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COLLEGE OF HEALTH SCIENCE

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Performance evaluation of graduating class of medical laboratory students and its associated factors on AFB smear microscopy in Addis Ababa Ethiopia.

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A Research thesis submitted to Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Science, Laboratory Management and Quality assurance speciality track.

February 2023

Addis Ababa, Ethiopia.

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

This is to certify that the thesis prepared by Eden Alamirew, entitled:

"Performance evaluation of graduating class of medical laboratory students and its associated factors on AFB smear microscopy in Addis Ababa Ethiopia" and submitted in partial fulfillment of the requirements for a Master of Science degree in Clinical Laboratory Sciences (laboratory management and Quality assurance track) complies with the regulations of the University and meets the accepted standards concerning originality and quality.

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Acknowledgment

I would like to express my sincere gratitude to the Department of Medical Laboratory Science, College of Health Science, and Addis Ababa University.

I would also like to express my sincere gratitude to my advisors Mr. Abay Sisay and Mr. Habtamu Molla from Addis Ababa University's Department of Medical Laboratory Science and Mr. Mengistu Tadesse for the valuable guidance they provided me throughout my work.

My sincere thanks go to each college and university staff on the study site that helped by giving information about study participants. Also, graduate medical laboratory students.

Above all, I thank almightily God for enabling me, and for the strength, courage, and wisdom he has given me throughout my study, and I would like also to cover my gratitude to my Husband.

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Acronyms/ Abbreviations

AFB	Acid Fast Bacilli
DOTS	Directly Observed Treatment, Short-course chemotherapy.
EPHI	Ethiopian Public Health Institute
EQA	External Quality Assessment
FMoH	Federal ministry of health
HFN	High False Negative
HFP	High False Positive
HIV	Human Immunodeficiency Virus
HPF	High Power field
LFN	Low False Negative
LFP	Low False Positive
NRL	National Reference Laboratory
NPV	Negative predictive value
PPV	Positive Predictive Value
TB	Tuberculosis
WHO	World Health Organization

Abstract

Background: TB sputum smear microscopy plays an essential role in initial TB diagnosis and follow-up. To have skilled laboratory professionals in AFB microscopy, it is crucial to have demonstrative competence among the professionals working on the examination of TB microscopes before the test report. There is limited research-based information on the performance evaluation of graduating class of medical laboratory students in this specific study area.

Objective: this study was to assess the performance of the graduating class of medical laboratory students in public and private academic institutions on AFB smear microscopy and its associated factors in Addis Ababa Ethiopia from April 2020 to April 2021.

Method: A cross-sectional study design was employed among the graduating class of medical laboratory students in 2 public and 4 private academic institutions. A standardized pre-validated 10 sputum TB smear slide panels for each academic institution were used then 106 undergraduate students were involved to assess the student performance by using convenient sampling techniques. The percentages of agreements and the different types of errors were calculated. The sensitivity, specificity, positive predictive value, and negative predictive value of the smear reading for participants were calculated. The strength of an agreement between a participant and the reference readers was assessed using kappa statistics. Logistic regression & Chi-square test was used to assess associations between different variables, a p-value <0.05 was statically significant.

Results: Among 106 evaluated students, 65(61.3%) were males and the mean age of the participant were 24.2±2.6 with the majority of the age group between 20 to 30 years. The overall performance score ranged from 40-100%, with 320/1,060 slides (32%) with minor errors and 224/1,060 slides (22.4%) with major errors. From the panel slide reading, the total number of students that scored greater than 80% is 22 (20.8%), and 84 (79.8%) students scored below 80%. The sensitivity, specificity, positive predictive value, and negative predictive value of reading smears were 51%, 84%, 76%, and 63% respectively. The strength of an agreement between participant result and the reference laboratory were moderate agreement Kappa value 0.51. In Multivariate analysis, the type of microscope and no practice session was affecting the student performance, this means a strong association with poor student performance (<80%) was p-value <0.05. The student performance was indicates a good attitude (91.7%) toward the AFB smear technique.

Conclusion: The performance of graduating batch students was very much lower than the recommended cut-off value of 80%. Factors contributing to decreasing student performance related to the allocation of practical time sessions and type of microscope. Accordingly, as an improvement project we recommend, students should take pre-service training (Internship) & active regulatory measures should be in place before student graduation.

Keywords: AFB microscope, competence, tuberculosis, Performance, Laboratory student.

1. Introduction

1.1. Background

Tuberculosis (TB) is a communicable and infectious disease that is a major cause of ill health. It is caused by the bacillus mycobacterium tuberculosis which is spread through coughing when people infected expel the bacteria into the air. One of the top 10 causes of death worldwide, and the leading cause of death from a single infectious agent. The lung is the primary organ affected by the bacteria (pulmonary TB) but can also affect other sites (extra-pulmonary TB) [1].

The WHO for tuberculosis control Directly Observed Treatment, Short-course chemotherapy (DOTS) relies on a network of laboratories that provide acid-fast bacilli (AFB) sputum smear microscopy. But, if the laboratory diagnosis is unreliable, all other activities will be affected. It is known that microscopy errors are likely to fail to detect persons with infectious TB who will then continue to spread infection in the community or unnecessary treatment for non-cases [2]. On the other hand, errors in reading follow-up smears can result in patients being placed on prolonged treatment, or in the treatment being discontinued prematurely. Therefore, quality assurance of AFB sputum smear microscopy is essential to reduce such types of problems [3, 4].

According to the WHO TB report, Ethiopia ranks 7th in the list of the world's 22 high-burden countries for TB with an incidence estimated at 379/100,000 for all forms of TB & 168/100,000 for smear-positive TB [5].

The quality Assurance (QA) system for sputum microscopy is aimed at minimizing the false positive and false negative results in the laboratory services. Performance assessment of acid-fast bacilli (AFB) sputum smear microscopy is a process that assesses peripheral laboratory performance by a higher-level laboratory, and it includes blinded rechecking on-site evaluation, and panel testing [6].

Tuberculosis diagnosis requires a functional laboratory set-up with quality diagnostic services and trained laboratory personnel. However, the performance of such laboratories depends on continuous monitoring and quality improvement mechanisms put in place. It is also important to understand the limitation of smear microscopy in the detection of tuberculosis. But the limited diagnostic capacity for TB in the countries remains a challenge to improving the case detection rate. When resources are extremely limited and technical expertise is insufficient to prepare smears, stained smear slides collected from the routine services at the reference laboratory may be used to develop test panel sets. Advantages of this method include low workload for the central laboratory, no requirements for special equipment, and the slide sets that can be prepared quickly. This method tests only the ability of technicians to correctly read and report smears [7].

Accurate, Reliable, and timely results from laboratory examination are crucial elements in the decision-making of TB diagnosis, competency in AFB microscopy of graduating medical laboratory students must

be assessed. Hence, this study aims to assess the performance of graduating medical laboratory students in detecting acid-fast bacilli from sputum AFB smears.

1.2. Statement of the problem

Globally the annually reported newly diagnosed TB cases were 7.1 million notified in 2019, up from 7.0 million in 2018 and a large increase from 6.4 million in 2017 and 5.7–5.8 million annually in the period 2009–2012, medical and public health laboratory services areas are critical components of national health systems and are central to disease diagnosis, treatment, prevention, surveillance, and outbreak investigations. Laboratory medicine generates knowledge that facilitates patient safety, improves patient outcomes, and leads to more cost-effective healthcare when properly used [8].

Ziehl–Neelsen (ZN) stained smear of un-concentrated sputum (direct smears) microscopy is the routine diagnostic method in resource-limited countries. This method is simple, efficient, and inexpensive in detecting TB bacilli but it's dependent on the performance of the laboratory personnel in identifying a low number of AFB from the stained sputum smear using a conventional light microscope. The sensitivity of this method is variable (20-60%) [9-1

False-negative errors could lead to failure to detect persons with infectious tuberculosis, who could continue to spread the disease in their communities; false positives could lead to unnecessary anxiety, exposure of patients to unwanted side effects of medications, and unnecessary expenditure [14].

Errors in sputum smear reading may be classified as false-negative and false positives errors. False-negative errors fail to detect persons with infectious TB, who could continue to spread the disease to the communities, false positives errors could lead to unnecessary anxiety and exposure of patients to unwanted side effects of medications. Unnecessary anti-TB treatment for non-TB cases predisposes the development of drug-resistant tuberculosis (MDR-TB). Therefore, a quality assurance (QA) system for sputum microscopy is important to minimize the false positive and false negative results in laboratory services [15].

