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Comparison of direct microscopy (ziehl neelsen) and light-emitting diode fluorescent microscopy) against Xpert MTB/RIF for the diagnosis of pulmonary tuberculosis and assessing facility infrastructure in Assosa general hospital Benishangul- gumuz region, Western Ethiopia

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This is to certify that the thesis prepared by Ashebir Legesse entitled: "Comparison of direct microscopy (ziehl neelsen and light-emitting diode fluorescent microscopy) against GeneXpert MTB/RIF for the diagnosis of pulmonary tuberculosis and Facility Infrastructure Assessment of routine TB diagnosis in Assosa general hospital Benishangul- gumuz region, Western Ethiopia." submitted in fulfillment of the requirements for the degree of Master of Science in Clinical Laboratory Sciences (diagnostic and public health microbiology specialty track)

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Table of Contents

I. Acknowledgement I

II. Lists of Figures V

III. Lists of Tables V

V. List of Abbreviations VI

Abstract VII

1. Introduction .... - 1 -

1.1. Background .... - 1 -

1.2. Statement of the problem .... - 2 -

1.3. Significance of the Study .... - 4 -

2. Literature Review .... - 4 -

3. Objective .... - 7 -

3.1 .Genera objective .... - 7 -

3.2. Specific Objectives .... - 7 -

4. Materials and methods .... - 7 -

4.1. Study Area .... - 7 -

4.2. Study Period .... - 9 -

4.3. Study design .... - 9 -

4.4. Source population ....- 9 -
4.5. Study Population....- 9 -
4.6. Inclusion and exclusion criteria ....- 9 -
4.6.1. Inclusion criteria ....- 9 -
4.6.2. Exclusion criteria ....- 10 -
4.7. Measurement and Data collection procedure....- 10 -
4.8. Sample size calculation and Sampling method....- 10 -
4.9. Study Variables....- 11 -
4.9.1. Dependent variable: ....- 11 -
4.9.2. Independent Variable:....- 12 -
4.10. Data collection ....- 12 -
4.10.1. Sample Collection and socio-demographic data....- 12 -
4.11. Laboratory Diagnostic Methods ....- 12 -
4.11.1. Gene Expert Assay....- 12 -
4.11.2. Ziehl–Neelsen staining....- 13 -
4.11.3. Fluor chrome staining ....- 13 -
4.12. Facility infrastructure assessment....- 13 -
4.13. Operational terms....- 13 -

4.13. Data Quality Assurance ....- 15 -

4.14. Ethical Clearance ....- 16 -

5. Results ....- 16 -

6. Infrastructure assessment based on Onsite supervision. ....- 24 -

7. Discussion....- 26 -

8. Limitation and Conclusion ....- 29 -

9. Recommendation ....- 30 -

10. References ....- 31 -

Annex I: participants information sheet [English version]....- 36 -

Annex II. Informed consent [English version] ....- 37 -

Annex III: participant’s information sheet [Amharic version] ....- 38 -

Annex IV: consent [Amharic version] ....- 39 -

Annex V: Result Report and interpretation AFB smear microscopy ....- 40 -

Annex VI: On-Site Evaluation Checklist ....- 41 -

Annex VII: SOPs for AFB Reagent Preparation ....- 45 -

Annex VIII. Assurance of Principal Investigator ....- 48 -

II. Lists of Figures

Figure 1:Demographic map of Assosa Zone, Benishangul Gumuz region, 2017 .... - 8 -

III. Lists of Tables

Table 1: Interpretation of Cohen's kappa .... - 14 -

Table 2: A description of study participants based on their sex and age groups................ - 17 -

Table 3: Magnitude of PTB based on sex and age category at Assosa General Hospital.2019 - 18 -

Table 4: Microscopic observation of AFB .... - 20 -

Table 5: Performance of FM smears microscopy in reference to Gene xpert........................ - 21 -

Table 6: Performances of ZN smear microscopy in reference to Gene xpert .... - 22 -

Table 7: Infrastructure quality supporters Variables of Tb diagnosis .... - 25 -

V. List of Abbreviations

AIDS---Immune Deficiency Syndrome

ART---Anti-Retroviral Therapy

DNA---deoxyribonucleic acid

HIV---Human Immune deficiency Virus

LED-FM--- Light Emitting Diode Fluorescence Microscopy

LJ---Lowenstein-Jensen Medium

MDR-TB--Multi Drug Resistance Tuberculosis

MTB---Mycobacterium tuberculosis

NPV---Negative Predictive Value

OI--Opportunistic Infection

PPV--Positive Predictive Value

PTB--Pulmonary tuberculosis

RIF---Rifampecin

RNA-- Ribose Nucleic Acid

rpoB--RNA polymerase gene

RT-PCR---Real-Time Polymerase chain Reaction

TB--Tuberculosis

WHO---World Health Organization

ZN---Zieh lNeelsen

Abstract

Background: Globally TB is associated with a lot of morbidity and mortality .More so in high burden like Countries like Ethiopia and other African Countries where high prevalence of HIV has further compounded this problem leading to resurgence of TB. Moreover, the emergence of MDR- and XDR-TB coupled with inefficient diagnosis is a major challenge to TB control leading to TB being one of the major public health problems in poor resource countries like Ethiopia.

Objective: This study aimed to evaluate performance of smear microscopy with X-pert/Rif and facility infrastructure assessment was carried out in Benishugul regional sate, Assosa General Hospital, western Ethiopia

Methods: Hospital based cross-sectional and descriptive study was conducted on a total of 331 sputum samples collected from Assosa General Hospital. The sputum samples were analyzed using gene expert, ZN and FM methods. Kappa value, Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of iLED-FM and ZN were calculated against the gene xpert. And real practice of current Tb laboratory diagnosis was assessed from nineteen periphery health centers

Results: The sensitivity, specificity, PPV and NPV of LED-FM was 97%, 100%, 100% and 100% respectively. The measure of agreement and percent agreement between the LED-FM and reference standard results were substantial with kappa value (k=0.99) and 99 respectively. The sensitivity, specificity, PPV and NPV of ZN when compared to Gene expert, were 81%, 100 %, 100 %, 99% respectively with percent agreement 89 and kappa value of 0.89

Conclusion: The overall performance of LED microscopy is superior compared to ZN TB microscopy. The sensitivity and specificity of LED is comparable with GeneXpert and use of combined LED –Gen-expert methods can increase case detection of PTB particularly in resource limited settings.

Key Words: Level of agreement, comparison with gene expert, LED Microscopy, Facility infrastructure assessment.

1. Introduction

1.1. Background

Mycobacterium tuberculosis is the second most common infectious cause of death in adults worldwide (HIV is the most common). The human host serves as the natural reservoir for M.tuberculosis. The ability of the organism to efficiently establish latent infection has enabled it to spread to nearly one-third of individuals worldwide **[1].**

Pulmonary tuberculosis (TB) is a major health issue in the developing world. Since it is spread by droplet infection, timely diagnosis, treatment and cure is of utmost importance. Patients, who are suspected of having tuberculosis, are referred for sputum smear microscopy to tuberculosis laboratories, where AFB microscopy using either ZN or FM techniques is performed **[2].**

Main reasons for using the ZN smear microscopy is its low cost, high specificity, does not require sophisticated equipment or high laboratory standards**[3] .**

The World Health Organization (WHO) has recommended scaling up the use of light-emitting diode fluorescent microscopy (LED FM) which is on average10% more sensitive in detection of TB compared to the conventional ZN-based light microscopy using culture as a gold standard **[4].**

Early diagnosis is imperative for early patient management and successful patient outcomes. False-negative results and misdiagnosis of TB suspects are common in developing nations, as most TB control programmed use Ziehl-Neelsen (ZN) smear microscopy, which has poor sensitivity and multiple visits are required that leads to higher default. Mycobacterium culture, although considered as the gold standard but is slow and usually takes 2-6 weeks time to yield a final result and requires proper infrastructure and technical expertise Also does not provide a rapid diagnosis therefore cannot be afforded in most of resource limited settings [**5].**

Microscopy is not as sensitive and specific as GeneXpert which is described as a molecular technique for MTB detection. However, in most nations, the conventional microscopy, culture and drug susceptibility testing (DST) method is used in TB diagnosis [6].

