists in TB detection can have significance importance for tuberculosis control activities and help to identify area of improvement for better and effective tuberculosis control program for Ethiopian Ministry of Health.~~.Moreover,the result of this study ~~this study is the second one concerning FM methods in Ethiopia; this~~ can be used as source of information and baseline data for different researchers who are interested to conduct further studies on the method.

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2. Literature Review

2.1 Random Blinded Re-checking

A study was conducted between October 2003 and September 2005 in Limu, Peru ten peripheral laboratories performing routine acid fast bacilli to test whether external quality assessment rechecking of AFB smears becomes more efficient with stratified Lot Sampling of treatment follow up smears. In two consecutiveyears~~,a~~, a lot sample of 36 treatment follow- up slides and 24 diagnostic slides were randomly selected and blindly rechecked. A second controller determined the final result for discordant slides. Feedback was provided to laboratory technicians during supervisory visits. More false negative errors were found in follow-up slides than in tuberculosis suspect slides 25Verses 3.This represented a yield of 3.5% in 720 follow up slides and only 0.6% in 480 diagnostic slides. Positive predictive value was high in both years. Excessive workload seemed to preclude raising the level of routine performance. This showed that EQA with stratified lot sampling of treatment follow-up slides proved very efficient and

effective for identifying laboratories with substandard and performance in a setting with low positivity rates in routine diagnostic smears [23].

Another similar study was conducted in India in 2016 to evaluate the results of a pilot study, where the random blinded rechecking for the entire state of Delhi at a reference laboratory. Slides from 25 Revised National Tuberculosis Control Program designated districts (200 peripheral microscopy centers) in Delhi were re-read after proper coding by all the Senior Tuberculosis Laboratory Supervisors (STLS) at an intermediate reference laboratory under proper supervision. Of 12 162 re-read slides, 204 discrepant results were found. Of these, 150 (1.23%) errors were attributed to the peripheral microscopy centers and 54 (0.44%) to STLS. High false-positive errors were observed at a frequency of 12(0.1%), and high false-negative errors at a frequency of 38(0.31%).Minor errors, i.e., low false-negative, low false-positive and quantification errors, were observed at frequencies of respectively 68(0.56%),17(0.14%) and 15(0.12%).This concluded that greater stringency in the supervision of random blinded rechecking at the district level is essential to make smear rechecking more efficient and effective[24].

Another study conducted in India examined a total of 4799 centrifuged smears of sputum samples were stained by the auramine phenol method and examined using LED FM; 564 systematically selected smears were subjected to blind rechecking without re-staining by controllers within month. The initial results of the readers were compared to those of the controllers. Discrepancies were resolved by a referee. The quality of LED FM was assessed by the referee using the culture result as gold standard. Among the rechecked smears, 1 (0.18%) high false-negative error was made by a reader, while 1(0.18%)high false-positive error and 19(3.37%) high false-negative errors were made by the controllers. The errors were resolved by culture. Smear results for 18 slides were not available due to AFB fading [25].

A study conducted in China to assess the effectiveness of blind rechecking applied to FM From 2003 to 2010,of all smears were randomly selected, relabeled and assigned to laboratory technicians and re-stained and re-examined. Low false-negative (LFN) errors (0.10–0.27%) were within the critical values, at 85% (1 year) and 90% (7 years) sensitivity. However, LFN error (0.28–0.62%) among re-checkers was prominent. There were also low false-positive (LFP)(0.13–

0.75%), but subsequent cultures showed these to be mycobacterium culture positive. This relatively poor re-performance among the re-checkers might be due to background fluorescence increase after re-staining and/or in efficiency of the rechecking procedure [26].

Retrospective study was conducted in Taiwan to evaluate the quality of sputum smear microscopy in nine laboratories. Rechecking of 981 readable slides in 2005 identified 3 (0.3%) high false-negatives, 164(16.7%) low false-positives and 26 (2.8%) low false-negatives; after training provided the corresponding errors were 3 (0.3%), 278 (28.6%) and 12 (1.3%) for the 972 slides rechecked in 2006.The study showed that technical training and EQA improved the quality of sputum smear microscopy services [27].

Another cross-sectional study was conducted in Tanzania to assess the quality of sputum smear microscopy for AFB detection and 600 randomly selected slides from peripheral laboratories were blindly rechecked by intermediate and central laboratories. The overall agreement in reading was 89.2%. The finding showed that there were poor performances and activities should be done to improve the quality of the microscopy service [28].

A study conducted in Kinshasa, Democratic Republic of Congo to evaluate the impact of external quality assessment guidelines for resource poor settings. A total of 741 slides were collected from the peripheral laboratories by using EQA guideline and rechecked by the national reference laboratory, there were 77 (10.4%) discrepant results. Discrepant slides were sent to supranational reference laboratory, 67 (87%) of these discrepant results were attributed to the peripheral laboratory and 10 (13%) were attributed to the national reference laboratory. The study showed that blind rechecking allows an unbiased and representative evaluation of the quality of sputum microscopy and identification of underlying problems [29].

A study conducted in Ethiopia to examine the applicability random blinded rechecking of LED-FM sputum slides before re-staining and after re-staining. LED-FM sputum smear slides that was done and read by microscopic centers were stored for 3-5 months in closed slide box, the negative and positive slides were sampled, and re- read at Bahirdar Regional Laboratory Health Research Centre in blinded fashion. Among 625 slides (525 negative and 100 positive) read and the false positive (FP) and false negative (FN) rates before re-staining was 2.2% for both and after re-staining the FN become 3.1% and that of the FP 1.5%. Twelve slides were labeled as FN

during before re-staining reading and after re-staining the FN slides reduced to 8 slides. Two slides were false positive and it increase to 3 slides after re-staining. In the second phase of RBRC piloting 3513 negative and 191 positive FM-LED slides in 64 health facilities randomized and there was only 1 FN and 2 FP slides [22].

Even though, there were no similar study on the method we are using a cross-sectional study that was conducted in Ethiopia in Southern Nations, Nationalities and Peoples' Regional state (SNNPR) from October 2000 to June 2002 to see the quality of sputum microscopic examinations for AFB. Two thousand two hundred and nine slides [54% (1,184) positive and 46% (1,025) negative slides] were collected from the peripheral laboratories and reread by regional laboratory. The overall false reading was 3.2% and the overall agreement with regional laboratory was 96.8% showing the nearly perfect level of agreement [30].

Similar study was conducted in Ethiopia in the Eastern Amhara region to see the performance of laboratories on AFB microscopy from October 2010 to April 2011. Seven hundred ninety nine stained smears were randomly collected for rechecking and a set of ten panel slides were sent to 21 microscopic centers to evaluate reading, staining and reporting performance of individuals. Out of 799 randomly selected slides, the overall agreement was 98.4% and the overall false reading was 1.6 %. Panel test scores were 100 %, 80-95 %, 60 % performed by 9, 11, 1 laboratories, respectively. The finding showed that there was acceptable performance in majority of microscopic centers [31].

A cross sectional study was conducted from July 08, 2013 to July 07, 2014 on Tuberculosis Laboratory Diagnosis Quality assurance among Public Health facilities in West Amhara Region, Ethiopia indicated that among 201 laboratories enrolled in this study, 47 (23.4%) laboratories hadmajorerrors,41(20.4%) laboratories had a total of 67 false negative and 29(14.4%) laboratories had a total of 68 false positive results. Specimen quality, smear thickness and evenness were found poor in 134 (66.7%), 133 (66.2%) and 126 (62.7%) laboratories, respectively [21].

2.2. On-site evaluation AFB

A study was conducted in India to assess the facilities by using on-site evaluation method with three rounds from January 2005 to December 2010. The Gujarat district tuberculosis centers

(DTCs) achieved an overall score of 86% (820/957) during the initial on-site evaluation visits which consistently improved to 88% (842/ 957) and 92% (885/957) during the two follow-up on-site evaluation visits along with sustenance and improvement in many important laboratory parameters [32].

In a related study done in Ghana to assess the situation of TB microscopy centers in the country and a total of 114 laboratories were visited between 2000 and 2001 to assess the smear preparation and reading ability. The overall reading agreement rate in reading was 73%. There were 13% false-negative rates and 14% false-positive rates. Most of the false results were high false-negative and false-positive. The study indicated that the need to improve the quality of TB laboratory services and to establish quality assurance system in Ghana [33].

Another cross-sectional study was conducted in Tanzania to assess the quality of sputum smear microscopy for AFB detection and 600 randomly selected slides from peripheral laboratories were blindly rechecked by intermediate and central laboratories. The overall agreement in reading was 89.2%. The finding showed that there were poor performances and activities should be done to improve the quality of the microscopy service [34].

A facility based cross-sectional study was conducted from February 1 to April 15, 2015, on 56 peripheral TB laboratories of Sidama zone, Southern Ethiopia to assess the quality ofTuberculosis Laboratory showed that the overall performance of the tuberculosis laboratories was 48.9%.The lowest score was recorded on training status (19.7%) and the highest score was recordedonbiosafetyandwastedisposal(69.4%).Inadequate performance also identified in infrastructure (28.1%), Standard Operating Procedure's(49.5%),Reagents and Equipment (43.5%), Maintenance of microscope(42.9%),External Quality assessment (46.8%),Internal Quality Control (25.6%) and data management (57.1%)[20].

A facility-based cross-sectional study was conducted in select health institutions in Mekelle City, Ethiopia, using a simple random sampling technique,18health facilities were included in the study revealed that of the 18 laboratory facilities, only seven (38.9%) had a legible AFB registration book. In three (16.7%) of the laboratories, heat fixation was not applied before adding primary staining reagent. In 12 (66.7%), the staining reagents had precipitates. Two laboratories had microscopes with mechanical stages that could not move freely on both axes.

Seven (38.9%) of the laboratories reported samples to be negative before examining all required fields. Most laboratories, 16 (88.9%) and 17 (94.4%), respectively, did not run positive and negative controls after new batch reagent preparation [35].

2.3. Panel testing

A study was done in India to assess the proficiency of Senior TB Laboratory Supervisors and district level Laboratory Technicians in sputum smear microscopy by using proficiency panel testing and on-site evaluation from January 2005 to June 2009. High level performance in ZN smear grading was found in district laboratory staffs with overall agreement level of more than 98%. The study showed that laboratory supervisor's proficiency should be quickly assessed by different quality assurance systems of sputum smear microscopy. The proficiency of district laboratory staff readers' was high level of precision and excellent consistency [36].

A study was done in Mexico to evaluate the implementation of proficiency testing in conjunction with a rechecking system for external quality assurance in tuberculosis laboratories in 2001. Different types of errors were encountered during the panel testing process and most common type of errors was quantification errors, followed by low false negatives (with 4.1% HFN, 0.9 % HFP, 5.7% LFN, 2.4% LFP and 12.3% EQ) [37].

A prospective cross-sectional study was conducted in Ethiopia Southern Nations, Nationalities and Peoples' Regional state (SNNPR) to evaluate the quality of TB smear microscopic examination from April 23 to June 26, 2012. Eighty one participants were selected, 11(13.6%) correctly reported all panel slides, 70 (86.4%) missed at least one slides. A total of 29.75% (241/810) errors were reported that include major errors of 2.22% (13 HFN; 5 HFP) and minor errors of 27.5% (25 LFN; 60 LFP and138 QE). The sensitivity and specificity of participants in detecting TB bacilli as compared to the reference reading were 91.97 and 80.0 %, respectively. Overall agreement of participants with the reference reading on TB detection was 95.18% (Kappa = 0.73). Agreement of the participants with reference reading in the detection of TB bacilli was good [38].

Similar study was conducted in west Amhara to see the quality of sputum smear microscopy in Public-Private Mix Directly Observed treatment laboratories in July 2013. 370 AFB panel slides were distributed and the result showed that 3.5% false reading and 96.5 % agreement with

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reference reader (Kappa = 0.92). Moreover, the consistency of reading scanty bacilli slides was lower (93%) compared to 1+, 2+ and 3+ bacilli. Based on panel testing results, PPM-DOTS site laboratories showed good agreement with the reference laboratory [19].