Various factors including infrastructure, work experience in sputum smear microscopy, and reagent supplies affect the quality of sputum smear microscopy [16]. Beyond those factors, the laboratory professional should gain the proper knowledge and skills in detecting TB bacilli before they are graduated from the universities. The accuracy and reliability of laboratory testing are critical to the success of TB control programs. It must be monitored to ensure the quality of the overall process, detect and reduce errors, and improve consistency between testing sites. The minimum number of acid-fast bacilli necessary to produce a positive smear result has been estimated to be 5000-10,000/ml of sputum at a concentration below 1000 AFB per ml of the sputum, the chance of observing the bacilli in a smear is less than 10%. With the inherent limitations of sputum AFB microscopy, the highest sensitivity achievable by microscopists in reading smears can be 95% [17].

Case detection through quality-assured laboratories is an essential element of the WHO STOP TB Strategy [18]. On the other hand, the country is still among the 22 high TB burden countries. This might be related to a high burden of undiagnosed or missed cases (30% of incident cases) as shown by individual reports in the country. Several documents recommend the need for training laboratory technicians to improve the quality of sputum AFB microscopy [19].

According to the study conducted by Gemechu et al in 2012, there were 19.1% of major errors and 117% minor errors among graduating batch students on AFB microscopy. Several documents recommend the need for training laboratory technicians to improve the quality of sputum AFB microscopy [20].

For this study to assess the performance of graduating medical laboratory students. Medical Laboratory Technology students learned in the academic institute should have a good competency in practical performance specifically on TB detection and grading which is currently a sensitive issue. As the National TB program recommends, for accurate TB detection and early treatment, immense work must be done on increasing the detection ability of Laboratory personnel. Comparability of laboratory test results depends on the standardization of all phases of laboratory testing, including pre-analytical, analytical, and post-analytical phases. Pre-analytical and analytical phases of laboratory testing aim to generate an accurate test result, while the post-analytical phase, when the clinician receives the test results, interprets them, and uses them to make diagnostic and therapeutic decisions - aims to reduce errors or bias associated with the hand-off from laboratory to the clinician. Therefore, this study provides a clue to academic institutions and particularly to policymakers to formulate programs that can fill the gap of the students.

1.3 Significance of the study

Quality is a key concern of academia across the globe and several efforts in multiple directions are made by administrators and academicians to induce quality components into the teaching-learning situation. Both public and private academic institutions are the sources of qualified laboratory personnel and the quality of education they are given will determine the performance of each laboratory personnel during their service as a professional.

Adequate skills of medical laboratory students in TB microscopic identification are very important and it indirectly indicates the quality of the educational system. The result of this study will determine the quality of education and the effectiveness of the teaching methods and addresses information to the ministry of education about the need for continuous and regular performance assessment of graduating medical laboratory students on ZN-AFB microscopy identification.

Moreover, this study helps and gives a clue to identify related gaps in detecting AFB under a microscope and reference information to decision makers and program managers who are working on this and that could improve TB case detection.

2. Literature review

2.1. Performance evaluation of TB globally

In a study conducted in America by the Pan American health organization, the smear microscopy quality assurance consensus group shows that common sources of false results of smear microscopy are caused at pre-laboratory under administrative issues; with false negatives caused by specimen quality, patient identification, specimen labeling, and transport condition, whereas false positive is caused by an error in specimen labeling and patient identification. False-negative results may also be linked to smear preparation, stain formulation, staining technique, microscope performance, and smear examination are some of the factors caused by technical aspects of the laboratory [21].

Fujiki A et al conducted a study in the Philippines Cebu province in 1997. 90% of rural health units participated in the quality control activity. The proportion of good-quality smears increased markedly, and the FP and FN rates did not change during the period, but most of the FN was observed among the scanty positives of the field reading and no FN was noted, among the heavy positives slide [22].

In Belgium Van Deun A et al did a study to define the efficiency of the number of microscopic fields screened and the sputum collection scheme used for diagnostic smear examination. Acid-fast bacilli were found in 99.6% of 1412 positive and 79.3% of 576 scanty slides in the first 100 fields. Examination of a third specimen yielded a maximum of 2.7% positives incrementally. The most efficient strategy, using three-morning specimens, yielded 94.2% positives on the first and 1.0% on the third sputum; although 10% of suspects did not return, only 1.5% of the positives were among them and more cases were confirmed and treated. The positive predictive value of a single positive or scanty smear was very high (99.2%) [23].

A study was conducted in India by Paresh et al in 2005 to assess the proficiency of Senior TB Laboratory Supervisors and district-level laboratory technicians in sputum Smear microscopy. The result indicates that there was a high level of concordance in ZN smear grading found between the microbiologist and district laboratory staff. District tuberculosis laboratory center readers reported an overall consistency level of more than 98% in ZN grade agreement. The tendency to over-grade the panel slides was much higher (more than 22%) as compared to under-grade (less than 2%) them in "correct slides". A high false positive error was not observed in this study [24].

A cross-sectional study was conducted by Selvakumar N et al. to assess the proficiency to read sputum AFB smears by senior tuberculosis laboratory supervisors under training at a reference Laboratory in India. 342 trainees participated in the study to read the same set of 15 to 20 Ziehl Neelsen-stained smears in a blinded fashion on days 1 - 15 of the training program. The sensitivity, specificity, positive predictive value, and negative predictive value of smear reading were 75%, 88%, 93%, 63%, 94%, 99%, 99%, and 89% respectively on day-1 and day –15. The sensitivity to read sputum AFB smears by fresh trainees with little or no experience increased from 75% to 94% during the carefully planned training program; the

specificity increased from 88% to 99%. This study concluded the importance of training in improving the microscopy results of AFB sputum smear [25].

2.2. Performance evaluation of TB in Africa & Ethiopia status

A study conducted in the Northern Province of South Africa in 2000 for the first round and second round respectively in 21 province laboratories showed that the overall performance of first-round laboratories was 85.5% and the second round was 95%. The false positive and false negative rate was 20.5% and 9.4% respectively. The sensitivity and specificity were 92.1% and 76.3% respectively. For the second-round quality assessment, the overall agreement between the peripheral laboratory and central laboratory was 97.4% and the overall false reading rate was 2.63%. The sensitivity and specificity of their performance were 96.5% and 100% respectively [26].

In 2012 Gemechu Y et al assess the performance of graduating batch students in the field of Medical Laboratory Science in detecting and quantifying Acid-Fast Bacilli using sputum smear microscopy. A total of 124 were tested by 5 stained slides with a known grade of acid-fast bacilli (AFB) from February to June 2012 in the three earliest universities. The mean score was 87.1% and the overall PT score ranged from 40-100%. There were 24(19.1%) major errors and 117 (81%) minor errors. The total numbers of students that report major error were 18(14.5%) and there was a total of 80(64.5%) students that report a minor error. The proficiency to read sputum smear by graduating batch students who have taken pre-service training was better than the rest who hasn't taken pre-service training. Thus, the study has highlighted the importance of training in improving microscopy results [20].

In 2015 Hailemariam H et al conducted a prospective cross-sectional study to evaluate the quality of TB smear microscopic examination in Hawassa. Among the 81 participants, 11(13.6%) correctly reported all panel slides, and 70 (86.4%) missed at least one slide. A total of 29.75% (241/810) error was reported that including major errors of 2.22% (13 HFN; 5 HFP) and minor errors of 27.5% (25 LFN; 60 LFP and 138 QE). The sensitivity and specificity of participants in detecting TB bacilli as compared to the reference reading were 91.97, 80.00, 87.30, and 86.92%, respectively. The overall agreement of participants with the reference reading on TB detection was 95.18% (Kappa = 0.73) [27].

Another cross-sectional study was conducted by Asrat H et al in Ethiopia at 81 laboratories from April to July 2015. Panel slides were prepared and validated at the National Tuberculosis Reference Laboratory. Overall, 324 (83.3%) participants scored ≥ 80%. Sensitivity for detecting tuberculosis bacilli was 84.5% and specificity was 93.1%. The overall percent agreement between participants and reference readers was 87.1 (kappa=0.72). All 10 slides were correctly read (i.e., scored 100%) by 80 (20.6%) participants, 156 (40.1%) scored 90% – 95%, 88 (22.6%) scored 80% – 85%, and 65 (16.7%) scored below 80%. There were 806 (20.7%) total errors, with 143 (3.7%) major and 663 (17%) minor errors. The overall performance of participants in reading the slides showed good agreement with the reference readers. Most errors were

minor, and the ability to detect tuberculosis bacilli can be improved by building the capacity of professionals [28].