Recently, molecular diagnostics are increasingly being promoted as TB diagnostic tools due to rapid turnaround time, high sensitivity and specificity of the techniques. Consequently, WHO recommend GeneXpert to be used as an initial diagnostic test in individuals suspected of Multi-Drug resistance tuberculosis (MDR-TB), extra PTB, HIV-associated TB and children suspected of TB Patients with presumed TB or MDR-TB would undergo testing with Xpert MTB/RIF. Xpert MTB/RIF could be performed as an initial test or as an add-on test after prior testing with microscopy. Following an Xpert MTB/RIF test, subsequent culture and drug susceptibility testing (DST) are recommended to monitor treatment progress and to detect resistance to drugs other than rifampicin [7].

1.2. Statement of the problem

The global burden of TB may be summed up in economic terms through a few brief computations. Given 8.4 million sick, according to the most recent WHO estimates, the bulk of them potential wage-earners, and assuming a 30% decline in average productivity, the toll amounts to approximately $1 billion yearly. Two million annual deaths, with an average loss of 15 years' income, add an additional deficit of $11 billion. Every twelve months, then, TB causes somewhere near $12 billion to disappear from the global economy [8]

Mycobacterium tuberculosis remains the most common opportunistic Infection (OI) and is the most common cause of death in HIV infected patients and also increased risk of both reactivation of latent TB infection and acquisition of new TB infection. Tuberculosis is the most common opportunistic Infection (OI) among HIV-infected individuals, and co-infected individuals are at high risk of death [9].

There were cases in all countries and age groups, but overall 90% were adults (aged ≥15 years), 9% were people living with HIV (72% in Africa) and two thirds were in eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). These and 22 other countries in WHO's list of 30 high TB burden countries including Ethiopia accounted for 87% of the world's cases.4 Only 6% of global cases were in the WHO European Region (3%) and WHO Region of the Americas (3%) [8].

Three methods are most often used in the diagnosis of TB and these are microscopy, culture and GeneXpert. Of these, microscopy is not as sensitive and specific as GeneXpert which is described as a molecular technique for MTB detection. However, in most nations, the conventional microscopy, culture and drug susceptibility testing (DST) method is used in TB diagnosis. There is however, a possibility of a misdiagnosis leading to a false positive or false negative result in cases where culture is done. Furthermore, culture is time consuming, as it requires about 2-6 weeks to get a result and also requires technical expertise [10].

In Ethiopia, most health facilities have limited capacities to diagnose TB. For instance, in 59% health facilities in Ethiopia, the only available diagnostic test was sputum smear microscopy, 2% have GeneXpert. According to center for strategic and international studies, nearly 48% of all active TB cases would have been missed while screened by sign and symptoms. Hence, the need to expand and utilize different diagnostic modalities in different context of Ethiopia plays great role in increasing case detection and control of TB [7].

Benishengul Gumuz region has 471 woreda health center including refuge clinics. From the total of 471 health center 21 of them do not have Fluorescence Microscopy and Five of them do not have function Fluorescence Microscopy and also only five Health facilities(Assosa General Hospital, Kamash Health centers ,Bullan (Metakal) health center ,Pawe Hopital and Assosa Regional Laboratory) have Molecular diagnosis services which most of woreda facilities used as referral center .This study also focuses on the assessment of real practice of direct microscopy along with gene expert in Assosa General Hospital and selected Woreda Health center, western Ethiopia.

1.3. Significance of the Study

Burden of the TB Globally and locally still major health problem but in order to investigate the causative agent of Micro bacterium mostly used techniques is direct microscopy. However different studies show that the performance of direct microscopy lower than from the advanced Gene xpert technique

In our study area diagnostic of micro bacterium tuberculosis mostly performed by direct microscopy due to lack of advanced MTB diagnostic techniques in the region. Therefore, these findings could provide alternatives fast and more sensitive methods that can improve TB care in the region.

2. Literature Review

Most systematic review articles and journal stated that the newly molecular diagnostic (Gene expert) has better performance has been evaluating both MDR TB and MTB from smear negative HIV patients and children while comparing with DM (direct microscopy). And the studies used conventional culture as golden standard which is not available in the resource constrains areas, because it is time consuming and requires Biosafety setup and trained laboratory personals [11].

The study conducted in Niger delta in two different institution Port Harcourt Teaching Hospitals (UPTH) and the Braithwaite Memorial Specialist Hospital (BMSH), showed that from total of 555 sputum samples was collected. The two methods explored were ZN - Microscopy and the GeneXpert assay method. Using GeneXpert 26.2% and 30.9% of the samples were positive for MTB in UPTH and BMSH, respectively. While microcopy detected 15.4% and 29.4% MTB samples from UPTH and BMSH respectively [12].

The same study conducted in Pakistan by Shagufta Iram et al showed M. tuberculosis (MTB) were detected by Xpert MTB/RIF test in 111 (45.3%) out of 245 samples. Of these, 85 (34.7%) were smear positive on ZN staining. Rifampicin resistance was detected in 16 (6.5%) patients.

Nine out of 19 pus samples (47.3%) were positive for MTB by Gene Xpert, 03 (15.8%) on ZN staining and 04 (21%) [13].

Similarly the study carried out in the University of Port Harcourt Teaching Hospital Rivers State, Nigeria total of Sample size used 600 samples collected from mixed population indicated with the GeneXpert MTB/RIF Assay which was compared with the conventional Ziehl-Nelson Smear Microscopy. Sixty-nine (11.50%) were Mycobacterium tuberculosis detected while 531 (88.50%) were MTB not detected. Also, fifty-eight (9.6%) were positive for Ziehl-Nelson (ZN) smear microscopy while 542 (90.33%) were ZN smear microscopy negative and five (0.83%) were invalid.

A proportional difference in sensitivity was only observed among MTB detected low (23.53%) against Smear positivity (10.0%). This is a remarkable advantage of GeneXpert that uses as low as 131 ml of sputum but a limitation for smear microscopy requiring a minimum of 10,000 ml of sputum to diagnose TB [14].

Another study conducted in Benin City revealed that 33 out of 33 ZN technique positive were GeneXpert technique positive while 26 out of 148 ZN techniques negative were detected positive by GeneXpert technique. The difference in the number diagnosed by GeneXpert is a source of public health concern to embracing this modern technique [1].

Study conducted in Ethiopia, Addis Ababa, from a total of 286 sputum samples analyzed by culture and LEDFM, 105 (36.71%) were positive by both methods; 7 (2.45%) were smear positive by LED-FM. The measure of agreement between the LED-FM and reference standard results were substantial with kappa coeffient (k=0.765) and p-value (<0.001) [7].

Similar study conducted in western Ethiopia, in Ambo hospital, concluded with sample size 340 LED-FM and Gene expert of these 340, two samples were rifampicin resistant by both Xpert and DST. The overall sensitivity, specificity, positive and negative predictive values of

LED-FM and Xpert were 77.8, 100, 100 and 96; and 93.3, 98, 97.5 and 98.9% respectively [15].

Smear examination is believed to be simple, cheap, quick, practical, and an effective case finding method for developing countries. Microscopic examination of Ziehl–Neelsen (ZN) or auramine stained specimen allows detection of most strains in less than an hour. The fluorochrome stain offers the advantage of greater sensitivity compared with carbolfuchsin method since a significantly larger area of the smear can be scanned per unit time with auramine fluorochrome stain. In addition, a positive fluorescent smear may be restained using the conventional ZN [16].

Similar to the above this study mainly focus on the comparison of Direct microscopy (Light and LED-FM) against gene expert (as a golden standard) Despite its lower sensitivity, smear microscopy remains the main diagnostic method for pulmonary tuberculosis (PTB)in resource-limited countries [10].