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2.4 Conceptual framework

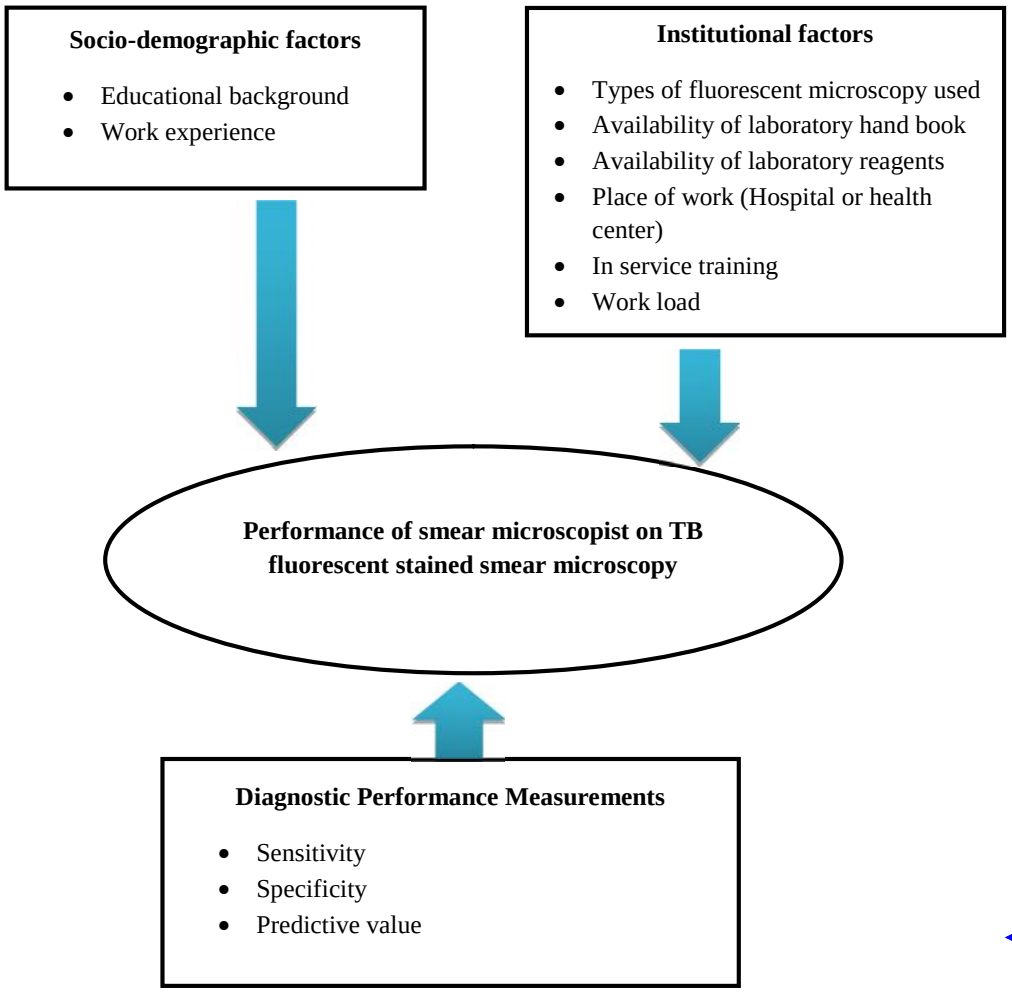

Figure 1: Conceptual framework of the study aimed to assess the performance of smear microscopist on TB fluorescent stained smear microscopy [21, 23]

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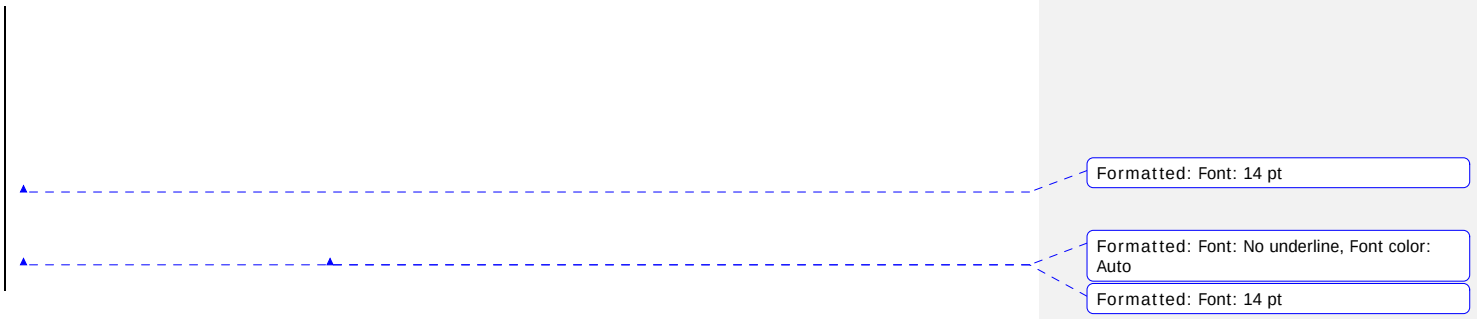
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3. Objective

3.1. General objective

To assess the Performance of TB Smear MicroscopistsonFluorescent Stained Smear Microscopy among health facilitiesin Eastern Region of Oromia, Ethiopia, 2018.

3.2 .Specific objectives

- To assess the performance of smear microscopist using Random Blinded Re-checking
- To identify the different gaps observed using on-site supervision regarding AFB Microscopy
- To assess the performance of smear microscopist through panel testing

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4. Hypothesis

HO: There is no difference among the performance of smear microscopists and reference readers on Fluorescent Stained Smear Microscopy among Health Facilities of Eastern Region of Oromia, Ethiopia

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5. Methods and Materials

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5.1. Study area

Oromia Regional state is one of the biggest ~~region~~Region among the nine regions ~~and two towns~~andtwo Administrative of Ethiopiawhich cover 359,619 square meters.Based on the

population projection undertaken in Ethiopia for all region at woreda level from 2014-2017,the region had an estimated population of 33,345,990 million ~~living in 20 zones and 18 special towns which is divided into 332 woreda(287 rural and 45 urban)~~ .The Oromia R~~r~~egional State Government health and health related profile had~~ve~~ one main Public Health Referral and Research Laboratory Centre, Two Sub-Regional Laboratory, Three Referral Hospitals, Three Teaching Hospitals, thirty Six General Hospitals, thirty eight Primary Hospitals and one thousand ten Health Centers .Among these~~isHealth Centers~~health facilitiesEeighty hundred sixty five were 865 ~~which is~~(79.36%)~~only~~ functional ~~by~~ now but~~and~~ the rest 226(20.7%)~~of them~~ was not ~~non~~ functional due to different reasons[39,40].

~~This study was conducted in~~Eastern ~~part of~~ Oromia Regional state ~~which~~ hadvef~~F~~our zone and four special town government administrative with a total population of 10,435,141[40].T~~The zonal health profile are One Referral Hospital, One Teaching Hospital, Seven General Hospitals, Six Primary Hospitals and Two Hundred Four Three diagnostics health centers. Among thus, t~~hise study was conducted on the health facilitiesthat was served as~~15 hospitals(1 Referral,1 Teaching Hospita,7 general and 6 Primary hospitals)~~ 15 that was served as TB EQA rechecking and 42 ~~-~~Diagnostics ~~HealthCenters~~Health Centers laboratories using Fluorescent stained smear Microscopy for TB case detection in Eastern Oromia Region which was included for TB diagnosis [41].

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~~**The study was conducted on laboratory professional found in Eastern Oromia regional state those using FM method for TB diagnosis which includedEast ,West Harage, East Shoaand ArsiZone. The Eastern Oromia region was the most populated regions among the Oromia region [40] and high number of health facilities found in the regions, and before now no similar study wasconducted. So that, this study was assisted to give clues on the performance assessmentof TB smear Microscopists on Fluorescent stainedSmear Microscopy among Health facilities of Eastern Region of Oromia,Ethiopia.**~~

5.2. Study design and period

A facility based cross sectional prospective study design was conducted from September to December 2018 G.C.

5.3. Population

5.3.1. Source population

All medical laboratory professionals working ingovernment healthfacilities utilizing fluorescent microscopy for TB detection in the~~The source populations were all smear microscopist working on fluorescent stained smear microscopy among Health Facilities of~~ Eastern Region of Oromia, Ethiopia.

5.3.2. Study Population

TB~~The study populations weres~~smear microscopist on Fluorescent stained smear microscopy among health facilities of Eastern Region of Oromia, Ethiopia, which fulfill the inclusion criteria.

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5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

TB Smear Microscopists on Fluorescent stained smear Microscopy among Health Facilities of Eastern Oromia Region, who were voluntaries to participate on the study, ever start participation on the TB-EQA and those available and use FM during study period was included in the study.

~~Smear microscopists on fluorescent stained smear microscopy among health facilities of Eastern Oromia regional state, Ethiopia.~~

~~*5.4.2. Exclusion Criteria*~~

~~Those TB diagnostic and rechecking laboratories that were providing diagnostic and rechecking service less than one year on the methods, participated yet on EQA and use ZN methods during the study period was excluded from the study.~~

5.5 Study variables

5.5.1 Dependent variables

- Performance of smear microscopist on TB F~~f~~luorescent stained smear microscopy.

5.5.2 Independent variables

- Educational background
- Work experience,
- Type of Fluorescent Microscopy used
- Availability of laboratory hand book
- Availability of laboratory reagents
- Quality of Laboratory reagents
- Place of work (Hospital or Health center) and
- In-service training on TB smear microscopy ~~of EQA Centers and diagnostics health facilities laboratories~~ was independent variables.

5.6. Measurement and Data collection

5.6.1. Sample size ~~calculation~~***calculation for panel testing***

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The sample size was calculated using single population proportion formula

$N = (Z\alpha/2)^2 \times P(1-P) = (1.96)^2 \times 0.5(1-0.5) = 384$

$d^2 0.052$

Where: n= sample size

Zα/2 = Z value at 95% CI [1.96]

p = prevalence = 0.5

d = Margin of error tolerated is (0.05%)

Adding 10% of non-respondent rate, the final sample size was 384 + 38 = 422 but the real data collected from Oromia Regional Health Berous human resource department indicated that there were 218 laboratories professional in the eastern Oromia Regional State in the FM site so that, all of them were considered as study participants.

5.6.2. Sample size calculation for Random blinded Re-checking

The sample size was determined based modified statistical sampling method called LQAS

It depends on

- Total annual negative slides processed previous year(ANSV)
- Slide positivity rates(SPR)
- Sensitivity (expected performance)

SPR=Total positive slide *100

Total slides processed in the previous year

Then, using table annexed in the annex part by considering Annual Negative Slides Volume and SPR on the column and row we got the number of total slides in the quarter both negative and positive.

~~The sample size of the study participant for panel slides participants by aiming to point out the determinate factors for the outcome of panel was determined by using single population proportion formula by considering P=0.5 since no such study was not conducted before in our setting. Level of significance = 0.05 Marginal of error (d) = 5% Z-score at 95% confidence interval = 1.96.The formula for calculating the sample size was~~

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$$(n)=\frac{Z(\alpha/2)^2 p(1-q)}{d^2}$$

$$=\frac{(1.96)^2 * 0.5(1-0.5)}{(0.05)^2}$$

$$=\frac{0.9604}{0.0025}$$

= 384.16, consider 10% non- resposerate 384+38=422 ~~Using correction formula, to adjust the sample size for small population.~~

~~So the above sample was taken from a relatively small population (let say N = 500) , the required minimum sample was obtained from the above estimate by making some adjustment (if the population is less than 10,000 then smaller sample size may be required).~~

~~nf= n~~

~~1+n~~

~~N~~

~~384~~

~~1+384~~

~~500~~

~~384*500~~

~~884~~

~~=217~~

~~Data fromOromia Regional Health Bureau Human resource and capacity building department indicated, the Eastern Oromia Region had 218 Laboratory Professional from recent profile of 2018 man power report and this data almost all similar to the number we calculated by correction formula .Then, the study participants for this study was, all the Laboratory professionals included in the study.~~

5.6.~~3~~2 Sampling methods

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- ✓ Convenient sampling technique wasused for both panel testing and on-site supervision to select a total 57 health facilities of Eastern Oromia Regional state.
- ✓ Systematic Random sampling method was used to collect the valid slide for Random Blinded Re-checking.