A cross-sectional study was conducted in Ethiopia by Mosissa L et al in 2016 to evaluate the external quality assessment of AFB smear microscopy performances and its associated factors in 32 selected private health facilities in Addis Ababa, Ethiopia. 2-scored 100%, 15 scored 80-95% & the remaining 15 scored 50-75% for overall proficiency test performance. There were 10 (3.15%) major errors and 121 (37.8%) minor errors. The sensitivity, specificity, PPV, and NPV of panel reading by microscopy centers were 89%, 96%, 96%, and 90% respectively. Out of 283 randomly selected slides for blind rechecking, 11 (3.9%) slides were interpreted falsely for AFB, with an overall agreement of 97.5%, a sensitivity of 88.4%, and a specificity of 99.3%. In terms of slide quality assessment, 71.6% of AFB slides were graded as good for evenness, cleanness, thickness, size, staining, and labeling. The performance score for AFB slide evenness was 56.9% (161 slides) and for labeling, the quality was 90.8% (257 slides); having a significant difference in slide quality (p-value < 0.05). The on-site evaluation indicated problems in terms of infrastructure, standard operating procedure, reagent quality, equipment maintenance, data management, and training issues. Most of the health facilities had poor maintenance schemes for microscopes (53.5%) and poor inventory management (25.0%) systems [29].

4. Objectives

4.1. General objective

- To assess the performance of evaluation of graduating class of medical laboratory students and its associated factors on AFB smear microscopy in Addis Ababa Ethiopia, from April 2020 to April 2021.

4.2. Specific objectives

- To assess the performance level of graduating medical laboratory students on AFB smear microscopy among graduating classes of MLS in a public and private academic institution.
- To identify the associated factors that hinder the performance of AFB smear microscopy among graduating classes of MLS in a public and private academic institution.

5. Hypothesis

HO: there is no difference in the performance of graduating class students and reference laboratory on AFB smear microscopy.

6. Materials and methods

6.1. Study area

The study was conducted in a public and private academic institution that has graduating class medical laboratory students in Addis Ababa. Addis Ababa is the capital city of Ethiopia. It's covering 527 square kilometers of area. The population density of Addis Ababa city administration was estimated to be near 6.5 million individuals per square kilometer [32]. It has an average elevation of 2,326 meters above sea level [33]. The city has 11 sub-cities and 116 Districts (Woreda). Addis Ababa is the educational and administrative center of Ethiopia. It is the site of Addis Ababa University (1950) and contains several private and public colleges and technical schools. There are four private (Rift valley University College, African Medical college, Yanet college, and Ekusta learning institution) and two public institutions that educate medical laboratory professionals (Addis Ababa University, college of health science school of medical laboratory & Kotebe metropolitan university (Minilik II health science college) providing medical laboratory sciences.

Menelik II Health Science College was established in 1949 as an Auxiliary health science training school, and it was the 1st nursing school in the history of the country. Currently, it's part of the health sector institution of the Addis Ababa city government. The college delivers different courses in junior clinical nursing, public health, midwifery, medical laboratory, anesthesia, pharmacy & radiography at the graduate & post-graduate levels [33].

Africa Medical College is a privately owned medical institution established in 2003/4 mainly to iron out the scarcity of health professionals in the country. Africa Medical College sets out its training program in four departments at the diploma level. These are clinical Nursing, Medical Laboratory Technology, pharmacy, and X-ray Technology [34].

Yanet College of Health Sciences was established in 2000 E.C Addis Ababa Ethiopia exclusively dedicated to the education of healthcare professionals. Five different levels of the department. These are clinical/compressive, Nursing, Medical Laboratory technician/Technology, and pharmacy [35].

The Ethiopian Catholic University of St. Thomas Aquinas "ECUSTA" managed by the LA SALLE Congregation seeks to become a reputable international training institution. Recognizing the need for strong and visionary African leadership, the former Ethiopian Prime Minister, Sir Hon. Meles Zenawi, in 1997 asked Pope St. John Paul II to establish a Catholic University in Ethiopia to address the acute educational needs throughout the country. The University was focusing mainly on health and medical services [36].

Rift Valley University began operations in October 2000 in Adama Town, with a total number of 154 evening program students, and five part-time faculty staff. In September 2003 Gotera branch campuses came into being [37].

6.2. Study design and period

An institutional-based cross-sectional study design was employed from April 2020 to April 2021.

6.3. Population

6.3.1. Source population

All medical laboratory students attend an academic institution in Addis Ababa.

6.3.2 Study population

For this study, the study population was medical laboratory graduating students at an academic institution found in Addis Ababa.

6.4. Inclusion and exclusion criteria

6.4.1. Inclusion criteria

- Public & private academic institutions that have medical laboratory departments and graduating class medical laboratory students.

6.4.2. Exclusion criteria

- Post-graduating medical laboratory students were excluded from the study.
- Graduating class students but did not go to graduate for different academic reasons.

6.5. Study variables

6.5.1. Dependent variable

- Performance level.

6.5.2. Independent variable

- Age
- Sex
- Type of microscope
- A number of times allocated for a practical session.
- Type of academic institution

6.6 Measurement and data collection

6.6.1. Sample size determination

In Addis Ababa, there are two public academic institutions (Addis Ababa University, college of health science, school of medical laboratory & kotebe metropolitan university) and four private academic institutions (Rift valley University College, Africa Medical College, Yanet College, and Ekusta learning institution) providing medical laboratory sciences courses. A total of 106 medical laboratory graduating class students are included in these public and private academic institutions.

6.6.2. Sampling technique

- A Convenient sampling method was used.

6.6.3. Data collection procedure

6.6.3.1. Panel slide preparation and distribution

Experts who have been qualified and certified in TB microscopy at the national tuberculosis reference laboratory, Ethiopian Public Health Institute (EPHI) was prepare and validate the panel slides with a protocol number of **EPHI5.15/952**. Both TB-positive and TB-negative sputum smears were used. Further concentration, as well as dilution of bacilli, was done after bleach concentration techniques (**Annex-I**). Experts interpreted the prepared Ziehl Nelson-stained AFB smears for the presence or absence of TB bacilli and the quantification of bacilli**.**

A set of 10 validated slides was distributed to participating medical laboratory academic institutions to ‘‘assess the reading and interpretation proficiency of graduating medical laboratory students; 50-70 minutes is allowed to complete the reading. The panel composition and bacilli load are five negative, two 1–9, one 1+, one 2+, and one 3+. The composition of test panels was standardized according to the WHO manual and Ethiopia Federal Ministry of Health Guidelines. 10-panel slides were used for each academic institution, which means a total of 60-panel slides for 6 academic institutions involved. The detailed panel slide preparation, validation procedure, and Ziehl Nelson- staining procedures are indicated (**Annex-II).**

6.6.3.2. Panel slides Interpretation

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

controller, low false positive (LFP) when negative smears are misread as scanty (1-9 AFB/100 field) and low false negative (LFN) when a scanty (1- 9 AFB/100 field) was misread as negative (Table 1).

Table 1A. Evaluation and interpretation of errors between participants and reference laboratory (31).

Participant result	**Reference laboratory result**	
	Positive	**Negative**
Positive	True positive	False positive
Negative	False-negative	True negative

Table 1B. Evaluation and interpretation of errors between participants and reference laboratory (31).

Results of participants	**Results of reference laboratory**				
	Negative	**1-9AFB/100fields**	**1+**	**2+**	**3+**
Negative	Correct	LFN	HFN	HFN	HFN
1-9 AFB/100fields	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

6.6.3.3. Scores for Grading

A set of 10 pre-validated slides, each slide worth 10 points, the total possible score is 100 and the passing score was 80% and above.

- Committing major errors like high false positive (HFP) and high false-negative (HFN) worth zero points whereas minor errors like low false positive (LFP), low false negative (LFN), and quantification errors (QE) worth 5 points [33].
- The demographic information and associated factors collection form is attached in **Annex III.**

Likert scale of measurement used for the attitude of AFB, each interview questions 1–5 points were scored.

- “5 point-strongly agree", "4 point -agree", "3 point -neutral", "2 point-disagree" and "1 point-Strongly disagree”. Then calculated the mean value and categorized based on the following scale:-
    - 1.00-1.80 Strongly disagree.
    - 1.81-2.60 Disagree
    - 2.61-3.40 Neutral
    - 3.41-4.20 Agree
    - 4.21-5.00 Strongly agree.