Therefore the study focuses on the strengthening and assessing infrastructure TB Laboratory diagnosis along with comparative study .The sample size is 484 based on single proportion formula based on the expected prevalence of pulmonary tuberculosis, kappa value, sensitivity, specificity, PPV and NPV will be calculated by using 2x2 table.

3. Objective

3.1 .Genera objective

To compare routine fluorescence microscopy and Zeihl Neelsen techniques against Gene expert for diagnosis pulmonary tuberculosis, among new cases attending Tb and ART clinic in Assosa General Hospital and assessing the facilities infrastructure in Benishengul gumuz regional state.

3.2. Specific Objectives

1. To assesses the level of agreement between fluorescence microscopy, Zn techniques and gene expert methods for the diagnosis of MTB
2. To determine the specificity ,sensitivity, positive and negative predictive value of smear Microscopy
3. Assessing the facility infrastructure of TB diagnosis in the Assosa woreda health center and Assosa General Hospital

4. Materials and methods

4.1. Study Area

The Study was conducted in Benishengul Gumuz Regional state Assosa city which is found in Beninshengul Gumuz Region locate at Western Ethiopia. Assosa town is the capital of Benishangul Gumuz Regional State (BGRS), which is one of the nine regional states comprising the Ethiopian federal structure. According to information obtained from municipality of the town, Assosa is a town founded in 1984, which is located in western extreme part of Ethiopia about 662 km from Addis Ababa in Benishangul Gumuz Regional State. So, the town could be taken as one of the border towns in the country and located at 90 km away from the Ethio-Sudanese border. It is situated on a flat plane at an average altitude of 1,550 meter above sea level (MWR, 2001).The town geographically located between 10̊ 00' and 10°03' north and between 34°35' to 34°39' east and lies on an area of about 982.5 hector. It

is surrounded by resettlement villages: in the North by Amba 8, in the South by Amba 38 and Amba 3, in the East by Amba 4 and in the West Komosha town .The area can be accessed through ground and air transportation. The ground access is via Ambo- Nekemte- Gimbi-Nejo-Mendi-Bambasi and then to the study area (Assosa). The road from Addis Ababa to Assosa is partly asphalt. The region has 471 woreda health centers and two General and primary Hospitals, Assossa General Hospital and Pawe General Hospital, Menge and Wanbera primary hospitals with one regional laboratory located in Assosa city [17].

The total population of the Assosa city in 2017 is 34,320 under the city there are 25 woreda health centers and 15 private clinics.

Figure 1: Demographic map of Assosa Zone, Benishangul Gumuz region, 2017

4.2. Study Period

The study was conducted from December 2018 up to April, 2019

4.3. Study design

A-Cross-sectional and observational descriptive study were conducted in Assosa General Hospital and around Assosa woreda.

4.4. Source population

- All patients visiting TB and ART clinic in Assosa Genera Hospital is source population for the study
- And all Laboratory health facility catchment site to Assosa General Hospital around Assosa woreda.

4.5. Study Population

- All patients having coughed more than two weeks and referring to Tb and ART Clinic in Assosa General Hospital was selected as study subjects and nineteen selected health facilities around Assosa woreda were also incorporated in the study group.

4.6. Inclusion and exclusion criteria

4.6.1. Inclusion criteria

The study was included those individuals potentially produce proper sputum sample and in compliance with sign and symptom of Tb included in this study. Laboratory personnel who were willing to participate and provided consent were included in this study.

4.6.2. Exclusion criteria

Patients on anti-Tb treatment and follow up cases were excluded from the study.

Patients that could not produce sputum were excluded from the study.

4.7. Measurement and Data collection procedure

A structured questionnaire with slight modification from that prepared by the WHO was administered to capture socio-demographic and clinical data for consenting patients before sputum sample collection. The second questioner used which assess the overall facilities infrastructure assessment to determine the ground peripheral laboratories towards diagnosis of Mycobacterium tuberculosis.

4.8. Sample size calculation and Sampling method

4.8.1. Method comparison

The sample size was determined using a formula of single population proportion[n=(Zα/2)2 P(1-P)/d2] based on the expected prevalence was to increase the required sample size non convenient sampling method and 95% confidence interval are used to calculate the sample size. This study calculated the sample size using a formula developed by Mallinckrodt B [18] for epidemiological studies as shown below

n=Z2 *P(1-P)/d2

Where, n = sample size

Z = Z statistic for a level of confidence

P = expected prevalence or proportion

d = marginal error

Given that expected prevalence of 50% was taken, assuming the marginal error d=0.05 which is half of p and Z is 1.96 at (95%CI).Therefore the sample size is calculated as follows

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

n =384

Assuming an attrition of 10 % then the study participants become 422 However, to increase precision we reached 484 of total sample size.

4.8.2. Sample size determination for Facility infrastructure Assessment :

Convenient sampling method was used for selection of facilities those 19 peripheral facilities were included for onsite evaluation.

4.9. Study Variables

4.9.1. Dependent variable:

Performance of LED microscopy and ZN including kappa statistics

Facility infrastructure assessment

4.9.2. Independent Variable:

3.3. Age, Sex and clinical sign and symptoms

4.10. Data collection

4.10.1. Sample Collection and socio-demographic data

Paired sputum samples were collected from 484, study participants using standard sputum collection tubes. All demographic data were collected using the structured questionnaires prepared for this study.

4.11. Laboratory Diagnostic Methods

4.11.1. Gene Expert Assay

Procedure

- The sputum samples were treated with sample reagent (SR) containing Noah and isopropanol provided as per the manufacturer’s instruction.
- The SR is added using a 2:1 ratio of the sputum sample, homogenized and incubated for 15 min at room temperature.
- From the treated samples 2 ml was transferred into multi chambered plastic cartridge preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing, deoxyribonucleic acid (DNA)extraction and hemi-nested real-time polymerase chain reaction (RT-PCR). The cartridge was loaded into the Xpert machine (GeneXpert®Dx System) and an automatic process completes the remaining assay steps.
- The results were visualized and printable in the view results window.
- Two internal quality control is in each Xpert MTB/RIF in each cartilage.
- The Xpert MTB/RIF assay includes two internal quality controls that verify specimen processing, success of PCR and cartridge integrity: Sample processing and Probe check control used respectively.

4.11.2. Ziehl–Neelsen staining

- ✓ Direct smears were made from the thick purulent part of sputum specimens, air dried, heat fixed, and stained by ZN staining method.
- ✓ The smears were scanned with an oil immersion objective and graded according to WHO guideline.
- ✓ The AFB smear was graded as per WHO guidelines: Scanty (1-9/100 fields), 1+ (10-99/100 fields), 2+ (1-10/ fields) and 3+ (>10/field). A person was taken as smear positive if at least one of the smears was graded scanty or higher

4.11.3. Fluor chrome staining

Smear for LED fluorescent microscopy were prepared accord-ing to standard procedures. Smears for FM were stained using auramin O. Briefly; smears were flooded with Auramin O for 20 min, destained with acid alcohol for 2 min, and then counterstained with methylene blue for one minute. With auramine O staining, Mycobacteria appear as bright yellow flu-orescent rods on a dark background. The slides were examined with primo star at 20× magnification. The presence or absence of AFB was reported using WHO/IUATLD guideline [8].

4.12. Facility infrastructure assessment

Peripheral health Facility assessments was carried out using standard on site supervision check lists

4.13. Operational terms

Sensitivity: the ability of a test to identify true cases; is the proportion of people with a disease who have a positive test for the disease.

Specificity: the ability of a test to identify true Negatives; is the proportion of people without the disease who have a negative test.

Negative Predictive Value (NPV):is the probability of not having the disease when the test result is negative (normal).

Positive Predictive Value (PPV): is the probability of the presence of the disease in a person with a positive (abnormal) test result. The proportion of cases identified by a test that are true case

On-site evaluation: is an ideal way to obtain a realistic situation and skills practiced in the laboratory. It provides an opportunity for immediate problem identification, corrective action and on-site retraining. It is used as part of an ongoing EQA process [19].