~~**All, 15 Hospitals and 42 Health Centers who were using FM method for TB identification was included in the study. For Random Blinded RecheckingLot Quality Assurance Sampling (LQAS)method was put into practice for collecting blindly all necessary sample slides with the assumptions of 95% CI, Zero acceptance number, 100% of Specificity and 80% of Sensitivity as per National TB EQA guidelines. Convenientsampling techniquewasused for validated panel testing slides distributionwhereas,On-site evaluation was conducted by structured checklist adopted from Adama Public Health Referral and Research Laboratory Centre (APHRRLC) and different reviewed literature.**~~

5.6.~~4~~3 Data collection Procedures

Data was collected from 57Health facilities in Eastern Oromia Region, Ethiopia using three External Quality Assessment (On-site evaluation, Blinded rechecking and Panel testing) used as data collection tools(Annex-I)

5.6.~~5~~4Laboratory Testing Procedure

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5.6.54.1.Random Blinded Rechecking

All hHealth facilities stored all slides sequentially in the provided slide boxes in the same order as they were listed in the laboratory register based on Ethiopian national TB-EQA guideline. Sample slides were collected blindly by trained laboratory personnel for this study using Lot Quality Assurance Sampling (LQAS) methods. Sample size (volume of slides) was determined based on modified statistical sampling method called Lot Quality assurance sampling (LQAS). This depends on the Annual Negative Slide Volume and Slide Positivity Rate of the previous years. After calculating the sample size collection of slide was undertaken with laboratory personnel or Woreda TB/Focal Personnel .The collected slides were put into an envelope and then writing the result and number of slide packed on the top of the envelop .Name, Quarter and Year of the respective laboratory was marked clearly at the back of the envelop and sealed finally before sending.After the slides arrived to rechecking laboratory TB-EQA laboratory Quality Officers or designated personnel handover the slide by checking the slide numbers with the hard copy result. Then, EQA site distribute the slide for assessment or rechecking to controllers as soon as all networked health facilities brought EQA slides. Controllers recheck slides using quality indicators criteria and write result of the slides and gap identified and strength observed during assessment on the provided format. Discordant slides between 1st controllers and examinees, re-read by 2ndcontrollers. Discrepant readings between the peripheral laboratory reading and the second reader were re-read and verified by third controllers. Finally, corrective action for the identified nonconformance and monitoring of the potential source of errors were investigated during the feedback visits by the regional laboratory personnel at site level (Random blinded Re-checking Annex-1C)[6].

5.6.54.2. On-site Eevaluation

On-site evaluation was conductedusing structured checklist customized from Adama Public Health Referral and Research Laboratory Centers (APHRRLC)
by senior laboratory professional who were technically equipped and took training on AFB Microscopy as data collectors from Zonal laboratoryfocal. The checklist contained:-Facility and ~~This was conducted using the structured checklist to assess the three phases of the Laboratory workflow and overall working condition.That wasInfrastructures, Standard~~Safety, Standard

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OperatingProcedures(SOP),Equipment's and Reagents, Reagent, Personnel and Training, Bio-safety and Waste disposal system as well as, Internal Quality Control(IQC) and External Quality Assessment on TB smear Microscopy(Annex-IB: On-site evaluation)[43].

5.6.5.4.3.Panel Testing

Specimen of known positiveand negative results was used to produce a collection positives slides with a consistent, determinedquantity of AFB per slide, as well as negatives withauthentic background material. Validation of the panel slide was required for ascertaining consistency of slides priors to use them. Alllaboratories involved in panel testing were received a standardized set of slides to minimize variation in expected results due to differences in smear consistencyproficiency testing(annex-IA)

Left over sputum samples were collected from Asella Teaching Hospital Laboratory for Gene-Xpert testing andtransported toAdama Public Health Referral and Research Laboratory Centers (APHRRLC) to inoculate on LJ (Lowenstein Jensen) and MGIT (Mycobacterium Growth Indicator Tube) to confirm for TB before using for panel slide preparation.After the samples were to be negative both by Gene-Xpert and Culture for MTB panel slides were prepared using negative and positive stock according to the PT manual in the annex-I by trained TB-Expertise laboratory professionals on panel slide preparation at APHRRLC in the Bio-safety cabinet at TB-Laboratory. Validation of panel slides were done every batch50-100 slides by taking 6slides and staining FM staining reagent to confirm the consistency among the panel slides by three Senior Laboratory Professionals. Prepared panel slides were dry by air in the Bio-safety cabinet. Thenwas doneby principal Investigator (PI) using electronic heating at 80^{o}C at for minute. After fixation 50%,slideswere stained with Auramine O and the rest 50% were unstained. The stained and unstained panel slides were stored separately in closed slide box and distributed to participating laboratories in line withAPHRRLC performs different activities by Zonal Laboratory Focal Personnel. Result of the panel slides were through postal office, direct handover to nearby EQA sites and couriers.Panel slides result were declared to panel participating laboratories through principal Investigator E-mail address until final feed-back result was aggregated and communicated to all participating health facilities.

~~***Those patient samples collected for routine TB diagnosis at Asella Referral and Teaching Hospital Laboratory by Gene X pert was used anonymously diagnosed to be negative by G***~~

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~~*expert for TB bacilli and then, sent to Adama Public Health Referral and Research Laboratory Centre for TB culture. Then after, it was cultured both on MGIT (Mycobacterium Growth Indicator Tube) liquid media and LJ (Lowenstein Jensen solid media) and read after 6 month and 8 month of cultivation respectively to confirm whether or not it was negative or positive for TB bacilli growth. However, positive sample for panel testing slide preparation was collected from sample leftover at Adama Public Health Referral and Research Laboratory Centre that was referred from health facilities for different laboratory test .So, after confirmed the bacilli load it was killed the bacteria by heating using autoclaving over the night and ,then panel slide preparation was done in collaboration with TB Expertise trained On PT slide preparation at APHRRLC and finally, panel slides were validated every 50-100 slides per batch and quantified by three senior laboratory technologists that were expertise on TB at Adama Public Health Referral and Research Laboratory Center). Specimen of known positive and negative results was used to produce a collection of positive slides with a consistent, predetermined quantity of AFB per slide, as well as negative slides with authentic background material. Validation of the panel slides was required for ascertaining consistency of slides prior to use them. All laboratories involved in panel testing were received a standardized set of slides to minimize variation in expected results due to differences in smear consistency (Annex IA: Proficiency Testing [43].*~~

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5.7~~Smear~~. SmearPpreparation and Sstaining

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Place 3ml of AFB positive specimen into a 50ml screw cap plastic tube, add one drop (approximately of 40% Formaldehyde per 1ml of sputum, vortex well, incubate for one hour at room temperature(25-30^{o}C),add 1ml of 4% NaOH, vortex thoroughly for 4-5 minutes, add up to 20ml of distill water, mix well, incubate in a water bath for 30 minutes at 56-60^{o}C,mix occasionally by inverting the tube during incubation, add distill water to a total volume of 40ml,mix by inversion; centrifuge @ 3000g for 20 minutes at room temperature (25-30^{o}C),decant the supernatant carefully; add 0.5-1ml of distill water to suspend pellets.

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Negative specimens (fresh specimens ,no more than 2 days olds ,will be preferred) which will be 5 ml or above, white to green, an AFB negative specimen with 20 or more white blood cells per field and the thickness of sputum will be watery(less mucous) specimens will be preferred to

increase consistency. Distribute 3-4 ml aliquots of AFB-negative sputum into 50 ml screw cap tubes, several good quality negative sputa will be pooled together and then split into 3 ml aliquots, add 1 drop (approx. 50 µl) of 40% Formaldehyde per 1 ml of sputum, vortex well, incubate for 1 hour at room temperature (25-30^{o}C), Add 1 ml of 4% NaOH (if the sputum is too thick, add up to 2 ml of NaOH solution that the final concentration of NaOH is always 1-2%), Vortex for 2-3 min, add up to 20 ml of distilled water, mix well, and incubate in a water bath for 10 min. at 55-60^{o}C

Concentrated smear for the auramine-O phenol method wasill be prepared from the sediment by taking1-3 drops with a wire loop, and smearing it on a microscope slide and dried in the air. In LED-FM smear staining the prepared slides by direct methods wasill be placed on a staining rack and electronic fixationheat fixed and stained with auramine-O phenol stain for 20 minutes, then rinsed briefly with gentle stream of water, flooded with 0.5% acid alcohol for 3 minutes then rinsed with water and counter stained with 0.51% Potassium Permanganate for 1 minute (Annex-III: SOP for FM Method) and (Annex-IV:SOP: SOP for FM reagent preparation)[42].

5.7.1Examination. Examination and Ggrading Ffluorescent Sstained Sslides

Fluorescent stained slides were examined using a light emitting diode (LED) fluorescent microscopy (primo star iLED, Zees Micro Imaging, Jena, Germany) with a 40× objective under a standard fluorescence UV filter viewing at least 40 fields. The tubercle bacilli seen as yellow luminous organisms in a dark field and the results were graded and recorded in a defined manner as per the guidelines of International Union Against Tuberculosis and Lung Disease(IUATLD) in a defined manner as per the guidelines of IUATLD [44] as well, in both cases smears were examined separately by two laboratory technologist without knowing the results of each other and in case of discrepancies smears were re-examined by the principal investigator.

Table 1: Validation logbook NaOH panel prepared slide (N=2180) at Adama public health referral and research laboratory center, Adama, Oromia region, Ethiopia, September-December, 2018.

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AFB Grade	Average Slides Taste Results									
	1	**2**	**3**	**4**	**5**	**6**	**Mean**	**Sum^{2}Diff**	**SD**	**M-2SD**
3+	120	125	127	129	122	125	124.6	147	5.4	115.2>0
2+	60	63	65	70	62	59	63.1	79	3.97	55.06>0
1+	150	190	165	220	210	140	179.1	5321	32.62	113.76>0
Scanty	15	19	25	12	10	8	14.8	199	6.31	2.38>0
Negative	0	0	0	0	0	0	0	0	0	0

If M – 2SD is > 0, then consistency is True (Sufficient)

Smear results: +3: >100 AFB/ field on average, +2: 10-100 AFB / field on average, +1: 30-1899 AFB / 30 fields, scanty: 1-29 AFB /30 fields and negative no AFB/ 30 fields.

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5.8~~Panel~~.Panel~~S~~lides Composition ~~a~~And Interpretation

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A set of 5 stained and 5 unstained validated panel slides were given for each microscopist and 50-70 minutes were allowed to complete the reading [44,45]. The panel composition and load of bacilli based on their grading were 1 slide 3+, 1 slide 2+, 1 slides 1+, 2 slides 1-29 /30 fields and 5 negative slides [44]. The resultwas ~~s were~~ expressed as correct, minor error or major error. Major errors were classified as high false positive (HFP) if a negative smears misread as 1+ to 3+ positive and high false negative (HFN) if a 1+ to 3+ positive smears was misread as ~~negative.Minor~~negative.Minor errors were classified as, low false positive (LFP) when a negative smears was misread as scanty (1-29 AFB/30 field) and low false negative (LFN) when a scanty (1- 29 AFB/30 field) was misread as negative andquantification error (QE) when there was a difference of more than one grade in a reading of positive smear between examinee and controller is indicated in Annex-V: Panel slide option [46, 47].

Scores for Grading

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A set of 10 panel slides, each slide worth 10 points, total possible scores were 100 (for 10 slides) and the passing score were 80 % and above. Committing major errors like high false positive (HFP) and high false negative (HFN) worth zero point whereas minor errors like low false

positive (LFP), low false negative (LFN) and quantification errors (QE) worth 5 points **(**Annex-VI: Evaluation and interpretation of errors) [44, 45].