6.7. Data quality assurance

Pre-analytical

- The sputum sample was collected in an appropriate sputum container and properly labeled.
- New clean frosted slides were used.

Analytical

- Reagents were filtered before use.
- ZN staining procedure was followed.
- Panel slide preparation SOP was strictly followed.

Post-analytical

- Pre-validated slides were distributed to participant universities in the slide box.
- The results of the students were kept in a safe place.

The stained smears with known grades of bacilli were prepared and validated by the National TB reference laboratory. Next to preparation and validation, the slides were arranged in ten sets and then packed for distribution to the participant.

6.8. Data entry, analysis, and interpretation

All data were entered into a Microsoft Excel (Microsoft, Inc., Redmond, Washington, United States) spreadsheet and exported to SPSS (version 25.0; SPSS, Inc., Chicago, Illinois, United States) for analysis. The percentages of agreements and different types of errors were calculated. The Chi-square test was used to assess associations between different variables. Then bivariate and multivariate analysis was done to see the association between outcome variables and independent variables. Among the baseline variables fitted to the binary regression model for bi-variable analysis $p<0.25$ was a candidate for multi-variable analysis. Finally, the level of significance was set at $p<0.05$. The strength of an agreement between participant readers and the reference readers is assessed using kappa statistics and the results were interpreted as a poor agreement (if $K<0.20$), Fair agreement (if K= 0.20 - 0.39), Moderate agreement (if K=0.40 – 0.59), Good agreement (if K= 0.60 – 0.79) and very good agreement (if K= 0.80 – 1.00) [34]. The qualitative data from in-depth and open-ended questions were organized, and categorized student responses were based on the Likert scale of measurement. Then summarized the findings and discussed by relating the findings thematically.

6.9. Ethical considerations

The study was obtaining ethical clearance from the Department of Research and Ethics Review Committee of Addis Ababa University, College of Health Sciences, Department of Laboratory Sciences with a protocol number of DRERC/645/21/MLS. The right of any individual not to participate or withdraw from the study at any time was fully respected. Data collection from each study participant was started after verbal consent is reached. During data collection, there was a high degree of confidentiality and informed consent was also obtained from each participant. No name or other identifiers on the questionnaire are found.

6.10. Dissemination of the result

The finalized paper of this study will be presented and submitted to Addis Ababa University, College of Health Sciences Department of Laboratory Science. A copy of this material will be given to participant universities. The finding will also be communicated to the Ministry of Health and the Ministry of technology and higher education. The result will also be disseminated through publication in peer-reviewed local and international journals and through presenting in relevant workshops, seminars, and scientific conferences.

6.11. Operational definitions

Academic institutions: the facilities accredited by HERQA to give education to the community.

Public academic institution: it is controlled and managed by a governmental organization.

Private academic institution: if it is controlled and managed by a non-governmental organization.

Graduating class students: students who will graduate together in the final year of completion of the study.

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

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

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

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

Quantification Error (QE): the difference of more than one grade in reading a positive slide between the examinee and controller.

Major error: error is considered the most critical mistake, it has high false negative/positive reports.

Minor error: errors that have minimum like low false negative/positive and quantification error.

Scanty: The term used to describe 1-9 acid-fast bacilli per 100 fields

Competency: Application of knowledge, attitude & skills in performance.

Performance evaluation: Systematically assessing student performance.

Ziehl-Nelson Stain (ZN): Acid-fast staining method using carbon fuchsin and methylene Blue.

Likert scale: a type of psychometric response scale in an in-depth interview specifies their level of agreement to a statement typically 5 points strongly agree (5), agree (4), neutral (Neither agree nor disagree) (3), disagree (2), and strongly disagree (1).

Specificity: Analysis able to distinguish TB from similar organisms under a microscope

Sensitivity: Analysis able to identify a small number (Scanty) of TB from positive slides.

Positive predictive value (PPV): It is the frequency of true positive slides among all positive slides.

Negative predictive value (NPV): It is the frequency of the true negative slide among all negative slides.

7. Result

7.1. Quantitative data analysis

7.1.1. Socio-demographic status of the participant

In this study, from 2 government and 4 private academic institutions, we have enrolled 106 students of which the majority, 65 (61.3%) were males and the mean age of the participant were 24.2±2.6 with the majority of the age group between 20 to 30 years **(Table 2.1).**

Table 2.1: - Socio-demographic status of study participants among graduating class of medical laboratory students in a private and public academic institution of Addis Ababa, Ethiopia, 2020/21.

Variable		**Frequency, (%)**
Sex	Female	41(38.7)
	Male	65(61.3)
Age group	18-21	65(61.3)
	22-25	30(28.3)
	26-30	11(10.4)
Total		106(100)

Most of the study participants 75(70.8%) were from a private academic institution. Most of the institution-located practical sessions related to bacteriology course 52(49.1%) were 2 secession/course (**Table 2.2**).

Table 2.2: - Status of an academic institution among graduating class of medical laboratory students in private and public academic institutions of Addis Ababa, Ethiopia, 2020/21.

Variable		**Frequency, (%)**
Type of educational facilities	Government	31(29.2)
	Private	75(70.8)
Type of Microscope	Olympus	63(59.4)
	ZEISS	28(26.4)
	Primo star	6(5.7)
	Unknown	9(8.5)
Practical time	2 session/course	52(49.1)
	3 sessions/course	36(34)
	No Session/course	18(17)
Total		106(100)

7.1.2. Student performance on AFB examination

Most students agreed on quality sputum smear preparation & examination of prepared AFB slides. These means were evaluating staining, size, thickness, cleanness, and evenness of the sputum AFB slide. The student evaluation on **"staining"** was observed under a microscope observed the color contrast, and the majority of 85/106 students were good staining. **"Size"** of smear or coverage of smear on the slide to looking at large area or field the student said good (100%) all are agreed. **"Thickness"** of the smear to says good if the slide is one layer or not multilayers, most of the students was says the thickness of the slide is good was 100/106. **"Cleanness"** was characterized by looking by necked eye & microscopically free from debris, & visible dirt, based on these criteria 106 students respond for the slide was good**. "Evenness"** was defined as the distribution of the smear symmetrically (consistent) coverage, almost half of the student responses was bad and were 56/106 **(Table 3).**

Table 3: - Students evaluated AFB slide characteristics among graduate MLS in Addis Ababa, Ethiopia, 2020/21 (n=106).

	Characteristics	Students respond (n=106)			
		Good		Bad	
		Frequency	%	Frequency	%
Pre-validated AFB slide	Staining	85	80.2	21	19.8
	Size	106	100	-	
	Thickness	100	94.4	6	5.6
	Cleanness	106	100	-	
	Evenness	50	47.2	56	52.8

From 4 private academic institutions, a total of 75 students were in the study. Among the participant 22(29.3%) scored 70-79%, 21(28%) scored less than 60-69%, 13(17.3%) marginal score (50-59%), and 6(8%) student score less than 50%. The mean score in this academic institution was 66.3% and overall PT results range from 40–100% **(Figure 2).**

From 2 government academic institutions, a total of 31 students were in the study. Among the participants 12(38.7%) scored 70-79%, 6(19.4%) scored less than 60-69%, 5(16.1%) scored less than 80-89%, 4(12.9%) marginal score (50-59%), and no student score less than 50%. The mean score in this academic institution was 72.9% and overall student performance results range from 50–100% **(Figure 2).**