Kappa Value (K): The fraction of the observed agreement not due to chance in relation to the maximum non-chance agreement when using a categorical classification of a variable [20].

Table 1: Interpretation of Cohen's kappa

Value of Kappa	Level of Agreement	% of Data that are Reliable
0-0.20	0 None	0-4%
0.21-0.39	Minimal	4-15%
0.40-0.59	Weak	15-35%
0.60-0.79	Moderate	35-63%
.80-0.90	Strong	64-81%
Above 90	Almost perfect	82-100%

4.13. Data Quality Assurance

The quality of the data were assured in the phases Pre analytical, Analytical and Post Analytical

Pre analytical: The patients were identified by using his/her request form and questionnaire data was collected by trained laboratory personnel. Then the patients were well instructed how to collect the sputum samples for both smear microscopy and Xpert MTB/RIF by senior technician assigned .The samples were collected in sterile and leak-proof containers [falcon tubes] all laboratory tests were performed using standard operational procedures.

Analytical phase: All the reagents were checked by both known positive and negative control samples based on the operating procedure standards of smear microscopy and then the patient sputum smear was prepared along with control slides.

GeneXpert sample assay

This assay is made up of a one-time use multi-chambered plastic cartridge, filled with liquid buffer and lyophilized reagent beads needed for processing of the sample, DNA extraction and hemi nested real-time Polymerase Chain Reaction (PCR). The cartridge contained lyophilized Bacillus globigii spores that served as internal sample processing and PCR control. The collected sputa were treated with NaOH and a sample reagent (SR) containing iso propanol. In a ratio of 2:1, the SR was added to the sputum and incubated at room temperature for 15 minutes. Target detection and characterization is performed in real time using a six-colour laser detection device. Molecular beacons using novel fluorophors and quenchers are used to detect hybridization to each of the five amplified target regions of the gene.

Validation of test results were done based quality control performance status that is positive control should read ;positive and negative control should negative and any discordant results checked by Regional referral laboratories.

Post Analytical: Based the protocol the results were transferred on AFB registration log and results were distributed to each ward after verified by senior Laboratory Technologists

For the onsite supervision assessment the personnel investigator check the necessary infrastructure and documents, retained records personnel file, availability necessary documents for TB diagnosis, how they validated the test results and traceability of the records .Finally the results appropriately completed on the check lists

4.14. Ethical Clearance

The study proposal was reviewed and approved by the departmental Research and Ethics review committee (DRERC no. 3888/18/MLS) of College of Health Sciences, Addis Ababa University and after permission, letter of support was sent to Assosa General Hospital and other health centers. Data collection was resumed after securing participant consent. All information was kept confidential and positive results were communicated to the respective clinicians for patient management.

5. Results

5.1. Demographic factors of study subjects

A total of 484 presumptive TB patients were included and 52.3 % were male participants and the male to female ratio was 1.1. to 1. The mean age 37.4 and standard deviation were 16.7 (Table 2.) Age group greater or equal to 36 years accounts the largest group of presumptive TB patients.

Table 2: A description of study participants based on their sex and age groups

Patient characteristics	Number of subjects		
Gender			
Male	253 (52.2%)		
Female	231(47.8 %)		
Total	484 (100%)		
Age group(years)	Male	Female	Total
15-19	21(8%)	27(12%)	48(10%)
20-24	31(12%)	33(14%)	64(13%)
25-30	47(19%)	36(16%)	83(17%)
31-35	39(16%)	45(19%)	84(17.3%)
>=36	115(45%)	90(39%)	205(42%)
Total	253(100%)	231(100%)	484(100%)

5.2. Magnitude of PTB based on GeneXpert, ZN and LED microscopy

Based on the gen-expert method, out of 484 presumptive PTB patients 38 patients (7.8 %) were positive for PTB while 446 (92.2%) were expert negative. One patient was positive for Rifampicin resistant. The magnitude of PTB among female patients was 18 (4.1 %). Age group greater or equal to 36 years accounts the highest proportion of PTB cases based on the gene expert method. (Table 3)

Table 3: Magnitude of PTB based on sex and age category at Assosa General Hospital.2019

Variables (Sex)	XpertTB/RIF positive	Xpert MTB/RIF Negative
Male	20(4.1%)	233(48%)
Female	18(3.7%)	213(52%)
Total	38(7.8%)	446(100%)
Age group (years)		
15-19	3(0.8%)	45(9.2%)
20-24	9(1.8%)	55(11.3%)
25-30	9(1.8%)	74(15.3%)
31-35	6(1.2%)	78(16.1%)
>= 36	10(2.2%)	194(40.1%)
Total	38(7.8%)	446(92%)
HIV status		
Positive	8(1.6%)	64(13.2%)
Negative	25(5.2%)	264(54.5%)
Unkowon	5(1.0%)	118(24.3%)
Total	38(7.8%)	446(92%)

On the other hand the ZN method detected 31 PTB patients resulting detection rate of 6.4 % (31/484) while the LED microscopic methods detected 37 PTB patients and the detection rate was 7.6 %(37/484)). Whereas from the total subjects involved in the study 8(1.6) positive) and 446 (92%) cases were negative for both HIV and MTB respectively.

5.3. AFB Microscopy

The presence of acid-fast bacilli in a smear reflects disease severity and patient infectivity. Therefore, it is important to record the number of acid-fast bacilli on each smear. After collection, the samples were decontaminated and processed according to standard protocol for smear preparation. Auramine O fluorescence microscopy technique performed and the slides were examined under 20 xs or 40x microscope objective for the presence or absence of acid-fast bacilli. Based on the number of bacilli load positive specimens were categorized into four groups. Among the positive cases, 13(35.1%) were 1+, 10(27.1%) were 2+, 6(16.2%) were 3+ and 8(26.6%) were scanty positive .Over all AFB results were summarized in table 4.

Table 4: Microscopic observation of AFB

Method of smear Microscopy		
iLED-FM	AFB Microscopy	Number of cases (%)
	Positive	37(7.6)
	Negative	447(92.4)
	AFB grading(n=37)	
	scanty	8(26.6)
	+1	13(35.1)
	+2	10(27.1)
	+3	6(16.2)
Zeehl Neelse	AFB Microscopy	Number of cases (%)
	Positive	31(6.4)
	Negative	453(93.6)
	AFB grading(n=31)	
	scanty	9(29)
	+1	10(32.2)
	+3	4(12.9)

5.4. Performance of LED and ZN Microscopy

From the total 484 samples analyzed by both gene xpert and LED-FM microscopy 37 (7.6%) were positive by both methods; one case was smear negative but gene xpert positive. Table 5.shows the two by two contingency table comparing presumptive sputum LED –Smear microscopy against the gene xpert as reference standard the overall sensitivity, specificity, PPV, and NPV for sputum acid-fast smear were 97%, 100%, 100%, and 100%, respectively. The kappa agreement between the two methods showed that (k=0.99)

Table 5: Performance of FM smears microscopy in reference to Gene xpert

Smear microscopy	X-pert Positive		X-pert Negative		Total
LED-FM					
Positive	37		0		37
Negative	1		446		447
Total	38		446		484
LED-FM	Sen	Sp	PPV	NPV	Kappa
	97%	100%	10%	10%	.99

***PPV=positive predictive value, NPV=Negative predictive value, Kappa=Level of agreement**

Sen=Sensitivity ,Sp=specificity

Kappa is $\frac{\text{Observed Agreement (\%)-Expected Agreement (\%)}}{\text{100- Expected Agreement \%}}$ [20]

Table (6) shows the two by two contingency table comparing between ZN TB microscopy and gene xpert the overall sensitivity, specificity, PPV, and NPV for ZN smear microscopy assay were 86%, 100%, 100%, and 99%, respectively with kappa agreement among methods showed that (k=0.89).Due to poor sensitivity of the method seven cases missed by ZN due to those of the results were detected by gen xpert having low bacteria density were detected