Reading

Examine one length of an auramine slide with a fluorescent microscope, using 200–250x magnification, to cover 30 fields in one length, equivalent to 300 fields at 1000x magnification. Alternatively, 400 x magnifications were used, covering 40 fields at this magnification. The results were recorded in the TB laboratory register, and recorded on the sample examination request form as well as forwarded to the person requesting the sample examination(Annex-VII: Reading and Reporting Microscopy smears)[43].

Slide storage

AFB smears were stored in consecutive numerical order without separating positive and negative smears. Blinding and re-storage was performed by an un-blinded principal investigator before dispatching the panel slides to health facilities.

Feedback Report

Feedback was sent back to participant laboratories through my email address after receipt of results. It was included both individual laboratory results, as well as aggregate performance for all laboratories. Reports were also included comments on performance, possible sources of errors and suggestions or requirements for remedial action [43].

5.9. Data Quality Assurance

~~The quality of data was checked by reviewing the checklist for coherence and completeness. In addition, the instrument is designed to measure all components of the variables and also use an existing instrument that is already tested in other studies and calibration/standardization of the measuring instrument is help to improve the validity of the study.~~Pre-testing of checklist for on –site evaluation was done on one Referral Hospital (Shashamane) and One Health Centers (ArsiNagelle) which is found in the West ArsiZone . The quality of data was checked by reviewing the checklist for coherence and completeness. Trainingwasgiven for data collectors

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and supervisor for one day on the data collection and managing systems by principal investigator.

~~*To ensure reliability during data collection, different strategies was employed.First, the measuring instruments were standardized. Second, four zonal laboratories focal personnel were trained for one day and participated as data collector. There was also daily supervision by the investigator during the actual measurements. In order to test the reliability of the instruments, internal consistency techniques wasapplied using Cronbach`s Alpha (Table 2).*~~

~~5.~~10.9.1. ~~Data~~ Data Entry, Storage and Management

All data were entered to E~~P~~PI Info version-7 and quality of the entire data was checked before doing the analysis for reliability and completeness. The datawas exported and analysis was performed using SPSS version 21.0.

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5.110. Data analysis and interpretation:

The percentage of different types of errors, Sensitivity, Specificity, Predictive Value ~~Positive predictive value (PPV), Negative predictive value (NPV)~~ of smear reading among~~by~~smear microscopist and reference readers~~TB EQAsmearmicroscopist~~ were~~as~~ calculated. The strength of an agreement among ~~between~~ participant readers and the reference readers were assessed using K~~k~~appa statistics.

5.121. Ethical considerations

~~For panel preparation, patient's leftover samples were collected from AsellaReferral and Teaching Hospital anonymously. All information of each TB EQA Centers and Diagnostics Health Facilities was kept confidential and used only for the study purpose and for improvement of AFB microscopy. The research proposal was evaluated and approved by the research and ethical~~Ethical clearance and approvalwas obtainedfrom~~committee of~~ Addis Ababa University college of Health Sciencesdepartment of Medical Laboratory Science ~~andreviewed and cleared by Institution of~~ Review Board Committee (~~I~~RBC)~~before the start of fieldwork.~~Permission letterwasoffered from ~~Addis Ababa University School of Allied health, Department of Medical Laboratory Sciences~~ORHB. ~~writes support letter to Adama Public Health Referral and Research Laboratory Center for the purpose of panel slide preparation and batch validation.~~Adama Public Health Referral and Research Laboratory Center wrote~~official letter~~ letter of support to respectiveZonal Health Office~~office~~ for their good cooperation and the Zonal and Woreda Health Office wrote similarly to the respective health facilities.~~Finally, before starting the data collection ethical clearance was obtained from each respective health facilities.~~

5.132. Dissemination of the result

First, final result of this paper will be forwarded to Addis Ababa University, Departmentof Clinical Laboratory science and will be presented as a thesis for partial fulfillment of MSc degree in Laboratory Management and Quality Assurance Specialty ~~Track. The~~Track. The results of this paper will also be disseminated to relevant bodies/stakeholders including the Federal Ministry of Health, Oromia Regional Health bureau, Adama Public Health Referral and Research Laboratory Center, and NGOs working on the program. Finally, efforts will be madeto

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disseminate to different stockholders by presenting in different scientific seminars, conferences, workshops, and will be published in reputable scientific journals.

5.143. Operational definitions

Random blinded rechecking: Is re-reading statistically valid samples of slides from peripheral sites and intermediate laboratory by controllers at a higher level to assess whether that laboratory has an acceptable level of routine performance or not.

High False Positive (HFP): A negative smear that is mis-read as 1+, 2+ to 3+ positive.

High False Negative (HFN): A 1+, 2+ to 3+ positive smear that is mis-read as negative.

Low False Negative (LFN): A scanty (1-29 AFB / 30 fields) smear that is mis-read as negative.

Low False Positive (LFP): A negative smear that is mis-read as a scanty (1-29 AFB / 30fields).

Major error: indicate gross technical deficiencies, and include both High False Positive and High False Negative errors.

Minor error: Occurrence of Low False Positive, Low False Negative or quantification errors.

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

Panel Testing: Sending stained and/or unstained smears from RRLsRegional Referral Laboratory (RRL) to Microscopic Centers(MCs) to check proficiency in AFB smear microscopy reading and reporting.

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

Scanty (Low Positive): Term used in this document to describe 1-29 acid-fast bacilli per 30 fields. 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 (diagnostic and monitoring) within a microscopy laboratory over a defined period of time.

Diagnostic laboratoryis alaboratory where clinical pathology tests arecarried out on clinical specimens to obtain information about the health of a patient to aid in diagnosis, treatment, and prevention of disease.

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6. Results

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In the current study,a total of 199participants involved in this study the response rate was 91.3% (199/218).

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~~In this study, 57 governmental health facilities were participated. Among them 42 of them were health centers and 15 of them were hospitals. Inthese health facilities 218 selected laboratory professionalswere found, among thus, 199 of them were participated in thestudy that makes the response rate to be 91.3%.~~

6.1 Socio-demographic characteristics of study participants

~~In this study, the majority~~Majority of the study participants 114(57.29%) of laboratory professionals were BSc holders. About 85(42.71%) of study participants had more than 9 years of working ~~experiences,while~~experiences, while the rest 67(33.67%), 29(14.57%) and 18(9.05%) had 6-8 years, 3-5 years and less than 2 years of working experience respectively(Table 2).

Table 2: Socio-demographic characteristics of laboratory professionals and profiles of their laboratories of Eastern region of Oromia, Ethiopia, February 2019

Variables	Level	Frequency (N=199)	Percentage

Educational background	MSc	2	1.0.0
	BSc	114	57.3.29
	Diploma	83	41.71.71
Working experience	≤ 2 years	18	9.0.05
	3-5 years	29	14.69.57
	6-8 years	67	33.767
	≥ 9 years	85	42.771
Trained on TB smear microscopy on FM	Yes	190	95.4.48
	No	9	4.64.52
Types of FM Microscopy used	LABO	2	1.0
	Primo Star	55	99.0

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6.2 On-Site Evaluation

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Structured checklist ~~that wasalready adopted~~was customized from Adama Public Health Referral and Research Laboratory Center was used to assess the status of facility and safety, SOP, job aids and standardized laboratory request form, adequate supply and availability of staining equipment and reagent for the ~~quarter,~~quarter, Bio-safety and waste disposal as well as internal quality control and external quality assessment.

6.2.1 Facility and Safety

In this study, 33(5~~8~~7.9%) of the laboratories had separate area for TB lab working, while,2~~0~~3(42.~~1~~%) didn`t have and from these laboratories 23(40%.~~4~~) had separate table for specimen receipt or smear preparation and microscopy reading and the rest 34(60.~~6~~%) did not had. All (100%) of rechecking and diagnostics laboratories had continuous power and running water supply. Almost all 56(98%) of laboratories had waste containers with lid /color coded/labeled for infectious and non-infectious. Segregation was the most common mechanism of waste disposal practiced by all (100%) of rechecking laboratories. About 55(96.5%) of health facilities used personal protective equipment's and only 8(14.03%) of these laboratories were cross-ventilated~~. Incineration was the most common method of waste disposal among all laboratories~~(Table 4).

6.2.2 SOP, Jjobaaid and Sstandardized laboratory request form

About 23(40.2%) laboratories had microscopic preventive maintenance regularly done and documented and SOP for smear preparation, staining and reading of TB microscopy. The majority, 50(8~~8~~7.9%) of health facilities had recent WHO grading chart for TB reading smear microscopy, job aids posted and used. Among health facilities 54(~~99~~958.5%) of them had standardized TB registration logbook and request form and more than three-fourth, 45(7~~9~~8.95%) of health facilities were not monitored TAT within 8 hours (Table 4).

6.2.3 Adequate supply and Aavailability of staining reagent and equipment for the quarter

Among health facilities all (100%) of them had frosted slides, filter paper, sputum cup, sprit lump, disinfectant, smearing and staining equipment, slide box, aura mine (0.1%),0.5% acid alcohol, (0.5%)potassium permanganate, sufficient ~~and~~slide box and there were no supply inconsistency during the study period. Among 15 hospitals, 10(6~~7~~6.6%) of the rechecking

laboratories didn`t had distilled water and 13(86.7%) rechecking laboratories didn`t had equipment's for preparation of stains reagent such as weighting balance and measuring cylinder. In this study,41(71.9%) of health facilities had FM working reagent prepared based on the working load, labeled and stored properly away from direct sunlight at correct place (Table 43).

6.2.4 Internal and External quality assessment

During this assessment, 31(5454 .3%) of health facilities had known negative and positive controlled slide, and 29(551 0.7%) of health facilities didn`t used IQC for new batch of stain.Amongstain. Among these health facilities, 36(6363 .3%) of IQC didn`t done at least once per week to check quality of stain. Among 15 rechecking laboratories sites in the Eastern Oromia region 2(1313 .33%) didn`t stored slide during On-site evaluation TB EQA (Table 4)

Table 3: Summarized on-site evaluation results of TB EQA rechecking and diagnostics laboratories with standardized checklist in Eastern region of Oromia, Ethiopia, February 2019.

	Items	Level	Frequency (N=57)	Percentage
Facility and Safety				
Separate area for TB lab working		Yes	24	42.1.1
		No	33	57.97.9
Separate table for specimen receipt or smear preparation and microscopy reading		Yes	23	40.40.4
		No	34	59.660.6
Power supply		Yes	57	100
Running water supply		Yes	57	100
Availability of waste container with lid/check color coded/labeled for infections and non-infections		Yes	56	98.2.2
		No	1	1.81.8
PPE used and practiced		Yes	55	96.596.5
		No	2	3.5
TB class rooms are cross-ventilated		Yes	8	14.0
		No	49	86.0
SOP, job aid and standard lab request format				

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Microscopic preventive maintenance regularly done and documented	Yes No	23 34	40.44 59.659.6
SOP for smear preparation, staining and reading of TB microscopy	Yes No	23 34	40.44 59.659.6
Grading chart TB smear microscopy, job aids posted and used	Yes No	50 7	87.77.7 12.33
Standardized TB registration book and request form used	Yes No	54 3	94.789.6 5.31.4
TAT monitored entioned within 8 hours	Yes No	12 45	21.1.05 78.978.95
Supply and Availability of Staining Reagent and Equipment for the Quarter			
Frosted slide, Sputum cup and Filter paper	Yes	57	100
Sprit lump, disinfectant and smear staineding equipment	Yes	57	100
Slide box, auramine (0.1%) and(0.5%) acid alcohol	Yes	57	100
0.5% Potassium permanganate	Yes	57	100
Distilled water	Yes No	12 45	21.11 78.98.9
Equipment for preparation of stains reagent such as weighting balance and measuring cylinder (EQA) slide only	Yes No	13 244	86.722.8 13.377.2
FM working reagent prepared based on the daily working load, labeled and stored properly away sunlight at correct place EQA	Yes No	41 16	71.91.9 28.18.1
Internal Quality Control and External Quality Assessment			
Lab had known negative and positive controlled slide	Yes No	31 26	54.44 45.645.6
IQC used for new batch of stain	Yes No	28 29	49.149.1 50.90.9
IQC used for at least once per week to check quality	Yes	21	36.86.8

of stain		No	36	63.2~~63.2~~
Lab participated on TB EQA Hospital	On-site Evaluation for	Yes No	13 2	86.7~~6.7~~ 13.3~~3~~
Lab participated on TB EQA RBR for Health Centers		Yes No	21 21	50 50
Lab documented EQA feedback		Yes	57	100

Generally, during on-site evaluation, 73.9% of laboratories had adequate facility and safety, 55.83% of them had SOP, job aid and lab request form, 59.4% of them had adequate supply and availability of re-agents and equipment's and 61.72% of them had practices of IQC and EQA (Figure 2).