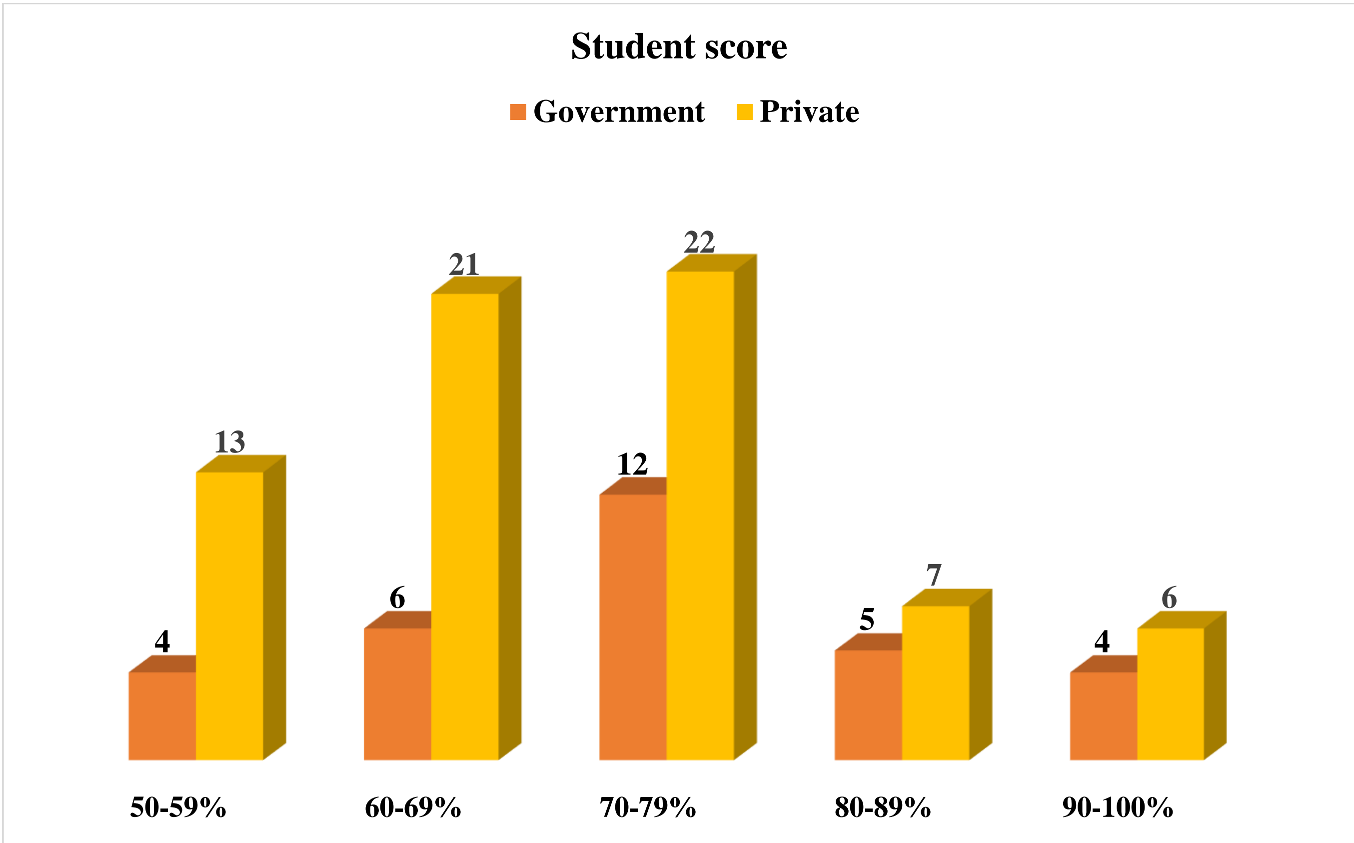

Figure 2: - Assessment of student TB sputum slide evaluation among government & private academic institution students score point, graduate MLS in Addis Ababa, Ethiopia, 2020/21.

Among 106 medical laboratory Science 2013/14 GC graduating batch students evaluated by pre-validated AFB slide, 22(20.8%) passing scored greater than 80%, and 84 (79.2%) scored less than 80%. The mean score was 68.2% and from 40-100% **(Figure 3).**

4).

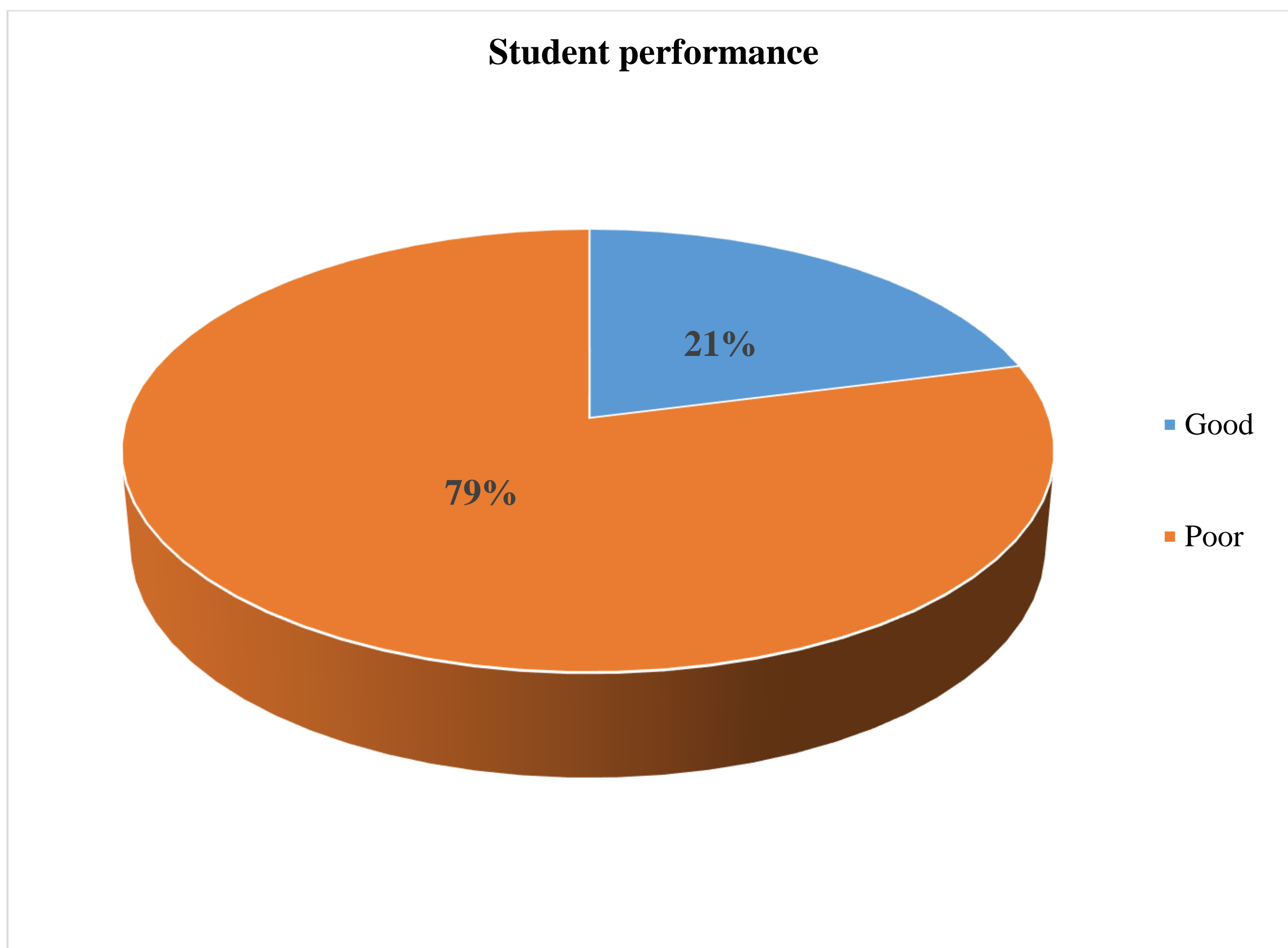

Figure 3: - Student status on TB sputum slide evaluation based on assessment for AFB smear microscopy WHO guidelines [30], Addis Ababa, Ethiopia, 2020/21.

7.1.3. Student AFB identification skill

From the panel slide reading, major errors (HFP and HFN errors) were observed, with HFN results being much more frequent than HFP results. Minor errors (i.e., LFP, LFN, and QEs) were observed in the majority of PT results QE being more frequently observed than LFP and LFN.

At 106 academic institutions graduated medical laboratory students were examined 10slides (PT-AFB smear-negative and positive slide) for each student then finally collect 1,060 PT slide test results from these 224 (2.1%) slide major errors 160 (1.5%) slide HFN and 64 (0.6%) slide HFP) and 321 (2.2%) slide minor errors 24 (0.6%) slide LFP, 101 (1.5%) slide LFN and 196 (1%) slide Quantification error) **(Table 4)**.

Table 4: - Frequency and types of errors for students evaluated by proficiency test among graduating medical laboratory students in Addis Ababa, Ethiopia, 2020/21.