Table 6: Performances of ZN smear microscopy in reference to Gene xpert

Smear Microscopy		**X pert**	**Total**
ZN	Positive	Negative	
Positive	31	0	31
Negative	7	446	453
Total	38	446	484
sensitivity	81%	PPV	100%
Specificity	100%	NPV	98%
Kappa	0.89	Kappa%	89

5.5. Summary of Smear Microscopy Performance

Based on Table 5.5: showed compares the results of ZN and fluorescence staining with the Xpert MTB/RIF test of the 38 positive Samples, 7(4%) were missed by ZN staining technique and 1 (2.8%) were missed by FM .Both fluorescence and ZN staining techniques showed a positive correlation with Xpert MTB/RIF diagnostic technique.37 cases positive with fluorescence microscopy also detected by gene xpert and 31 smear positive with ZN also from the total positive cases the three methods were agreed with 31 and 434 cases which were positive and negative respectively , it could also be observed that all the samples that were negative by Xpert MTB/RIF test were also negative by both ZN and Fluorescence staining. both fluorescent and ZN staining had a stronger correlation when compared to gene xpert (kappa=0.98, $p\leq0.001$ versus kappa=0.92, $p\leq0.001$) respectively. When we concluded the performance of direct microscopy methods based on Percent level of Agreement Statistics indicated the smear microscopy methods had almost perfect agreement when compared to gene xpert.

The diagnostic performance of ZN to that of FM. Fluorescence staining showed a positive Correlation with ZN staining technique (kappa=0.90, $p\leq0.001$). It could be seen that 2 (35.2%) of the samples that Were positive by fluorescence microscopy were missed by ZN staining whereas there was no sample that was positive by the ZN staining was negative by FM. The two tests had the same specificity (100%); however, the sensitivity of fluorescent staining (92%) was higher than that of ZN staining (81%)

6. Infrastructure assessment based on Onsite supervision.

Along with comparison study also a descriptive and Observational study was conducted to evaluate the infrastructure required for the diagnosis pulmonary tuberculosis by using on site supervision check list. The study comprised 19 health facilities Based on table 6.1 among About 19 laboratory personnel from 19 health facilities were assessed From this , 12 were diploma holders, the remaining were Degree holders. The average e experience of the laboratory personnel was four years. Among the 19 laboratory facilities, only five (26.3%) them were gene xpert service site in the region; eight (42.1%) of the facilities were no separated area of the TB work. Five(26.3%) facilities were no functional fluorescence microscopy and most of them have poor storage condition of reagents .During the study period appropriate documents and records were not available ;fourteen facilities (73.6%) have no trained of using internal quality control and from nine health facilities(47.4%) proper sputum collection were not verified

Table 7: Infrastructure quality supporters Variables of Tb diagnosis

Parameter Available	Facilities	
	Yes	No
Gene xpert	05(26.3%)	14(73.7%)
Separate laboratory room	11(57.8%)	8(42.2%)
Regular electric power	13(68.4%)	6(31.6%)
Regular Water Supply	13(68.4%)	6(31.6%)
Run IQC slides parallel with routine	15(78.9%)	4(21.1%)
Availability of SOP, Guide line and manual	15(78.9%)	4(21.1%)
Functional light microscopy	14(73.7%)	5(26.3%)
ZN reagent	16(84.2%)	3(15.8%)
LID reagent and Microscopy	14(73.7%)	5(26.3%)
Lens cleaning tissue paper	14(73.7%)	5(26.3%)
IQC reagents	15(78.9%)	4(21.1%)
Good storage condition of reagent	03(15.8%)	16(84.2%)
Checking quality of sputum	10(52.6%)	9(47.4%)
Having new frosted slide	11(57.8%)	8(42.2%)
Having personnel protective equipment	19(100%)	0(0%)
Weigh Balance	03(15.8%)	16(84.2%)
Diploma holders laboratory technician	12(63.2%)	7(36.8%)

7. Discussion

The lack of TB conventional bacteriological laboratory services in many resource-limited setting, in different remote and shortage of resource, forced many of the health systems of the country to depend uniquely on the acid-fast smear detection as the only laboratory support available for diagnosis. Mycobacteria cultures are the gold standard for TB diagnosis but were not performed for the patients in this study. Because it requires laboratory infrastructure that is not widely available in most countries with a high burden of TB and test results take up to 3 months to obtain. Treatment gaps in between the patient going to be tested for TB and receiving the test result can lead to high levels of default during the diagnostic processes is not the standard of care for TB suspects at our center [21].

A key gap in the fight against TB is the availability of cheap, and accurate diagnostic tests that can be used in resource-limited settings. The development of nucleic acid based tests has provided novel avenues for generation of highly sensitive point-of-care tests. The current model that has been rolled out world-wide is the GeneXpert, which has transformed the TB diagnostic landscape in well equipped settings, but which has significant limitations in resource-poor settings [22].

In comparison with smear microscopy and gene Xpert, from all the MTB tested cases (n=484) for 446 cases were a Negatives by smear microscopy whereas from 38 cases were positive. Among the total positive MTB detected by gene xpert thirty one(81.6%) cases Zeehl Neelsen smear positive and thirty seven(97.4%) of them were positive by Fluorescence microscopy where as one missed by FM and seven cases were missed by ZN smear microscopy. So the overall sensitivity, specificity, PPV and NPV of the ZN direct microscopy when compared to gene xpert were of 81%and 100%,100% and 99% level of agreement of ZN(K=0.89) .And the performance of FM techniques showed that [sensitivity=97%, specificity= 100% ,PPV= 100%, NPV= 100% with kappa value (k=0.99).

Our study revealed that the specificity and sensitivity of smear microscopy was significantly higher than when compared with the study conducted in different areas however the sample size, sample types and different reference standards were used. The conducted in Pakistan revealed that lower specificity and sensitivity of ZN smear microscopy (77.7% and 91.4%) respectively when compared to present study similarly the sensitivity of smear microscopy in the tertiary hospital in north India which had 46% lower than when compared to our study [3, 23].

And also study conducted in Egypt was demonstrated that lower sensitivity, specificity, ppv and NPV of smear microscopy (86.43, 99.66, 99.18 and 93.85) respectively so as small sample had used (n=2018) [24].

Another cross sectional study conducted in Kenya (Nyamira County Referral Hospital) showed that lower sensitivity and higher specificity of ZN smear microscopy (26.4%) and (98.2%) respectively despite small sample (366) size had used when compared to the above study [25].

In an ideal situation, a diagnostic technique should be100% specific and 100% sensitive. Our results indicated that both the ZN and FM techniques showed similar specificity while compared to Ghana; among direct microscopy methods, fluorescence microscopy strong correlation (k=0.864, with p<=0.001) with gene xpert whereas ZN techniques moderately (k= 0.584) and FM (0.765) correlated with GeneXpert, therefore the level of agreement nearly comparable with our study however the sample size of this study lower than the above study [16].

The study conducted by Muia PK, et al. revealed that the performance of ZN smear microscopy (sensitivity=81.1%, specificity=84.3%) higher than study conducted in Tanzania, Indonesia and Rome with different sample size had used but lower than present study [26].

The level of agreement between ZN smear microscopy with Gene xpert conducted in Bayelse state, Nigeria which showed fair agreement (k=0.55) much lower in this study this due the former study used very low sample size with retrospective study design [27].

The study conducted in Rome showed that better level of agreement between ZN smear microscopy and Gene Xpert(k=0.75) when compared to the others studies while the kappa value of ZN (Z=0.89) our study has still the better level of agreement [28].

Zahoor Detal etal Stated that the specificity of gene xpert and smear microscopy had comparable specificity (98.67% and 99.56%), which is almost similar with our study (specificity=100%) also concordant with the study conducted in North Thailand (specificity=100) [23, 29].