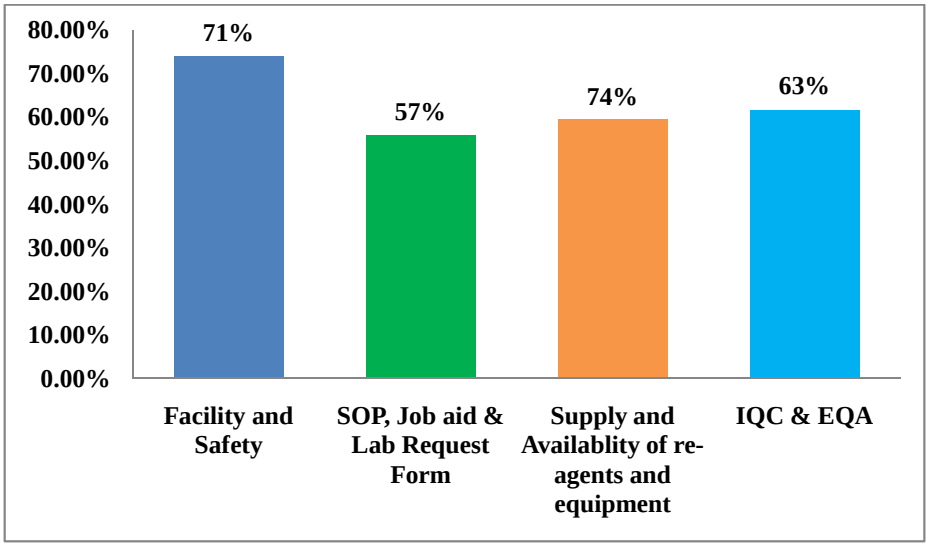

Figure 2~~:Summarized~~: Summarized on-site evaluation results of TB EQA rechecking and diagnostics laboratories with standardized checklist in Eastern region of Oromia, Ethiopia, February 2019.

6.3 Panel Testing

Based on Ethiopia TB EQA guidelines [43],~~performance of participant in proficiency testing should be ≥80% to get a passing score.Among 199 participants, the overall performance of the study participants showed that 96(51.9%) participants correctly read all the ten slides, 65(35.14%) got 80-85% and the rest, 24(12.97%)participants scored 85-95% .The performance of the participant based on their health facility showed that; Hospital laboratory professionals achieved the passing score (≥80%) than health centerlaboratory professionals. On the other hand, the number of laboratory professionals that scored≥ 80% were higher in MSc and BSc holders than diploma holders were.~~in this study, the overall performance of professionals scored ≥80% was 165(83%).The overall sensitivity and specificity in detecting TB bacilli were 90.54 and 94.03 respectively and the rest 34(17%) was below the 80%.The overall percent agreement of participant readers with reference readers were 92.21%(Kappa=0.755) which was god agreement.125(63%) of the participants were correctly read all the ten slides,23(12%) got 90-95%,17(8%) participants scored 80-85% and the rest 34(17%) scored below 80%

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~~A total of 1990 validated slides were read by study participants and the overall Sensitivity and Specificity of in detecting TB bacilli were 90.54% and 94.03% respectively.~~

~~The overall all percent of agreement of participant readers with reference readers were~~**~~92.21~~**~~%(kappa=0.755). Percent of the health center readers with reference readers were~~ **~~83.85~~**~~%(kappa=0.71) slightly lower than Hospitals readers~~ (table.4).

Table4: OverallSensitivity, Specificity, Predictive Values and Agreements of participants with reference readers in detecting TB bacilli in health facility of Eastern Region of Oromia, Ethiopia, February 2019.

Facility Laboratory Professional	Reference Readers				Sensitivity	Specificity	PPV	NPV	Percent Agreement	Kappa
		Positive ~~*1.1.1*~~	Negative	Total						
Hospital	Positive	657	16	673						
	Negative	35	638	673	94.94	97.55	97.62	94.79	96.21	0.80
	Total	692	654	1346	~~*1.1.2*~~	~~*1.1.3*~~	~~*1.1.4*~~	~~*1.1.5*~~	~~*1.1.6*~~	~~*1.1.7*~~
Health Centers	Positive	281	41	322						
	Negative	63	259	322	81.69	86.33	87.27	80.43	83.85	0.71
	Total	344	300	644						
Overall	Positive	938	57	995						
	Negative	98	897	995	90.54	94.03	94.27	90.15	92.21	0.755
	Total	1036	954	1990						

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From a total of 1990~~examined~~ slides examined there were 185(9.3%) total errors~~committed~~committed. From these~~,~~25(2.26%) major errors and160(8.04%) minor errors. Of 25(2.26%) major error errors;15(0.75%) were high false negative and 10(0.5%) high false positive errors. Of 160(8.04%) minor errors, 83(4.17%) were low false negative and 47(2.36%) were low false positive and the rest 30(1.51%) were quantification errors (Table 6).

~~As it was clearly indicated;~~Among the total errors committed (9.3%) during panel testing,6.63% of total errors were committed at Health Centers level and the rest 2.7%of total errors were committed at~~belongs to~~Hospitals. Among6.63 %of total errors committed at Health c~~C~~enters level, 5.73 %were minor errors and0.9% major errors. On the other hand, among 2.7 %of total errors committed by Hospitals;0.35% was major errorand 2.35% were categorized under minor ~~errors.~~errors. Among 199 Laboratory professional participated on panel testing 79(39.7%) of the were from Health centers and the rest 120(60.3%) were from Hospitals but when we had seen the proportion of errors committed between Health centers and Hospitals laboratory professional ,the laboratory professionals in Health centers(6.67%) committed higher errors than Hospitals(2.7%)table.5.

Table5: Type of errors committed by study participants during detecting TB bacilli in TB EQA rechecking and diagnostics laboratories of different health facility of Eastern Region of Oromia, Ethiopia, February 2019.

Health Facilities	Types of error (N=1990)										Total Err~~~~	
	Major Error				Minor Error							
	HFP		HFN		LFN		LFP		QE			
	N	%	N	%	N	%	N	%	N	%	N	%
Hospital	1	0.05	6	0.3	29	1.46	15	0.75	2	0.1	53	2.7
Health Center	9	0.45	9	0.45	54	2.71	32	1.61	28	1.41	132	6.63

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Total	10	0.5	15	0.75	83	4.17	47	2.36	30	1.51	185	9.33

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6.5 Random Blinded Re-checking

Among thestudy ~~participant~~participant health ~~laboratories, blinded~~facilities, blinded rechecking was conducted only in 34(59.65%) MCs~~,~~ of ~~them;~~them; which was 13(22.8 ~~38.24~~%) Hospitals and 21(36.84 ~~61.74~~%) Health centers. The remaining 23(40.35 ~~40.35~~%) health facilities do not store slides and we could not conduct blinded rechecking. From 842 ~~random~~ blindly selected and rechecked slides~~,~~,78(9.26%) and 764(90.74%) were reported to be positive and negative by microscopy centers respectively but when we saw the controller result ~~and~~ 80~~80~~(9.5~~9.50~~%) and 762(90.5~~2(90.5~~%) ~~were~~ reported as positive and negative ~~by the controller~~ respectively.

Out of the 842 re-checked ~~slides~~slides, 834 slides were concordant and the rest 8slides were discordant ~~agreement was observed81(9.62%) positive and 761(90.38%) negative slides. However, disagreed on 8(%) slides~~ means that 5~~1~~(0.6 ~~0.1~~%) false negative and 3~~7~~(~~0.~~0.4 ~~8~~%) false positive results were identified by controllers.

In general the overall S~~s~~ensitivity, S~~s~~pecificity, PPV and NPV for blinded rechecking ~~were 95.1~~were 93.75%, 99.6~~08~~%, 96.15~~1.67~~% and 99.35~~47~~%respectively andreading ~~with kappa value of 86.2%~~among slides were99.05% (Kappa statistics 0.89) this showing a very ~~good~~good Agreement.

~~reading agreement.~~

~~From sampled health facilities of Eastern Oromia region, 842 slides were collected for RBR based on lot quality assurance sampling techniques, among these 834 slides were concordant and the rest 8 slides were discordant.~~

Smear quality were also assessed during the visual re-examination of the sampled smears slides with average 651(77.3%) had proper smear size, 460(54.6%) had proper thickness, 687(81.6%) had proper staining quality~~,711~~, 711(84.4%) had good cleanness of smears and 669(79.5%) had good evenness of smear.

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Table 6: Evaluation and interpretation of RBR of microscopic centers and controllers among health facilities in Eastern Oromia region, Ethiopia, February 2019 (N=34 MC participated on EQA)

Concordant	Discordant				Total
	Major Error		Minor Error		
	HFN	HFP	LFN	LFP	
834	-	3	1	4	842

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Table.7: ANSV and SPR of smear microscopy among the studies laboratories in Eastern Oromia Region, Ethiopia, 2018.

Number of slides	SPR<5%	SPR=5-10%	SPR>10%	TOTAL
ANSV	N (%)	N (%)	N (%)	N (%)
<301 Slides	3(5.3%)	24(42.11%)	8(14.04%)	35(61.4%)
301-500slides	1(1.75%)	12(21.1%)	2(3.5%)	15(26.3%)
500-1000slides	2((3.5%)	5(8.8%)	0(0%)	7(12.3%)
>1000slies	0(0%)	0(0%)	0(0%)	0(0%)
Total	6(10.53%)	41(71.93%)	10(17.54%)	57(100%)

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NB: ANSV-Annual Negative Slide Volume, SPR-Slide Positivity Rate

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7. Discussions

~~This cross sectional study aimed to evaluate the performance of TB smear microscopists working at EQA rechecking and diagnostic health facilitieson the status of the respective laboratories were using Panel testing for TBmicroscopists, Random Blinded Rechecking and using structured checklists for On site evaluation.~~

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In this study the overall total errors committed were 9.3%.Ofthese total errors 2.36% was LFP ; the result of this study was lower than studies conducted both in Mexico (2.4%) and Ethiopia (7.4%).Concerning HFP errors in this study it contributed to 0.5% of the total errors, which was still lower than both Mexico (0.9%) and Ethiopia (0.6%).On the other hand ,in this study 4.17% of the total errors were categorized under LFN ,the result was lower than Mexico (5.7%) but higher than Ethiopia (3.1%).Regarding HFN,found 0.75% of the total errors were lower than both Mexico(4.1%) and Ethiopia (1.6%)~~In this study, 0.57% of LFP error was committed; the result of this study was higher than studies conducted in India (0.14 %) but lower than in Taiwan (16.7%) [24,27]. Concerning HFP errors, in this study it contributed to (0.36%) % of the total errors, which was still higher than both India (0.1%) and Taiwan (0%). On the other hand, in this study 0.12%% of errors were categorized under LFN, the result was lower than both India (0.56%) and Taiwan (2.8%). Regarding HFN, in this study found no contribution to the total errors, for both India (0.3%) and Taiwan (0.3%).~~ The possible reasons for the discrepancy might be the method was new in the study area as well as newly introduced at country level, more effort should be done to increase level knowledge and awareness laboratory professional through supportive supervision, Regular External Quality Assessment and Continuous refreshment training~~The possible reasons for the discrepancy might be due to the length of period and the number of slides collected for Random Blinded Rechecking. The other possible reason might be due to lack of consistent refreshment training and lack of technological advancement was among the factors affecting the proficiency of the TB smear microscopies detection among health professionals~~ [24,27].