Results of participants	Total number of slides prepare to be examined	Results of reference laboratory	Student answer (n=1,060slide)					
			Correct	Type of error				
				QE	LFP	LFN	HFP	HFN
Slide No 1	6	**Negative**	93	-	6	-	7	-
Slide No 2	6	**Scanty**	13	62	-	31	-	-
Slide No 3	6	**Negative**	82	-	5	-	19	-
Slide No 4	6	**Negative**	76	-	5	-	25	-
Slide No 5	6	**Negative**	93	-	2	-	11	-
Slide No 6	6	**(+2)**	17	30	-	-	-	59
Slide No 7	6	**Negative**	99	-	5	-	2	-
Slide No 8	6	**(+1)**	15	23	-	-	-	68
Slide No 9	6	**Scanty**	9	27	-	70	-	-
Slide No 10	6	**(+3)**	19	54	-	-	-	33
Total	**60**		**516**	**196**	**23**	**101**	**64**	**160**

Out of 1,060 slides examined by 106 graduating class of medical laboratory students, Most of the results were "True negative" results 443/1,060 slides reported then followed by "True positive" results accounted for 269/1,060 slides reported. The sensitivity, specificity, positive predictive value, and negative predictive value of reading smears were 51%, 84%, 76%, and 63% respectively. The percentage of agreement between participants with reference laboratory results was 67% and the strength of agreement between participant results and the reference laboratory was assessed by using kappa value were a Moderate (0.40 – 0.59) agreement K value 0.51 (**Table 5**).

Table 5: - Performance status of student performance among graduating class of medical laboratory students in a private and public academic institution of Addis Ababa, Ethiopia, 2020/21.

Student result	Reference Laboratory		Total	Sensitivity	Specificity	PPV	NPV	Accuracy	Kappa value
	Positive	Negative							
Positive	269	87	356	51%	84%	76%	63%	67%	0.51
Negative	261	443	704						
Total	530	530	1,060						

NPV; Negative predicted value, PPV; Positive predicted value

7.1.4. Association of student performance points with independent variables

This study indicated that student performance in this study except for the type of microscope was not statistically significant ($p>0.05$) affected by any independent variables listed in table 6. However, student performance is significantly affected by the type of microscope ($x^2 = 6.947$; $p= 0.044$) (**Table 6**).

The prevalence of poor student performance (Less than 80% score) was 79%. In Multivariate analysis, each type of microscope [AOR: 0.595, (95 % CI: 0.470-0.754) p-value < 0.001], [AOR: 0.253, (95 % CI: 0.176-0.362) p-value < 0.001], [AOR: 0.369, (95 % CI: 0.242-0.562) p-value < 0.001] were Olympus, ZEISS, & primo star respectively, and no practice session [AOR: 0.260, (95 % CI: 0.126-0.535)] were factors independently associated with poor student performance. The student who had a type of microscope was 0.6 times more likely to score poor performance level than those who had a good performance level. Students who had no practice session/course were 0.3 times more likely to perform with poor score value than those who had 2/3 practice session/course (**Table 6**).

Table 6: - Association factor of independent variables with student performance score points among graduate medical laboratory students in Addis Ababa, Ethiopia, 2020/21.

Variables	Category	Student performance		Student status					
		Poor	**Good**	**Chi-square (X^2)**	**p-value**	**COR (95%) CI**	**p-value**	**AOR (95%) CI**	**p-value**
Gender	Male	52 (49.1%)	13 (12.2%)	1.460	0.227	0.681(0.219-2.119)	0.507	0.526(0.155-1.783)	0.302
	Female	32 (30.2%)	9 (8.5%)			1		1	
Age group	18-21	51 (48.1%)	14 (13.2%)	0.170	0.918	0.886(0.717-1.095)	0.262	0.426(0.045-4.017)	0.456
	22-25	23 (21.7%)	6 (5.7%)			0.454(0.103-2.010)	0.155	0.574(0.067-4.930)	0.613
	26-30	10 (9.4%)	2 (1.9%)			1		1	
Educational facilities	Government	22 (20.8%)	9 (8.5%)	1.375	0.241	0.326(0.101-1.046)	0.060	0.445(0.122-1.620)	0.219
	Private	62 (58.5%)	13 (12.2%)			1		1	
Type of Microscope	Olympus	51 (48.1%)	12 (11.3%)	6.947	**0.044**	2.089(1.011-4.318)	**0.047***	0.253(0.176-0.362)	**0.001****
	ZEISS	25 (23.6%)	3 (2.8%)			1.240(0.189-8.126)	**0.030***	0.369(0.242-0.562)	**0.001****
	Primo star	3 (2.8%)	3 (2.8%)			9.725(0.287-10.936)	0.054*	0.595(0.470-0.754)	0.065
	Unknown	5 (4.7%)	4 (3.8%)			1		1	
Practical time	3session/course	28 (26.4%)	8 (7.6%)	3.325	0.190	1		1	
	2session/course	42 (39.6%)	10 (9.4%)			2.433(0.370-15.999)	0.355	0.126(0.015-0.241)	0.254
	No Session/course	14 (13.2%)	4 (3.8%)			0.892(0.309-2.577)	**0.034***	0.260(0.126-0.535)	**0.001****

COR: Crude odd ratio, AOR: Adjusted odd ratio, Factors significantly associated with P-value <0.05*, <0.001**:

7.2. Qualitative part of the study

7.2.1. Thematic review of student response on AFB smear

We did a qualitative research design for supporting our quantitative data for our research finding the AFB staining principle, procedure, reagents, examination, and identification. The interview was taken with 26 volunteer graduate MLS students from 6 academic institutions. Different academic institution students' have different ideas on AFB stating procedure uses, Majority of students argued that 2 credit hours (160 hours) practicum did not like as academic lecture and practice.

About AFB

- The majority of students were saying about *“AFB, starting from the term of AFB "Ziehl-Neelson (Acid fast Bacilli-AFB)” staining method, one of a bacterial microorganism, air born disease person to person transmitted through air droplet from an infected person to an exposed person”*. But 1 student gives an incorrect answer about AFB.

Principle of AFB staining

- Most students responded about AFB stating procedure was used for staining mycobacteria which are hardly stained by Gram‘s staining method. *“Sputum smear by applicator stick oval shape on slide dry then flooded a slide with a solution of carbon Fusin and heat–fixed and heated until steam rises then wait some minutes add acid alcohol to remove the primary dye, and last add counter staining dye”*.
- Finally, *“AFB color was red, and background of staining was blue color looking under a microscope*”. But some student tells *“about gram stain principles when asking about AFB staining principles asking”*.

Purpose of AFB staining other than other bacterial staining procedure

- All most ¾ of the student respond about different this AFB staining and other types of staining procedures choice for these types of bacteria, *"it is an alternative way of staining to identify the bacteria without of these nothing different other types of staining procedures or not special for AFB identified bacteria detect by using gram staining"*. Six students were *“Once the Mycobacteria is stained with carbon fusion then cannot be decolorized by acid, so named as Acid-Fast Bacilli”*.

Chemicals/dyes are used in AFB staining.

- Most of the respondent was uses chemicals or dyes procedures *“main reagents use 3dyes those are carbolfuchsin, 3% Acid alcohol, methylene blue was as a primary stain, decolorize & counterstain respectively”*. And some students “*mention gram stain dyes”*.

Describe the function of each of the following in the AFB primary stain, Decolorize & Counterstain

- The majority of student says, “*each primary stain was used to stain all cells, and microorganisms (Once the Mycobacteria is stained with carbon fusion then cannot be decolorized by acid), Decolorize was used to the smear is sufficiently decolorized & Counterstain was used to gives background stain and for decolorized bacterial species”.*

What is the purpose of the heat/steam during the acid-fast staining procedure?

- *Most of the students are gives answers about the purpose of heat on AFB staining procedure “the main feature of mycobacteria is their extraordinarily high lipid content in the cell wall contains Mycolic acid. So, the heating which facilitates penetration (entrance) of the primary stain into the bacterium”.*

Why are the organisms that resist decolorization by acid alcohol?

- Almost all students responded, "*mycobacteria their high lipid content in the cell wall contain Mycolic acid to uses impermeability to stains, acid fastness, and unusual resistance to killing by acid and alkali”.*

Colour of AFB in acid-fast staining

- Most student academic institution says was *a "Red colour with straight or slightly curved rods, occurring singly or in a small group”.* And the minority of student*s "yellow background with red straight or slightly curved rods”*

Which times of objective were used to look for AFB?

- All students say *“using 100x objectives for identification or examination of bacterial”.*

Purpose of the oil immersion objective

- Almost half of the students "*knew about the purpose of oil immersion add on using 100x objectives because of to prevent light dispersed out of the objective and targeted through the objective.*

Explain the reporting system of AFB.