Afsar et al. performed study including 483 samples they found the sensitivity, specificity, PPV and NPV 53%, 100%, 100% and 98% respectively of ZN smear microscopy with almost the same sample size this study had comparable performance with our study [30].

In Argentina high yield samples were collected (n=6,968) to evaluate the performance of both LED-FM and ZN smear microscopy the both had the same specificity (99.9%) but the former had better sensitivity this also similar with our study that have the same specificity (100%) but different sensitivity [31].

Study conducted in Pakistan by Khan AS revealed the sensitivity and specificity of LED-FM compared to GeneXpert 40% and 100% respectively this was in concordance specificity with our study but much lower sensitivity due to different clinical specimen (EPTB) had used [32].

The specificity and PPV of LED-FM smear microscopy(100%) of our study agree with Gelalcha G (2017) in western Ethiopia (Ambo) and by Dzodanu EG et al(2019) in University of Cape Coast, Ghana [15,16].

Study conducted in Addis Ababa by Konjit Ababa showed the specificity of both LED-FM and ZN smear microscopy (100%) similar with our study where as the sensitivity much lower than present study (Zn=45.8%, LED-FM=62.5%) this due to the Addis Ababa study used concentrated sputum samples and study participants were HIV positive and our study subjects included irrespective of HIV status [33].

In contrasts Meseret W. et al .stated and also supported by other studies recently, fluorescence microscopy had increase the sensitivity (10% higher) when compared with ZN*)* [34].

Despite the introduction and recommendation of molecular based diagnostic devices as line probe assay and GeneXpert for diagnosis of TB, AFB test is still used in poor resource countries like Ethiopia and also other African country due to inadequate infrastructure and access to basic facilities like regular water and power supply which are necessary for sustainable use of these devices [35, 36].

Finally the on site evaluation of our study revealed that Lack of reagents, supplies, favorable working environment and AFB related technical problems were identified in the peripheral laboratories in this study and this also identified in study conducted in eastern part of Ethiopia by Ayana et al.(2015) [37].

8. Limitation and Conclusion

Mycobacterium cultures were not available to confirm PTB diagnosis.

*A*nother Rifampcin susceptibility test and other molecular techniques were not done

Not all EQA methods were used for full quality assessment of the peripheral facilities due lack of resource

In conclusion, even though this study have some limitation , our study showed that direct smear microscopy methods have better level of agreement when compared with X-pert smear microscopy and LED –FM has better performance than ZN techniques .

9. Recommendation

As much the Advanced and Culture tests are expensive, Benishengul regional state government should be encouraged to procure more of these machines to help earlier and prompt start of treatment to improve TB out come in high burden TB-HIV co infection settings to robust WHO 2035 end of TB in sustainable development goal. Promoting and evaluating concurrent training on TB diagnosis for Laboratory professionals. Providing capacity building to build consistence infrastructure of TB laboratory diagnosis to enhance the Over quality system of the laboratory Further studies are needed by considering the gold standard culture

10. References

1. Chinedum OK, Emwiomwan A, Ifeanyi OE, Babayi A .Comparative Analysis of Ziehl-Neelsen and GeneXpert Techniques for the Diagnosis of Tuberculosis in Human Immune-Deficiency Virus Positive Patients in Benin City. Ann Clin Lab Res 2017; 5(4): 208.

2. Noori MY, Ali F, Ali Z, Arshad T, Asif M, Sharafat S. The Ratio of Smear-Negative Tuberculosis in Sindh: An insight using Fluorescence Microscopy. J Liaquat Uni Med Health Sci. 2017; 16(02):76-9. doi: 10.22442/jlumhs.171620510

3. Munir MK, Rehman S , Aasim M, Iqba R, Saeed S. Comparison of Ziehl Neelsen Microscopy with GeneXpert for Detection of Mycobacterium Tuberculosis. Journal of Dental and Medical Sciences 2015;14(11):56-60.Available from www.iosrjournals.org

4. Seni J, Kidenya BR, Anga M, Kapesa A, Meda, JR, Mutakyawa R etal. Incremental detection of pulmonary tuberculosis among presumptive patients by GeneXpert MTB/RIF® over fluorescent microscopy in Mwanza, Tanzania:an operational study, PATH - Tanzania, Dar es Salaam National Institute for Medical Research, Healthcare in Low-resource Settings 2015; volume 3:5011

5. Agrawa M, Bajaj A, Bhatia V, Dutt S. Comparative Study of GeneXpert with ZN Stain and Culture in Samples of Suspected Pulmonary Tuberculosis Journal of Clinical and Diagnostic Research 2016;10(5): DC09-DC12

6. Biadglegne F, Sack U and Rodloff AC. Multidrug resistant tuberculosis in Ethiopia efforts to expanddiagnosticservices.TreatmentandcareAntimicrobialResistanceandInfectionControl 2014;3:31.Availablefromhttp://www.aricjournal.com/content/3/1/3

7. Taddese BD, Desalegn DM, Misganaw AS, Kitila KT, Balcha HM et al. Comparison of GeneXpert against Light-Emitting Diode Fluorescent Microscopy for the Diagnosis of Pulmonary Tuberculosis in Addis Ababa, Ethiopia. J Microb Biochem Technol 2018; 10: 12-15. doi: 10.4172/1948-5948.1000388

8. WHO | Tuberculosis (TB) 2018. Available online: http://www.who.int/gho/tb/en/ (accessed on 16 December 2018).

9. Global TB report. Switzerland 2018.

10. Bhirud P, Joshi A, Hirani N, Chowdhary A .Rapid laboratory diagnosis of pulmonary tuberculosis. Int J Mycobacteria 2017;6: 296-301.Avalialable from http://www.ijmyco.org on Friday, August 16, 2019, IP: 196.190.255.6

11. Fiberesima M G, Akio B, Okwelle SPC, Ibiju W, Wokoma V, Omakwelle GC et al ."Utilization of GeneXpert for Point of Care Testing of Tuberculosis: A Bench Mark Approach over Ziehl-Nelson Smear Microscopy". EC Pulmonology and Respiratory Medicine 2018; 7(2) : 65-71

12. Ejeh EF , Undiandeye A, Akinseye VO, Okon KO, Kazeem HM, Kudi CA et al .Diagnostic performance of GeneXpert and Ziehl Neels on microscopy in the detection of tuberculosis in Benue State, Nigeria. Alexandria Journal of Medicine 2018; 54:529-533. Available from http: //www.elsevier. com/locate/ajme

13. IramS, Zeenat A, Hussain S, Yusuf NW, Aslam M. Rapid diagnosis of tuberculosis using XpertMTB/RIFassayReportfromadevelopingcountry.PakJMedSci2015;31(1):105110doi: http://dx.doi.org/10.12669/pjms.311.6970

14. Ngozika WG, Obioma A, Appollus JN. Evidence-based comparison of molecular-GeneXpert and microscopic method in the diagnosis of mycobacterium tuberculosis among subjects in the limited resource setting of Niger delta .J Microbial Exp.2018; 6(2):98–102.