In this study, the overall agreement in reading was 92.21%.Similar cross-sectional study was conducted in Tanzania and Taiwan showed that the overall agreement in reading was 89.2% [34 and 27] respectively. The result of this study was slightly better than Tanzania and lower than Taiwan, but not remarkeable.so, the finding showed that there were poor performances and different activities should be done to improve the quality of the microscopy service.

~~In this study, among 842 total slides collected for Random Blinded Rechecking from EQA center and diagnostics Health facilities in Eastern Region of Oromia, 834(99.05%) were concordant~~

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~~1(0.12%) were FN and 7(0.83%) were FP. The result of this study was better than a study conducted at Bahirdar Regional Laboratory and Health Research Centers in which FN and FP was 2.2 %and 1.5% [22]. The possible reason for the different might be due to inconsistencies of IQC measures, poor smear microscopic , poor smear thickness, poor evenness and size of smear and cross-contamination, yellow precipitation and use of re-agents exposed to direct sunlight~~

~~.~~ In this study, among 842 total slides collected for Random Blinded Rechecking from EQA center and diagnostics Health facilities in Eastern Region of Oromia, 834(99.05%) were concordant and the rest 8(0.95%) were discordant i.e. 5(0.6%) were FN and 3(0.4%) were FP. The result of this study was better than a study conducted at Bahirdar Public Health Referral and Research Laboratory Centers in which FN and FP was 2.2 %and 1.5% [22]. The possible reason for the difference might be due to inconsistencies of IQC measures, poor microscopic smear, poor smear thickness, poor evenness and size of smear and cross-contamination, yellow precipitation and use of re-agents exposed to direct sunlight.

~~In this study when we assess slide using quality indicators such as smear size, smear thickness, staining quality, neatness and evenness, proper smear size was found to be 77.3%, while 81.6% and 54.6% were proper smear thickness and proper staining respectively. The result of this study was slightly different from finding in Taiwan in which; 62.6%, 48.6% and 86.9% were proper smear size, proper smear thickness and proper staining respectively [27]. The possible explanation for the slight difference might be due to some technical errors such as lack of proper patient counseling on how to bring proper sample, smear might be made from non-purulent part of the specimen and lack of in adherence to SOP might be the possible reason for the differences.~~In this study, proper smear size was found to be 77.3%, while 81.6% and 54.6% were proper smear thickness and proper staining respectively.The result of this study was slightly different from finding in Taiwan in which; 62.6%, 48.6% and 86.9% were proper smear size, proper smear thickness and proper staining respectively [27].The possible explanation for the slight difference might be due to some technical errors such as lack of proper patient counseling on how to bring proper sample, smear might be made from non-purulent part of the specimen and lack of in adherence to SOP.

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~~In this study, the overall agreement in reading was 90.4%. Another similar cross-sectional study was conducted in Tanzania to assess the quality of sputum smear microscopy for AFB detection and 600 randomly selected slides from peripheral laboratories were blindly rechecked by intermediate and central laboratories. The overall agreement in reading was 89.2% [34]. Another study from Taiwan found that the overall level of agreement in reading was 99.1 [27]. The result of this study was slightly better from Tanzania and lower from Taiwan, but not remarkable. Generally, the finding showed that there were poor performances and activities should be done to improve the quality of the microscopy service.~~

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In this study, 6(10.53%) of laboratories were found to have below 5% of SPR. Both low SPR and ANSV were found in 3(5.26%) of the participant ~~laboratories.These~~laboratories.These findings were relatively lower as compared to the report from a study done in West Amhara Region of Ethiopia [21] which showed that 47% of diagnostic centers were found to have low SPR (< 5%) and ANSV (< 301 slides).On the contrary, ~~rate of low~~ low rate<5% SPR and ANSV<301slides from this study was higher than that reported from a study done in New Delhi [24] which revealed that 2.9% of participating diagnosticcenters had both low SPR (< 5%) and ANSV (< 301 slides).WHO's recommendation for laboratorieswith such poor performance is to intensively assess their performances in order to minimize false negative or ~~false positive results or should discontinue providing AFB diagnostic service if they wouldn't be improved otherwise~~minimizing unnecessary referral of non-cases for tuberculosis [44].

~~In the present study, the overall achievements during onsite evaluation were 62.7%.The study conducted in East Amhara, Ethiopia showed lower (69.2%) performance than our finding [31]. Comparative overall score (86%) were observed in a study conducted in India with the current study [24] but have higher score (>85%) for presence of properwaste disposal system (Incineration), power supply, water supply, slides, filter paper, spirit lamp or Bunsen burner, disinfectants, and re agents. The most common problems encountered during this study were shortages of staining re-agent preparation equipment (balances and measuring cylinders and lack~~

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~~of distilled water) and lack of adequate working area (separate and cross ventilated TB laboratories).~~In the present study, the overall achievements during onsite evaluation were 62.7%.The study conducted in East Amhara, Ethiopia showed higher (69.2%) performance than our finding [31]. Comparative overall score (86%) were observed in a study conducted in India with the current study [24] but have higher score (98%) for presence of correct waste disposal system (Incineration) and no inconsistency in electric power ,water , supplies, and laboratory reagents.

Another similar study conducted at West Hararge Zone and in Sidamo,SNNPR; the overall performance of on-site evaluation was 53.56% [48] and 48.9% [20] respectively. The result of this study was better than the findings from West Hararge and Sidamo Zone. The possible reasons behind might be due to the difference in study period, lack of facility and safety and inconsistencies of IQC measures performed per batch as well as per week.~~In this study, during on site evaluation, 85% of rechecking and diagnostic laboratories had bio safety and waste disposal system, 73.9% had adequate infrastructure, 59.4% had adequate supplies and re agents, 65% were participated in EQA, 49.1% performed IQC per batch and 40.4% had SOP and regular microscopic maintenance. The overall performance of on site evaluation for this study was 62.7%. In another similar study conducted at West Hararge Zone of Oromia regional state, 83.6% of laboratories had adequate supplies and reagents, all (100%) of health facilities were participated in EQA, 36.4% had done regular microscopic preventive maintenance, 31.7% and 16.4% of health facilities had facility and safety and performed IQC per batch respectively [48]. The overall performance of on site evaluation on West Hararge was 53.56% [48]. In another similar study conducted in Sidamo, SNNPR; 69.4% of laboratories had bio safety and waste disposal system, 49.5% had SOP, 46.9% were participated in EQA, 43.5% had adequate supplies and re agent, 42.9% had regular microscopic preventive maintenance, 28.1% had infrastructure and 25.6% had performed IQC per batch [20]. The overall performance of on site evaluation was 48.9% [20]. The result of this study was better than the findings from West Harerge and Sidamo Zone. The possible reasons behind might be due to the difference in study period, lack of facility, safety, and inconsistencies of IQC measures performed per batch as well as per week.~~

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Laboratories internal quality control practice in this study was lower (61.72%) than the study done in West Amhara, Ethiopia; which 83.1% [19] was. In this study, all most all of facilities had uninterrupted power and water supply whereas in West Amharait was reported lower supply of power and water which was 56.7% and 18.4%, respectively [21]. In general, the current on-site assessment findings were promising and continuous support is needed to fill those identified gaps.

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~~In this study, from 1990 examined slides there were 9.3% total errors committed, which account 1.26% major errors and 8.04% minor errors and the study conducted in Mexico showed that 5% and 20.4% major and minor errors committed respectively[37].So, our study was lower as compared to that done in Mexico.Since the method is new in this study area, more efforts should be done to increase the level of knowledge and awareness of laboratory professionals through closed supportive supervision, Regular External Quality Assessment and continuous refreshment trainings.~~

In our study the overall sensitivity and specificity was 90.54 and 94.03% were respectively but the study conducted in Hawassa Ethiopia showed higher sensitivity (91.97%) and lower specificity(80.0%)[38].On the other hand, both sensitivity (96.5%) and specificity (96.4%) were higher in west Amhara,Ethiopia report[19] than our study. The performance in the present study was slightly lower than similar studies conducted in in West Amhara,Ethiopia and higher in sensitivity than in Hawassa. This probably due to the method was different from that both in Hawassa and West Amhara that of in present study .

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8. Strength and Limitations

8.1 Strength

- To my knowledge, this study was the second ~~research~~ onein Ethiopia concerning FM next to BahirdarPublic Health Referral and Research Laboratory and Center that wasdone as in line with Heal-TB Ethiopia using RBR as pilot test before staining andafter staining[6]
- Slides validation~~s~~ were carried out by three senior laboratory technologists who ~~was~~ had training onTB expertise ~~on EQA slide~~panel slide preparation and validation at TB department in Adama Public Health Referral and Research Laboratory Center before dispatchto participant laboratories.

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8.2 Limitations

This study was few limitations

- Difficult to get sufficient literatures both locally and internationally to make adequate comparisons ,since limited studies were conducted on this topic
- This study was conducted only in public health facilities so; it does not illustrates the private health institutions
- Lack of experience on doing such kind of study

- ~~This study has a few limitations;~~

- ~~Since limited studies were conducted in this topic, it was difficult to get both local and international literatures to make adequate comparisons.~~
~~This study was conducted only in public health facility so; it does not illustrate the private health institutions.~~
- ~~Lack of experience conducting research~~

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9. Conclusions

Based on the results of this study, the following conclusions were made;

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- Due attention should be given for FM External Quality Assessment since the method is applied recently and not familiarized as that of ZN.

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- Even if, the overall performance were remarkable errors should require correction and close follow up.
- Detection of errors had serious consequences that all steps to improve the diagnostic performance of laboratories should be mandatory.
- The number of health facilities participating on EQA were very low from time to time this should need attention and incorporate all health facilities fulfill the criteria as per Ethiopia TB_EQA guide line.

~~The overall performance of rechecking and diagnostic laboratories in reading indicated that there was good agreement with the reference readers (89.1%) and the overall facilities assessment results were (62.53%). Besides, the total errors committed were 4.32% and majority of them were minor errors and the presence of this errors are alarming for TB control program and due attention should be given for FM- EQA method since the method is applied recently and not familiarized as that of ZN method.~~

~~This revealed that, even if the overall performance are remarkable, errors should require correction and close follow up as TB is a global treat these days due to MDR and XDR TB, detection error have serious consequences that all steps to improve the diagnostic performance of laboratories should be mandatory.~~

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10. Recommendations

Based on the finding of the study the following recommendations were forwarded for future actions;

- Even if the agreement among laboratory professionals were remarkable and promising, much effort should be made by laboratory professionals ~~need to up grade their educational level~~ to maximize the level of agreement.
- Even though, the overall performance of on-site evaluation was relatively better than other study conducted in different area, some variables were scored below the average, therefore, ORHB should give due attention in collaboration with FMOH and other stakeholders in order to fill the gaps identified by making the necessary interventions
- Errors among laboratory professionals arise due to different reasons, such as lack regular supportive supervision at diagnostic laboratory level, inconsistencies of IQC measures and irregularities of EQA participation. ThereforeAdama PHRRLC and EQA Centre should give corrective actions as per the EQA guidelines.
- This study was conducted in governmental facilities only but, Public private health facilities should be included in order to have more tangible and generalizable results.