- The majority of academic institution students said *"When no AFB are seen after examining fields report the smear as 'No AFB seen', but any correct Red color with straight or slightly curved rods, occurring singly or in small groups are seen report the smear as AFB positive and indicate the number of bacteria present as follows: When looking and count was 1- 9 AFB /100 fields report the actual number, 10 – 100 AFB/100 fields report +1, 1 – 10 AFB/field report +2, and more than 10 AFB/field report +3".* Minority of students interchanges between the group to groups of reporting like "*when no AFB are seen after examining 100fields report the smear as "Negative", and AFB are seen after examining 100fields report the smear as "positive" not reported +1, +2, +3".*

7.2.2. Likert scale analysis for qualitative data

This section reports the interview questions answered by 26 student participants. Table 7 showed the information collected and analyzed based on students' attitudes towards theoretical ZN staining procedures based on a 5-point Likert Scale, (1=Strongly Disagree, 2=Disagree, 3=Neutral, 4=Agree, 5=Strongly agree). The mean is very significant. From 1.0 to 1.8, it means strongly disagree. From 1.81 to 2.60, it means to disagree. From 2.61 to 3.40, it means neutral, from 3.41 to 4.20, it means agree; from 4.21 to 5.0, it means strongly agree. Findings from Table 7 revealed the below responses from the students for each of the aspects in the table mean greater than 3.40 except for AFB staining other than other bacterial staining procedures. This assessment indicates an attitude of student performance related to the ZN staining procedure for TB identification mean value was good (3.40-5.00), which was 91.7% **(Table 7)**.

Table 7: - Qualitative data based on Likert scale measurement among graduate medical laboratory students in Addis Ababa, Ethiopia, 2020/21.

No	Variable	N	Likert scale (#, %)					Result			
			5	4	3	2	1	Mean	Min	Max	Interpretation
Q1	What does it mean AFB	26	20(76.9%)	5(19.2%)	-	-	1(3.8%)	**4.65**	1	5	**Strongly agree**
Q2	Principle of AFB	26	5(19.2%)	15(57.7%)	-	5(19.2%)	1(3.9%)	**3.69**	1	5	**Agree**
Q3	Purpose of AFB staining other	26	5(19.2%)	1(3.8%)	-	10(38.5%)	10(38.5%)	**2.27**	1	5	**Disagree**
Q4	Chemicals/dyes are used in ZN staining procedure	26	10(38.5%)	5(19.2%)	-	10(38.5%)	1(3.8%)	**3.50**	1	5	**Agree**
Q5	The function of each of the following in the ZN dye	26	5(19.2%)	15(57.7%)	-	-	1(3.8%)	**4.08**	1	5	**Agree**
Q6	Purpose of the heat/steam during the ZN procedure	26	15(57.7%)	11(42.3%)	-	-	-	**4.58**	1	5	**Strongly agree**
Q7	Organisms that resist decolorization by acid	26	10(38.5%)	16(42.7%)		-	-	**4.38**	1	5	**Strongly agree**
Q8	Color of AFB	26	26(100%)	-	-	-	-	**5.00**	1	5	**Strongly agree**
Q9	The objective used to look for AFB	26	26(100%)	-	-	-	-	**5.00**	1	5	**Strongly agree**
Q10	Purpose of oil immersion	26	11(42.3%)	5(19.2%)	-	5(19.2%)	5(19.2%)	**3.46**	1	5	**Agree**
Q11	Express GOOD or BAD smear	26	10(38.5%)	11(42.3%)	-	5(19.2%)	-	**4.00**	1	5	**Agree**
Q12	Reporting system of AFB	26	15(57.7%)	5(19.2%)	-	3(11.5%)	3(11.5%)	**4.00**	1	5	**Agree**

NOTE: 5-strongly agree, 4-agree, 3-neutral, 2-dis-agree, 1-Strongly disagree

8. Discussion

Every laboratory technologist must be trained in sputum AFB microscopy. It is also desirable to know the proficiency of smear reading by the laboratory technologists before they are assigned the responsibility of sputum AFB microscopy. Both, false positive and negative results have serious implications. Since students graduating from higher institutions going to serve the community, retention of proficiency in sputum smear microscopy is most important.

Overall, 22(20.8%) students' score above 80% and 84(79.2%) students score below 80%. According to the international EQA guideline, PT scores below 80% are considered unacceptable performance. In this study, students examined 1,060 PT slides and there were 320 slides (30.2%) of students who report minor errors and 224 slides (21.3%) of students who report major errors. The most frequent minor error was QE which accounts for 196 slides (13.5%) of the total errors reported by the student's examination of slides. The main problem associated with this type of error (Quantification error, low false positive and negative). As we will be relying on TB smear microscopy for the foreseeable future, quality assurance of smear microscopy is of utmost importance to National Tuberculosis Control programs. All errors were defined as a quantification error (QE), a low-false-negative (LFN) result, a high-false-negative (HFN) result, a low-false-positive (LFP) result, or a high false-positive (HFP) result according to the international EQA classification. EQA results were interpreted by using the most stringent criteria listed in the guidelines, suggesting that any major error (HFP or HFN result) is unacceptable performance, as well as the least-stringent criteria, suggesting that any HFP result, more than three LFN results, and one or two HFN results define unacceptable performance [17]. The study conducts in other parts of the Ethiopian government's higher educational institutions the result different from this study 103(83.1%) students scored above 80% and 21(16.9%) students score below 80%. The main problem associated with this type of error (is high false positives and negatives) [20].

In this study, the student PT slide examination 516/1,060 (51.6%) slide correctly reported a PT result. A total of 1,060 slide PT result with 54.4% (544/1,060) error was reported that including major errors of 22.4% (160 HFN & 64 HFP) and minor errors of 32% (101 LFN, 23 LFP & 196 QE). The sensitivity, specificity, NPV, and PPV of students in detecting TB bacilli as compared to the reference reading were 51%, 84%, 63%, and 76%, respectively. The overall agreement of the student's result with the reference reading on the PT result was moderate (Kappa = 0.51). In another study conducted to evaluate the quality of TB smear microscopic examination in Hawassa on 81 participants, 11(13.6%) correctly reported panel

slides, A total of 29.75% (241/810) error was 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%, respectively. The overall agreement of participants with the reference reading on TB detection was 95.18% (Kappa = 0.73) [23]. Another study was conducted in Ethiopia, 324 (83.3%) participants scored ≥ 80%. Sensitivity was 84.5% and specificity was 93.1%. The overall percent agreement between participants and reference readers was 87.1 (kappa=0.72) [27]. The overall compare our result with another study's performance of participants in reading the slides showed poor agreement with the reference readers. Most errors were minor because the type of microscope statically significantly with less than 80% score students and to increase the ability to detect tuberculosis bacilli can be improved by building the capacity of microscope utilization & practice TB identification.

9. Limitation of the study

- We face a major challenge with students were not volunteer for interviews during these studies.
- Lack of trust in the result of this finding to utilize for HERQA re-accreditation process on evaluation of student or interview and looking availability laboratory and asking some laboratory instrument like a microscope.
- To our knowledge, there are not enough published similar studies conducted globally and nationally.

10. Conclusion and recommendation

10.1. Conclusion

From this study, AFB smear evaluation to read sputum smear by graduating students who have scored pass point ≥80% accounted for 20.8% (22/106). There were 21.1% (224/1,060) major errors & 30.2% (321/1,060) slide minor errors (The majority of minor error was categorized as quantification error (196/1,060slides)) which was reported by the student who participated in AFB smear microscopy identification. Based on this finding there was a gap in the counting and grading report system of AFB identified under the microscope by students.

The student attitude based on student interview response was 91.7%, this indicates good attitude on AFB smear technique, and factors contributing to decreasing student performance related to the allocation of practical time and type of microscope. Thus, the study has highlighted the importance of training, quality of microscope, and practical time in improving the microscopic identification of AFB.

10.2. Recommendation

- The first step to improving the effectiveness of AFB microscopy networks is increasing the ability of laboratory technologists that read sputum smear microscopy to diagnose tuberculosis.
- Since our findings indicate that students had poor competency in reading and grading reports on AFB. We recommend that students should take pre-service training before they go to serve the community.
- Gives more attention to practical sessions during student practicum or team training programs (TTP) must be given a great concern and more practical session set students, these means techniques related topics gives parallel with the theoretical part of the course.