15. Gelalcha AG, Kebede A and Mamo H .Light emitting diode fluorescent microscopy and Xpert MTB/RIF® assay for diagnosis of pulmonary tuberculosis among patients attending Ambo hospital, west central Ethiopia .BMC Infectious Diseases (2017) 17:613DOI 10.1186/s12879-017-2701-5

16. Dzodanu EG, Afrifa J, Acheampong DO, and Dadzie I. Diagnostic Yield of Fluorescence and Ziehl-Neelsen Staining Techniques in the Diagnosis of Pulmonary Tuberculosis. A Comparative Study in a District Health Facility Tuberculosis Research and Treatment Volume 2019; Article ID 4091937, 6 pages https://doi.org/10.1155/2019/4091937

17. Bekele G characterization of Assosa Town, Benishangul gumuz Regional state, School of Earth Science, Hydrogeology stream 2017;p(8)

18. Mallinckrodt B, Abraham WT, Wei M, Russell DW. Advances intesting the statistical significance of mediation effects.J Couns Psychol 2006; 53:372–378

19. Federal Democratic Republic of Ethiopia Ministry of Health, Ethiopian Public Health Institute Guidelines for External Quality Assessment of AFB Smear Microscopy in Ethiopia 2016 January ; Second Edition:13-16 pages

20. MaryL,HughM.Intrreliability:thekappastatistics.DepartmentofNursing,Nationaluniversity ,Aerocourt,SanDiego,California.BiochemicalMedica2012;22(3):27682.Availablefromhttps://www.biochemiamedica.com/assets/images/upload/xml_tif/McHugh_ML_Interrater_reliability.pdf

21. Ba F, Rieder HL .A comparison of fluorescence microscopy with the Ziehl Neelsen technique in the examination of sputum for acid-fast bacilli. Int J Tuberc Lung Dis 1999; 3: 1101-1105

22. Enarson DA, Rieder HL, and Arnadottir T. Management of tuberculosis: Aguide for law income countries2000; 5th ed. Paris: IUATLD

23. Zahoor D, Farhana A, Kanth F, Manzoor M. Evaluation of smear microscopy and GeneXpert for the rapid diagnosis of pulmonary and extra pulmonary tuberculosis in a tertiary care hospital in North India.. Int J Res Med Sci 2018; 6(5):1756-60. doi: http://dx.doi.org/10.18203/2320-6012.ijrms20181774monary

24. Elsayed AE ,Mishad Y, Mahmoud MA., El-hosiny IM ,Tawab AA, Ghanem A. Mohamed GA. Comparison between the Use of a GeneXpert Device and Conventional Diagnostic Methods in the Detection of Mycobacteria Tuberculosis and Its Resistance to Drugs . The Egyptian Journal of Hospital Medicine 2018; 73 (9): 7404-7411

25. Ondimu TO, Grace MO, Samson AO, Asito SA. Comparative Study of Smear Microscopy, Gene Xpert and Culture and Sensitivity Assays in Detection of Mycobacterium tuberculosis on Sputum Samples among Tuberculosis Suspected Cases in Nyamira County Referral Hospital. Mycobacteria 2017; 7: 249. doi:10.4172/2161-1068.1000249

26. Muia PK, Ngugi MP, Mburu DN. Performance of GeneXpert Assay in Detecting Pulmonary Tuberculosis and Rifampicin Resistance in Patients Attending Kitui County Hospital, Kenya . Journal of Tropical Diseases 2017; 5: 246. doi:10.4172/2329-891X.1000246

27. Jumbo J, Ikuabe PO and Ambakederemo TE. Level of agreement between Ziehl Neelsen (ZN) microscopy and Gene Xpert in the diagnosis of Pulmonary Tuberculosis amongst Smear Negative PTB Suspects and Smear Positive Suspected MDR PTB Patients, Nig. Del Med J 2017; 1(3):7-15

28. Sauzullo I, Rodio DM, Facchinetti S, Puggioni G, De Angelis M, Goldoni P at et al. Diagnostic accuracy of Xpert MTB/RIF versus smear microscopy in the early diagnosis of tuberculosis in the real life of the " Umberto I" Hospital in Rome. New Microbiological 2016; 39(4): 304-306

29. Imaz M, Allassai S, Aranibar M, Gunia A, Poggi S, Togneri A etal. Performance of LED fluorescence microscopy for the detection of acid fast bacilli from respiratory samples in Peripheral laboratoriesinArgentina.Biomedical2017;37:16474.doi:http://dx.doi.org/10.7705/biomedica.v37i2.326

30. Pinyopornpanish K, Chaiwarith R, Pantip C, Keawvichit R, Wongworapat K, Khamnoi P et al. Comparison of Xpert MTB/RIF Assay and the Conventional Sputum Microscopy in Detecting Mycobacterium tuberculosis in Northern Thailand. Tuberculosis Research and Treatment Volume 2015; Article ID 571782, 6 pages. Available fromhttp://dx.doi.org/ 10.1155/2015/571782.

31. Afsar I, Gunes M, Er H and Sener AG. Comparison of culture, microscopic smear and molecular methods in diagnosis of tuberculosis. Atatürk Training and Research Hospital, Department of Microbiology, Izmir, Turkey. Official journal of the Spanish society Chemotherapy2018; 31(5): 435-438

32. Khana AS, Ali S, Khand MT, Ahmeda S, Khattak Y, jabbar A etal. Comparison of GeneXpert MTB/RIF assay and LED-FM microscopy for the diagnosis of extra pulmonary tuberculosis in Khyber Pakhtunkhwa, Pakistan, Brazilian journal of Microbiology, 2 0 1 8.49: 909–913

33. Getachew K, Abebe T, Mihret A and Malkamu G .Performance of LED fluorescence microscopy for diagnosis of pulmonary tuberculosis in HIV positive individuals .Tuberculosis research and treatment 2015; Article id 794064, 6 pages. Available from http://dx.doi.org/10.1155/2015/794064

34. Maru M, Seman I, Bezu Z, Negash M, Melku M, Gize A, Shibabaw A, Meseret W. et al. Agreement between Direct Fluorescent Microscopy and Ziehl-Neelsen Concentration Techniques in Detection of Pulmonary Tuberculosis in Northwest Ethiopia, J Health Sci 2017; 27(5):459. doi: http://dx.doi.org/10.4314/ejhs.v27i5.3

35. Sımsek H .Requirement of Quality Assessment for Modern Tuberculosis Laboratory Services. Refik Saydam National Public Health Agency. National Tuberculosis Reference Laboratory, Ankara, Turkey. Mycobacteria Diseases 2011;1:104.doi:10.4172/2161-1068.1000101

36. Cheon SA, Cho HH, Kim J, Lee J, Kim HJ, Park TJ. Recent tuberculosis diagnosis toward the end TB strategy. Journal of Microbiological Methods 2016; 123: 51-61. Available from www.elsevier.com/ locate/jmicmeth

37. Ayana DA, Kidanemariam ZT, Tesfaye HM and Milashu FW. External quality assessments for acid fast bacilli smear microscopy in eastern part of Ethiopia. BMC Res Notes (2015) 8:537 8:537.DOI 10.1186/s13104-015-1478-0.

Annex I: participants information sheet [English version].

Principal Investigator: Ashebir Legesse

Addis Ababa University College of Health Sciences, department of medical laboratory science.

Purpose: Comparison of direct microscopy (Ziehl neelsen and light-emitting diode fluorescent microscopy) against GeneXpert MTB/RIF for the diagnosis of pulmonary tuberculosis and Facility Infrastructure Assessment of routine TB diagnosis in our hospital

Procedures to be carried on: you are invited to participate in the study after giving your consent and by giving the requested sample for investigation.

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study and there is no additional time required from you to stay during study.

Benefits of the study: There will be no financial benefit to you. But the result of this project will help you and other people living in Benishangul-Gumuz Region.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you later on change your mind and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

Annex II. Informed consent [English version]

I, the undersigned individual, am oriented about the objective of the study. I have informed that all of my information will be kept confidential and used only for this study. Your signature below indicates that you have read /or listened, and understand the information provided for you about the study. Before you sign, please understand purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have any question.

I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study.