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~~The finding of this study indicated that the overall performance of on-site evaluation was relatively lower. Therefore, Oromia Regional Health Bureau should give due attention in collaboration with FMOH and other concerned stakeholders in order to fill the gaps such as equipment's and periodic maintenance of laboratory instruments by making the necessary interventions.~~

~~Errors among laboratory professionals arise due to different reasons, such as, lack of consistent refreshment training, lack of supportive supervision at diagnostic laboratory level, inconsistencies of IQC measures and irregularities of EQA participation. Therefore, Adama Public Health Referral and Research Laboratory Center and re-checking laboratories should give corrective actions such as, consistent refreshment training and regular supportive supervisions as per the EQA guidelines.~~

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~~Finally, since this study was conducted in governmental facilities of Eastern Oromia Regional Sate, Public private facilities should be included in order to have more tangible and generalizable results. In addition, further researches should also be conducted using mixed approach since the current study was conducted only using quantitative approach.~~

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Appendixes

AnnexI: AFB Microscopy Checklist Adapted from Adama Public Health Referral and Research Laboratory Center

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ANNEX: IA Proficiency Testing Formats

Annex A1: Panel Test Result Reporting format for TB smear microscopy

Name of PT provider**:** _____

Result submission Date: _____

Round: _____

Complete the following spaces carefully; your result will only be valid if the form below is completed

Region: _____

Facility Name: _____

Date sample receipt at the laboratory; _____

Received by: _____

Date tested: _____

Date sent to PT provider: _____

S.No	Slide code	Staining method	Result	Remarks
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Tested by _____ signature _____

Contact address of PT provider

Tel_____

Fax_____

Email_____

PT Result Accession log book

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	To be completed by PT provider	
	Date Received:	Received by:
	Form complete: Y/N Date entered in Database:	Data Entered by:

Annex A2: Feedback format of Panel testing for TB Smears Microscopy

Round: _____

Region: _____

Health facility Name: _____

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Smear	Reported result	Expected result by PT provider	Error type	Score
T1				

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T2					
T3					
T4					
T5					
T6					
T7					
T8					
T9					
T10					

Total score: _____

Percentage: _____

* Performance: _____

* Acceptable if total percentage score is >80%

Annex I: B On-Site Evaluation Checklists and formats

Annex B1: On-Site Evaluation Checklist

I. General Information

Name of Laboratory	
Type of laboratory	
Code for the laboratory	
Number of laboratory personnel	
Name of Head/Quality Officer of the Laboratory	
Laboratory Head/Quality Officer Phone Number	

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Laboratory Head /Quality Officer Email	
Institution of supervisors	
Name of Supervisors	
Date of Visit	

II. Current Visit Particulars

Fill the findings as a labeled option 'Y'-Available, 'N'-Not available and NA- not applicable for the sites to be visited. In case of quantitative questions write the exact number.

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S.No	Item	Findings			Remark
1	Facility and safety	Y	N	NA	
	Separate area for TB Laboratory work				
	Separate tables for specimen receipts/smear				
	Power supply				
	Running water supply				
	Waste containers with lids				
	Waste disposal by segregation Autoclave/disinfection/buried				
	Incinerators available				
	General order/cleanliness				
	Personnel protective equipment's used (gloves, aprons, masks and hand washing facility etc.)				
2	Personnel and training				
	Number of Laboratory personnel trained in TB microscopy smear and EQA	Zeihel Nelson			
		Fluorescent microscopy			
	Number of laboratory personnel not trained on TB microscopy and EQA	Zeihel Nelson			
		Fluorescent microscopy			
3	Manuals ,Standard Operating Procedure, Job Aids				
	Is there Standard Operating Procedure for smear preparation, staining and reading				
	Does grading chart and TB smear microscopy job aids posted and used?				
	EQA protocol and training manual available and followed				
	Is there sufficient EQA forms				
4	Adequate stock and supply of staining reagents for a quarter and availability of equipment				
	Slides	Y/N			
	Lenses tissue	Y/N			
	Filter paper	Y/N			

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	Sprit lamp or Bunsen burner		Y/N	
	Smearing /staining equipment (staining racks,loops,applicatorsetc)		Y/N	
	Slide boxes		Y/N	
	AuramineO		Y/N	
	Acid alcohol		Y/N	
	Potassium permanganate		Y/N	
	Distilled water		Y/N	
	Equipment for preparation of stains /reagents such as balance (for weighing reagents),measuring cylinders etc.		Y/N	
	Equipment and facility for preparation of panel testing		Y/N	
	Number of FM(functional)			
	Number of FM(non-functional)			
5	Internal Quality Control And External Quality Assessment			
	IQC are used for each new batch of stain		Y/N	
	IQC are used for at least once every week for checking the quality of stain		Y/N	
	Are all slides stored properly in slide box for Random Blind Rechecking		Y/N	
	Control smears are used for each new batch		Y/N	
	Peripheral Laboratory participated in External Quality Assessment	Proficiency testing	Y/N	
		Random blind Rechecking	Y/N	
		On-site evaluation	Y/N	

Note: A copy of this summary has to be given to the visited laboratory

I. General Information

Name of Laboratory	
Date of visit (dd/mm/yy) E.C	
Name of supervisor	
Supervisor Phone Number	

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II.Summary of current visit

A.Operational/adminstrativeproblems_____

_____

_____

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B.Techincalproblems_____

_____

_____

C.Overall remarks_____

_____

_____

D.Action required

Issues		Cause(s)	Corrective action(s) for Each cause

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Name Laboratory Representative_____Sign_____Date_____

Name Supervisor Sign _____ Date _____

Annex I: C Random Blind Re-checking Formats

Annex C1: blind rechecking slide collection form

Region_____Zone_____woreda_____Peripheral laboratory_____

Quarter_____Year_____Date of collection_____

- Quarterly performance data collection form

Type of slide		Number
Diagnostic	Negative	
	Positive(1+ and above)	

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		Scanty	
Follow-up		Negative	
		Positive (1+ and above)	
		Scanty	
Total			
TB suspects		Positive	
		Negative	
Total			

- Slide collection form to be submitted with slides

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S. No.	Slide	Result of Peripheral Laboratory Including grade for positive	S. No.	Slide No.	Result of Peripheral Laboratory Including grade for positive

Name of Periphery laboratory representative _____signature_____ Date_____

Name of slide collector (TB supervisor) _____signature_____ Date_____

NB: Slides to be collected and data filled by TB supervisor, sealed in an envelope marked as RBRC smears with name of Peripheral Laboratory, address & date. The re-checking laboratory Quality officer keep this form with facility results and write slide number on annex C2 (next) and provide to first controller.

Annex C2: Worksheet for Blinded Rechecking of Peripheral Laboratory Slides (to be used by

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first controller)

Peripheral Laboratory: _____ Zone _____ Woreda _____

Region _____ Quarter ____ Year: _____Date of received _____

Tick appropriate column or write letter as indicated below table.

S. N	Slide No.	Specimen		Staining		Size		Thickness				Evennes		AFB result / Graded by		
		≥.10 WBC/ field	<10 WBC/	Good	Poor (U/O)	Good	Poor (B/S)	Good	Poor (K/N)	Clean	Not clean	Good	Poor	Peripheral laboratory	1st controller	2nd controller
		1		2		3		4		5		6				

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Improvement area addressing summary form

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Overall summary (please tick appropriate alternative):		
	Needs improvement	
	Yes	No
Specimen quality		
Smear size		
Smear thickness		
Smear evenness		
Staining		
Cleanness		
AFB result		

Key:

- If staining is poor write 'U' for under-decolorized, 'O' for over-decolorized
- If the size is poor write 'B' for too big, 'S' for too small
- If the thickness is poor write 'K' for too thick, 'N' for too thin
- Any error for 1 to 6 above in two or more slides indicates the need for improvement
- Write the results with grade for positive smears.
- Any error in results/ grading in two or more slides indicate the need for improvement
- Please carefully review all discordant slides with the Laboratory Personnel

Annex C3: Second controller results form:

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Lab slide no	Result 1	Result 2	Second controller result	Remark

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Annex C4: Summary and feedback form to peripheral laboratory *

Region: _____Zone _____ _____Woreda:

_____

Name of Peripheral Laboratory: _____

_____Quarter_____ Year: _____

Name of Re-checking laboratory

_____

_____

Summary for Error type

*Totals reported results on the samples (nos.)					
Positive (+1 to +3): _____		Scanty: _____		Negative: ____	
Summary of errors identified (nos.)					
HFP		LFP	HFN	LFN	QE

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***To be obtained from** Annex C2

Concordance Rate of the laboratory

		Peripheral lab	Rechecking center	Total
Positive slides				
Negative slides				
Total				

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Conclusion

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_____

_____

_____

Recommendation

_____

_____

_____

Name of Re-checking laboratory QO _____

Date_____ sign_____

Name of Re-checking laboratory Head _____

Date_____ sign_____

Table1.5: Calculation of SPR in each laboratory (Example)

Peripheral laboratories	**Slides / year**	**Positive / Year**	**Negative / Year**	**SPR** = Positive slides / total slides x 100
A	1500	200	1300	**13.3%**
B	2550	351	2199	**13.8%**
C	1990	156	1834	**7.8%**
D	1006	72	934	**7.2%**
E	2005	141	1864	**7.0%**
Total	9051	920	8131	**10.2%**

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Table 1.6: Recommended Annual Sample Size (80% sensitivity, 100% Specificity and '0' Acceptance number)

Number of negative slides in the MC in a year	Slide positivity rate (SPR %)				
	2.5[5]-4.9	5.0[2]-7.49	7.5-9.9	10-14.9	>15
	Annual sample size of both positive and negative slides (quarterly sample size[3] in parenthesis)				

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301[4]-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)

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Annnex:II. Preparation of Panel Testing Slides with Known Contents

1 .Introduction

This procedure is a self-explanatory laboratory method for producing multiple test slides from AFB positive and negative samples. Your laboratory staff should read and understand both the procedure and the testing protocols before developing test slides. This procedure has been reproduced/validated in state and national laboratories. If your laboratory has difficulty in producing slides that meet the requirements for consistency you should either: 1) review the procedure with special attention to the steps of heating and re-suspension; or 2) select patient specimens with less mucus. The sample development procedure requires materials that are routinely available in a national or regional reference laboratory in a low-income country. If your 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. Your laboratory should demonstrateproficiency 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.

NaOH method (ref Dr. Nguyen Ngoc Lan, Pham Ngoc Thach Hospital, Ho Chi Minh City, Vietnam and Dr. Alex Sloutsky, Massachusetts Dept. Health)

1. Materials Required

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

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

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

Amount: 3 ml or more;

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

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

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

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

Amount: 5 ml or more;

Color: white to green;

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

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

2. Preparation of AFB Positive Stock

a) Place 3 ml of AFB positive specimen into a 50 ml screw cap plastic tube.

If volume of the specimen is more than 3 ml, aliquot it into separate tubes

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

c) Incubate for 1 hour at room temperature (25- 30^{o}C).

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

e) Vortex thoroughly for 4-5 min.

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

g) Incubate in a water bath for 30 min. at 55-60^{o}C, mix occasionally by inverting the tube during incubation. If there is no water bath available, boil a beaker of water, cool to 90-95^{o}C and place

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the tube in the beaker for 20-25 min. It is important to maintain the incubation temperature in the 55-90^{o}C range.

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

i) Centrifuge @ 3,000 x g for 20 min. at room temperature (25-30^{o}C).

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

Note: It is advisable to avoid specimens containing impurities (food remains etc.) However if the impurities are still found in the sediment after it is dissolved in distilled water, filter the specimen through the gauze and re-centrifuge it.

3. Preparation of AFB Negative Stock

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

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

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

d) Incubate for 1 hour at room temperature (25-30^{o}C).

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

f) Vortex for 2-3 min.

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

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

4. Evaluation of Positive Stock Preparations

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

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

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c) Use a well leveled surface for drying the smears. Positive stock: It is optimal to have concentration 50-60 AFB per microscope field

5. Dilution Procedure

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

0 AFB/100 fields: negative

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

10-99 AFB/100 fields:

1+ 1-10 AFB/field:

2+ >10 AFB/field: 3+

b) Choose suitable AFB concentration on a case-to-case basis within suggested range. For better results, however, it may be recommended using

20 AFB/field for 3+ smears,

5 AFB/field for 2+ smears,

50 AFB/30 fields for 1+ smear, and 5 AFB/30fields for “exact” smears.

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

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

e) For calculation of the dilution factor use the following formula: **N = (DC / AC) * A**

Where:

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

DC - is desired AFB concentration.

AC - is actual AFB concentration.

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

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

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

Procedural notes:

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

2. 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/ 100 fields for further dilution to "exact" count smear.

6. Prepare and Validate Batches of Slides

a) Using diluted stock preparations, prepare slide batches (50-100 slides per batch is recommended).