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Annexes

Annex I - Panel preparation and Validation procedure NaOH Method

1. Materials Required

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

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

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

1. Place 3 ml of AFB-positive specimen into a 50 ml screw-cap plastic tube. If the volume of the specimen is more than 3 ml, aliquot it into separate tubes.
2. Add 1 drop (approx. 50 µl) of 40% Formaldehyde per 1 ml of sputum, vortex well.
3. Incubate for 1 hour at room temperature (25- 30oC).
4. 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%.
5. Vortex thoroughly for 4-5 min.
6. Add up to 20 ml of distilled water, and mix well.
7. Incubate in a water bath for 30 min. at 55-60ºC, mix occasionally by inverting the tube during incubation.
   - If there is no water bath available, boil a beaker of water, cool it to 90-95ºC, and place the tube in the beaker for 20-25 min.
   - It is important to maintain the incubation temperature in the 55-90^{0}C range.
8. Add distilled water to a total volume of 40 ml, and mix by inversion.
9. Centrifuge at 3,000 x g for 20 min. at room temperature (25-30ºC).
10. 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, before 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

1. Distribute 3-4 ml aliquots of AFB-negative sputum into 50 ml screw-cap tubes.
2. Note: Several good-quality negative sputa can be pooled together and then split into 3 ml aliquots. Sputa should be checked for AFB before pooling.
3. Add 1 drop (approx. 50 µl) of 40% Formaldehyde per 1 ml of sputum, vortex well.
4. Incubate for 1 hour at room temperature (25-30ºC).
5. 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%).

6. Vortex for 2-3 min.
7. Add up to 20 ml of distilled water, and mix well.
8. Incubate in a water bath for 10 min. at 55-60°C.

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 it to 90-95°C, 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

1. If the foam has formed on top of the stock solution, pipette the contents from beneath the foam into a fresh tube.
2. Using a standard microbiological loop make 2-3 test smears (approx. 1x2 cm in size) from the suspension for evaluation of the stock preparations.
3. Use a well-leveled surface for drying the smears.

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

5. Dilution Procedure

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

0 AFB/100 fields	Negative
1-9 AFB/100 fields	The exact number of AFB
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 the suggested range. For better results, however, it may be recommended to use 20 AFB/field for 3+ smears, 5 AFB/field for 2+ smears, 50 AFB/100 fields for 1+ smears, and 5 AFB/100fields for “exact” smears.

C) Make 3-4 ml of each suspension to be able to generate enough 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 number 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 the number of drops of positive sputum to be added.

DC - is the desired AFB concentration.

AC - is the actual AFB concentration.

A - is the number of drops in each volume that was estimated during calibration.

Example: -

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

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

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

Procedural notes

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

1) Using diluted stock preparations, prepare slide batches (50-100 slides per batch are 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.

2) 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.

The validation Log for AFB Panel testing slide batches can be used to record results for the test slides and determine if the 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 is recommended that laboratories prepare 50-100 slides so that sufficient slides are available to put duplicate samples in test slide sets.

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

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

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

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

$$x = \frac{\sqrt{\mathrm{n\Sigma x2} - (\mathrm{\Sigma x})2}}{\mathrm{n(n-1)}}$$

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 [29,30].

7. Prepare Panel Testing Sets

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

Annexes II - Ziel Nelson staining for sputum smear

Principle:

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

Method

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

Result: The AFB is stained bright pink

Annex III. Demographic information and result collection form

1. Facility Code: _____________
2. Age: ______
3. Sex: (Male / Female)
4. Type of university (PUBLIC/PRIVATE)
5. Availability of side laboratory (available / Not available)
6. Type of microscope writes the type………………………….
7. Type of staining method used………………………………
8. The number of times allocated for practical session……………………………………………………......

Slide No.	Result	Staining		Size		Thickness		Cleanness		Evenness	
		Good	Poor (U/O)	Good	Poor (B/S)	Good	Poor (K/N)	Clean	Not clean	Good	Poor

- If the staining is poor Write **U** for under-decolorized, and O for Over-decolorized.
- If the size is poor Write **B -** for too big, **and S** -for too small.
- If the thickness is poor write **K** – for too thick, **N** – for too thin

Interview Questions for qualitative study

1. What does it mean AFB?
2. Principle of AFB
3. Purpose of AFB staining other than other bacterial staining procedures.
4. Which chemicals/dyes are used in AFB staining procedure?
5. Describe the function of each of the following in the AFB stain.
   5.1.Primary stain
   5.2.Decolorizer
   5.3.Counterstain
6. What is the purpose of the heat/steam during the acid-fast staining procedure?
7. Why are the organisms that resist decolorization by acid and alcohol?
8. The color of acid-fast bacteria in acid-fast staining is ___________________________
9. Which times of objective are used to look for acid-fast bacilli?
10. Describe the purpose of the oil immersion objective.
11. How to express a GOOD or BAD smear?
12. Explain the reporting system of AFB staining.

Annex IV: Information sheet and Verbal Consent form (English and Amharic versions)

Background Information: My name is Eden and currently I am a master's student in clinical laboratory sciences in the medical laboratory management track. I am doing research undertaken by Addis Ababa University, School of Medical Laboratory sciences.

Aim of the study: The study aims to evaluate the performance of graduating class medical laboratory students in the detection of AFB under a microscope.

Benefits of the study participant: The study can allow participants to know their ability in AFB smear microscopy.

Risks and Complications of the participant: The study has no risks and complications for the participants.

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

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

I hope that you will be honest in answering questions and reading 10 stained slides:

Do you agree to answer the following questions and read 10 slides to the best of your ability?

Yes (---------------------) No (-----------------------)

If your answer is yes, please continue responding to a few questions and reading 10 stained slides.

Thank you for participating in this important study.

Participant signature: ________________ Date: ______________________

Annex V: Information Sheet (Amharic Version)

መግቢያ ሀሳብ፡እኔ ኤደን አላምረው የተባልኩ ባሁኑ ሰዓት በአዲስ አበባ ዩኒቨርስቲ በክሊኒካል ላቦራቶሪ ሳይንስ የሁለተኛ ዲግሪ ፕሮግራም በክሊኒካል ላቦራቶሪ ማኔጅመንት እና ኳሊቲ አሹራንስ ትምህርት ክፍል እየተከታተልኩ እገኛለሁ።

የጥናቱ አላማ: የላቦራቶሪ ተመራቂ ተማሪዎችን ቲቢባክቴሪ የመለየት ብቃታቸውን ለመገምገም ነው።

ሊያጋጥም የሚችል ጉዳት፡በጥናቱ ላይ የሚሳተፉ ተማሪዎች ላይ የሚደርስ ምንም አይነት ጉዳት የለም።

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

በጥናቱላይያለመሳተፍመብት ፡በጥናቱ ላይ ለመሳተፍ ሙሉበሙሉ በበጎፍቃደኝነት ላይ የተመሰረተ ነው፤ አለመሳተፍም ሆነ ጥናቱን በፈለጉት ስዓት ማቋረጥ ይችላሉ ፤ ነገርግን የእርስዎ በጥናቱ ላይ መሳተፍ የተፈለገውን ውጤት ለማግኘት በጣም ጠቃሚ ነው።

በግልፅነት እና በታማኝነት የምንሰጥዎትን 10 ስላይዶች እንደሚያነቡልን ተስፋ እናደርጋለን።

የምንሰጥዎትን 10 ስላይዶች ለማንበብ ፍቃደኛነዎት?

አዎ ( ) አይደለሁም ( )

መልስዎ አዎ ከሆነ ፤ እባክዎትን የሰጠንዎትን 10 ስላይዶች ማንበብ ይቀጥሉ።

በዚህ ጠቃሚ ጥናት ውስጥ ስለተሳተፉልን በጣም እናመሰግናለን።

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

Annexes VI Dummy table

Evaluation and interpretation of errors between participants and reference laboratory

Results of participants	Results of reference laboratory				
	Negative	1-9AFB/100fields	1+	2+	3+
Negative					
1-9 AFB/100fields					
1+					
2+					
3+					

Sensitivity, specificity & predictive values of the microscopy for panel slides

Graduating result	Reference Laboratory		Total	Sensitivity	Specificity	PPV	NPV	Kappa value
	Positive	Negative						
Positive	TP	FP						
Negative	FN	TN						
Total								

Declaration

Assurance of Principal Investigator

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

Name of the student: **Eden Alamirew** (BSc, MSc candidate)

Date ______________________________ Signature ____________________

Approval of Advisors:

Mr. Abay Sisay (MSc, Ph.D. fellow)

Date_____________________________ Signature ______________________

Mr. Habtamu Molla (MSc, Ph.D. fellow)

Date ____________________________ Signature ________________________