Note: If laboratories are proficient in developing consistent slides, then developing many slides from fewer samples will help to save time. Heat fixed slides should last for months if stored in a cool/dry location.

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

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

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

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

Slide test results (columns 1-6) each column represents the number AFB/30fields for ~~6~~six separate slides selected for the sample and preferably read by 2-6 different technicians. For high positives (2+ or ~~3+)~~3+), the technicians may estimate the number AFB/30 fields by selecting a sufficient number of representative fields. For low positives (exact count AFB/30 fields and 1+) and AFB negatives slides the technicians should read a minimum of 300 fields per slide and record the average number AFB/30fields.

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

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

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

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

Mean [M] minus 2 standard deviations [SD]

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

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

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

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

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

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Annex III: Standard Operating Procedurefor FM Method

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Principle of the Procedure

The property of acid-fastness of mycobacteria is based on the presence of mycolic acid in their cell wall. The primary stain (~~auramine~~aura mine) binds cell wall mycolic acids. Intense decolo~~u~~rization (strong acid) does not release the primary stain from the cell wall of AFBs so they will have the fluorescent bright yellow colour of auramine. Counter stain (Methylene blue or Potassium Permanganate) provides contrasting background. Fluorescent stains are usually organic substances which absorb ultraviolet light and remit part of the energy as light of longer wavelength which can be observed through the eyepiece as fluorescence. When exposed to ultraviolet light, the fluorescent bacilli areperceived as brightly coloured organisms against a dark background.

Assay Procedure

Preparation of smear

1. Label the slides properly using a unique NTRL register number
2. Place the labeled slides, the samples and the wire loops/applicator sticks in the BSC
3. Match each slide with the corresponding sputum or sample container.
4. Proceed to smearing, taking the labeled slides and opening containers one by one; do the smearing from the center of the slide outwards making small coil-like movements.
5. For a direct sputum smear select a small portion of purulent or muco-purulent material with the applicator stick/wire loop and transfer it to the slide; if a stick is used, break it in two pieces and use the ragged ends for dissecting sputum and for smearing.

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6. If a smear is prepared after specimen decontamination, the concentrated material has to be transferred to the slide with a sterilized loop or Pasteur/transfer pipette.
7. If a loop is used, it has to be sterilized before re-use by heating until it becomes red-hot
8. Spread the material carefully over the middle area of the slide equal to about 2x1 cm using repeated coil-like movements, without touching the margins of the slide.
9. Make the smear as even as possible by continuing this process until no thick parts remain. Removeexcessmaterial with the second stick and discard in the biohazard bag.
10. The thickness of the smear should be such that a newspaper can be read through the dried smear held about 10 cm above it (slide is translucent).
11. Warm the slides on the Fisher Slide Warmer (60^0C to 70^0C) in the BSC for at least two hours to dry and fix the smear.
12. If slide warmer is not functional, air dry the smear and when dry, fix the smear by passing the slide three times slowly through the flame of a spirit lamp, or quickly through the flame of a Bunsen burner (until the slide back part is hot but can still be touched without burning), smear upwards; do not overheat or else the AFB staining will be poor.

Staining procedure

1. Place the slides on the staining rack over a sink.
2. It is a must to keep distance of at least 1 cm between every ~~slide.Otherwise~~slide. Otherwise, there is a possibility that acid- fast bacilli might float off one slide and become attached to the next slide.
3. Cover the smears completely with 0.1% ~~auramine~~aura mine solution (with simultaneous filtration by pouring through a small funnel with filter paper held over the slides).
4. Do not heat.
5. Leave for 20 minutes.
6. Wash the slides well with distilled or running water.
7. Pour 0.5% acid alcohol solution over the slides.
8. Allow to react for 3 minutes.
9. Gently rinse each slide with distilled or tap water
10. Repeat decolorization if macroscopically visible stains are still present.

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11. Flood smear with 0.5% Potassium permanganate counter stain solution for 1 minute. Time is critical because counterstaining for a longer time may quench the acid-fast bacilli fluorescence
12. Wash off with distilled or running water.
13. Stand the slide on edge to drain, and air dry on the slide rack out of the direct sunlight. Recording Because flour chrome-stained smears are examined at magnifications of 200x to 400x, the number of AFB can roughly be divided by a factor 10 or 5, respectively (depending on the objective) to make them equivalent to fields seen on examination of fuchsin-stained smears at 1000x.

Standards for reagents and equipment's

1.Standards for Reagent

A. ~~Auramine~~Aura mineO(AO)

- Chemical name: ~~Auramine~~Aura mineO (Merck, lot number ---------, a~~A~~nd Expiry date ---------)
- Chemical Formula: C17H21N3
- Molecular Wt: 267.373
- Color: Yellow
- Dye content: Approximately 85%

B. Acid Alcohol

- 96% Ethanol
- 37% Hydrochloric acid

C. Potassium permanganate

- Chemical name: Potassium permanganate (ROTH)
- Chemical structure: Kmno4
- Molecular Wt: 158.04
- Dye content: 99%
- Appearance: Purple solid, dissolves in water to give deep purple solutions

D. Shelf life of prepared reagents

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- 0.1% ~~Auramine~~Aura mine~~O~~ - one month
- 0.5 % HCL -6 month
- 0.5 % potassium permanganate- 6 month

E. 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 ~~Auramine~~Aura mine~~O~~, Acid alcohol, P~~p~~otassium permanganate reagents should in addition have the name of the person preparing the reagent. Freshly prepared reagents should not be mixed with old stock.

- Supply and Equipment:

  I. Slides:

  - Size: 76 mm x 26 mm,
  - Thickness: 1.3 mm
  - Edges: Polished
  - Sealed in a moisture absorbing descant pack

  II. Balance: balance, with a sensitivity of 0.1 g

  III. LED Fluorescence microscope

- Primo star LED microscope, fixed –holder stage drive Refilled 18,D=0 including
- LED illumination
- 4 position nose piece, tilted backwards
- Mechanical stage 75 x30 ,drive right and specimen holder with spring clip left
- Binocular tube 300/20
- Eyepieces 10x/18
- Objectives plan –A Chromate D=0 without cover glass ,10x,20x,40x and 100x oil
- Condenser 0.9/1.25
- External power unit 100-240 VAC/50 6 Hz/30VA with country specific adapters
- Dust cover

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Annex-IV: Preparation of stains and reagents (~~auramine~~aura mine method)

A~~a~~.~~0.1%Auramine~~0.1% Aura mine

The reagents for preparing 1 liter 0.1% ~~Auramine~~Aura mine staining solutions are the following

~~Auramine~~Aura mine O(solution 1)	Amount	Grade
1%Auramine O~~o~~	1g	Certified
95% ethanol	30ml	Technical

Phenol (solution 2)	Amount	Grade
Phenol crystals	30.0g	Analytical
Distill water	870ml	

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

1. Dissolve 1g ~~Auramine~~Aura mine, certified grade in100ml Alcohol (ethanol or methanol)
2. Dissolve 30g of phenol (analytical grade) in 870ml distilled or purified water
3. Mix (1) and (2) and thorough mixing for about one hour on a magnetic stirring plate is recommended, but the solution should not be heated
4. Label the bottle "0.1% ~~auramine~~aura mine", add date and sign with initials. The date first opened has to be mentioned. The stock and working solutions have to be kept in dark bottles, or better in a cupboard. Working solutions should not be used over 1 month.

b. Decolorizing solution (0.5%HCL)

The required reagents to prepare 1liter of 0.5% acid alcohol are the following

Chemicals	Amount	Grade
Fuming hydrochloric acid	0.5ml	Technical

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95% ethanol	100ml	Technical

1. Add 0.5 ml Hydrochloric acid, technical grade in to 100 ml of Ethanol

2. Label the bottle “0.5 % Acid alcohol", add date and sign with initials. The date first opened has to be mentioned. Stocks and solutions should not be used over 6 months.

C.0.5%Potassiumpermanganate

The required reagents to prepare 1liter of 0.5% Potassium permanganate are the following

Chemicals	Amounts	Grade
Potassium permanganate	5g	certified
Distilled water	1000ml	

1. Dissolve 5g of potassium permanganate, certified grade in 1000mlof distilled water.

2. Label the bottle “0.5% Potassium permanganate ", add date and sign with initials. The date first

opened has to be mentioned. KMnO4 is explosive, therefore, avoid contact with combustible materials. Stocks and solutions should not be used over 6 months.

E. Supplies and equipment’s

- balance, with a sensitivity of 0.1 g
- brushes to clean bottles before reuse
- dark amber glass bottles or plastic bottles, Filter paper
- Flasks, capacity at least one liter, funnels
- stirring plate with heating and magnetic stirrer Spatula
- Measuring cylinder
- Water bath (optional)
- Timers
- Forceps
- Slide box
- QC log~~book~~

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~~Known~~ Known +1 positive and negative slides

Annex V: Option for panel slide compositions

Option 1	Option -2	Option-3	Option-4
1 Slide Graded 3+	1 Slide Graded 3+	1 Slide Graded 2-3+	1 Slide Graded 2-3+
1 Slide Graded 2+	1 Slide Graded 2+	2 Slide Graded 1+	2 Slide Graded 1+
1 Slide graded 1+	2 Slide Graded 1+	3 Slide Graded Scanty	4 Slide Graded Scanty
2 Slide Graded scanty	3 Slide Graded scanty	4 Negative Slides	3 Negative Slides
5 Negative slides	3 Negative slides		

Annex VI: Evaluation and interpretation of errors between controllers and microscopists

Result of Microscopist	Result of Controller (NTRL)				
	Negative	1-29AFB/30F	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
1-29AFB/30F	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

Reading, Recording and reporting of FM smear

1. Keep stained smears in the dark (boxor folder) until reading, and read as soon as possible
2. Place smear facing upwards onto stage (subdued lighting is preferable for reading the slides and dark room is not required for LED/FM.
3. Focus the smear with 10x objective by turning the course adjustment
4. Adjust the distance between the ocular lenses until both the right and left images become one
5. Examine slides using 20x objectives
6. Confirm if necessary with 40x objective

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7..scan the stained smear systematically at least one length has to be scanned before reporting a negative, corresponding to 30 high-power fields and taking 1-2 minutes (20x-40x objectives. Systematic examination of smear.

8. Acid fast bacilli appear long, slender, slightly curved rod, bright yellow or green (may vary in staining intensity) against dark background.

9. Quantify AFB in the fields; and record and report results according to the standard grading and reporting table

10.Store all smears in slide box for External quality assessment

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Annex VII: Reading and Reporting of microscopy smears

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IUATLD/WHO Scale(1000x field=HPF	Microscopy system		
	Bright field (1000x magnification: 1 length = 2 cm = 100 HPF)	Fluorescence (200–250x magnification: 1 length = 30 fields = 300 HPF)	Fluorescence (400x magnification: 1 length = 40 fields = 200 HPF)
Result			
Negative	Zero AFB/1 length	Zero AFB/1 length	Zero AFB/1 length
Scanty	1–9 AFB/1 length or 100 HPF	1–29 AFB/1 length	1–19 AFB/1 length
+1	10–99 AFB/1 length or 100 HPF	30–299 AFB/1 length	20–199 AFB/1 length
+2	1–10 AFB/1 HPF in at least	10–100 AFB/1 field on average	5–50 AFB/1 field on average

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	50 fields		
+3	>10 AFB/1 HPF in at least 20 fields	>100 AFB/1 field on average	>50 AFB/1 field on average

Reporting

- Use the laboratory number to find the correct patient Request form
- Immediately record the result in the Request Form
- Use red pen for positive results
- Date and sign the Laboratory Request Form
- Transfer the result to the Laboratory Register

~~**Release the completed Laboratory to the request clinician as soon as.**~~

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Annex VIII: Declaration

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The undersigned declares that this proposal complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

MSc. candidate: AmanNebiAyatoo (BSc.)

Signature:_____

Date of submission:_____

This thesishas been submitted with our approval as advisors.

Advisor:KassuDesta (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: AbaySisay (BSc, MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia

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