Based on the above information I agree to participate in the research

Signature: ____________________ Date: ___________________

Name of Data collector ____________________________ Signature ___________

If you have any question you can ask the principal investigator

Principal investigator Ashebir Legesse [Msc candidate]

Mobile: 0911071443

e-mail.ashebirlegesse1@gmail.com

Annex III: participant's information sheet [Amharic version]

ጥናቱን የሚያጠናው ፤ አሸብር ስቶሰ

የጥናቱ አላማ፡ የቲብ ፤መመሪመሪያ፤መሠሪያዎች፤አሠራርና ጥራት ስመስጠበቅ ¾ሚራÇ፡ በመሠሪያዎቹ፤ መሀል ፤ ያስዉን የዉጥት ፡ መማሳሰል፤ ከሆስፒተሉ ፤በተሰያዪ ፤ክፍል ፤ወደ ላብራቶሪ የሚላኩት፡ የአክተ ናሙና ፤ስይ ጥናት፤ መከናወን፡ይሆናል፡፡ ተተጠጠ

በጥናቱ ዉስጥ አረሰ- ተሳታፊ ስመሆን ፈቃደኛ መሆንዎን በስምምነት ሲያረጋግጡ • እና ናሙናዉን ስምርመራ መስጠት ሲችሉ ብቻ ይሆናል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት

በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡በጥናቱ ምክንያት የሚያባክኑት ተጨማሪ ጊዜም አይኖርም፡፡

ለ ጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ተሳታፊ በመሆንዎ ምንም አይነት የገንዘብ ክፍያ አያኙም ነገር ግን ጥናቱ ስሎችንም ሆነ ስክልሉ ናዋሪዎች ጥናቱ የጎላ ጥቅም ይኖሮወል፡፡

የመረጃ ሚስጥራዊ አጠባበቅ:የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡

በጥናቱ ላይ ያለመሳተፍ መብት አለዎት፡ በጥናቱ ለመሳተፍ ከወሰኑ በኋላም ሃሳቦዎትን መቀየር ቢፈልጉ እንኳዋን ውሳኔዎትን በማክበር

በማንኛው ሰዓት ማቋረጥ ይችላሉ፡፡ ይህ ደግሞ ተሳትፎን ቢያቋርጡም የምርመራ ውጤትዎን ይሁን ከምርመራ በኋላ ማግኘት የሚገባዎትን የህክምና ግልጋሎት ሁሉ ማግኘት ይችላሉ፡፡

Annex IV: consent [Amharic version]

ከእኔ የሚወሰደዉ ናሙና ለጥናቱ አላማ ብቻ እንደሚዉል ተረድቻለሁ። ሁሉም

መረጃዎች እና የናሙና ዉጤቱ ምስጢራዊ መሆኑን ተገንዝቤአለሁ። በጥናቱ ላይ በመሳተፌ

ምንም የገንዘብ ክፍያ እንደማላገኝ ተረድቻለሁ። በጥናቱ ያለመሳተፍ እንዲሁም በማንኛዉም

ጊዜ የማቋረጥ መብት እንዳለኝ አዉቄአለሁ።ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ።

እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼያለሁ።

ፊርማ ----------------------------------ቀን-------------------------------------

መረጃውን የሰበሰበው ግለሰብ ስም--

ፊርማ -------------------------

የዋና ተመራማሪው አድራሻ

አሸብር ለገሰ

ኢ-ሜይል ashebirlegesse1@gmail.com

ስልክ፣ 0911071443

Annex V: Result Report and interpretation AFB smear microscopy

ZN grading scale using 100x oil immersion

Report	AFBs seen
Negative	No AFB seen in at least 100 fields
Actual Number	1–9 AFB / 100 fields
+1	10–99 AFB / 100 fields
+2	1–10 AFB / field in at least 50 fields
+3	More than 10 AFB / field in at least 20 fields

FM Grading Scale using 40X Objective

AFB seen	(400 magnification; one length=40 fields=200 HPF in bright field microscopy)
Negative	No AFB seen in at least 40 fields
Actual number	1-19 AFB per 40 fields
(1+)	20-199 AFB per 40 fields
(2+)	5-49 AFB per field in at least 20 fields
(3+)	More than 50 AFB per field in at least 8 fields

Annex VI: On-Site Evaluation Checklist

I. . General Information

Name of Laboratory:	
Code for the laboratory:	
Number of laboratory personnel:	
Head of Laboratory :	
Laboratory Head Phone Number	
Date of Visit:	
Supervisor:	

II. Current Visit Particulars

Fill the findings as a labelled 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.

S/N	Item	Findings			Remark
1	**Facility and safety**	Y	N	NA	
	Separate area for TB laboratory work				
	Separate tables for specimen receipt/smear preparation/ microscopy				
	Power supply				
	Running Water supply				
	Waste containers with lid				
	Incinerator available				
	General order/cleanliness				
	Personal Protective Equipments used and practices(glove, aprons, hand wash, etc)				
2	**Personnel and Training**				
	Number of Laboratory Personnel trained in TB Smear microscopy: Zeihel Nelson				
	Number of Laboratory Personnel trained in TB Smear microscopy: Florescent microscopy				
	Any change in trained laboratory personnel staff since last supervisory visit				

3	**Manuals, Standard Operating Procedure, Job Aids**				
	Is there standard operating procedure for smear preparation, staining and reading?				
	Grading chart, 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	
	Lens Tissue	Y / N	
	Filter paper	Y / N	
	Spirit lamp or Bunsen burner	Y / N	
	Immersion oil	Y / N	
	Disinfectants	Y / N	
	Smearing/staining equipment (staining racks, loops, sticks etc)	Y / N	
	Slide boxes	Y / N	
	Carbolfuchsin	Y / N	
	Auramine O		
	Methylene Blue	Y / N	
	Acid alcohol	Y / N	
	Distilled water	Y / N	
	Equipment for preparation of stains/ reagents such as balance (for weighing reagents), measuring cylinders etc	Y / N	
	Equipment and facility for preparation of panel testing	Y / N	
	Number of Bright field Microscopes (functional)	Y / N	
	Number of FM (nonfunctional)		

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	

	All peripheral laboratories* are visited twice a year by RRL/Sub-RRL/EQA centers	Y / N	
	Are all slides stored properly in slide box for random blinded rechecking?		
	Control smears are used for each new batch of stain	Y / N	
	Control smears are used for at least once every week for checking the quality of stain	Y / N	

III. **Summary of current visit:**

A. Operational /administrative problems (pending as well as new)

B. Technical problems (pending as well as new)

C. Action Required

Issues	Cause(s) for each issue	Corrective action(s) for each cause

D. Overall remarks

____
____
____
____
____
____
____

RRL supervisor:

Name ______________________________

Signature __________Date_________________

Peripheral Lab. Representative

Name ______________________________

Signature _____Date___________________

Annex VII: SOPs for AFB Reagent Preparation

1. Aura mine 0.1% Staining Solution Preparation

Preparation.

To ensure solutions are fresh, laboratories examining low numbers of smears should prepare smaller volumes

Solution A

Step	Action
1.	Add 1000ml of ethanol (or methanol) to a one-litre glass flask
2.	Add 10.0g of Auramine powder, mix until dissolved completely Note: Do not use heat since this can inactivate the Aura mine
3.	Label "**1.0% auramine in alcohol**", date and initial
4.	Store in a dark bottle in a cupboard at room temperature (expiry 12 months)

Solution B

1.	Dissolve 30g of phenol crystals in 900ml distilled water, mix
2.	Label the bottle "3% phenolic solution for auramine", date and initial
3.	Store in a dark bottle in a cupboard at room temperature (expiry 12 months)

Preparation of 0.1% auramine solution

1.	Add 50ml of solution A (1% auramine in alcohol) to a 500ml dark glass bottle
2.	Add 450ml of solution B (phenolic solution for auramine) and mix
3.	Label the bottle “0.1% auramine”, date and initial
4.	Store in a cupboard at room temperature (expiry 2 months) Note:Filter auramine solution when applying to smears or filling bulk staining containers.Perform a Quality Control check and record results in the QA log book.

2. **Acid alcohol 0.5% Staining Solution Preparation**

Preparation

Step	Action
1	Add 5ml Hydrochloric acid, technical grade in to 995 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.
3	Store in a dark bottle in a cupboard at room temperature (expiry 12 months)
4	Perform QC and record in the QA log book

3. 0.5 Potasium permanganate Staining Solution Preparation(Quenching solution)

Preparation

Step	Action
1	Dissolve 5g of potassium permanganate, technical grade in 1000ml of distilled water
2	Label the bottle “0.5% Potassium permanganate “, add date and sign with initials. The date first opened has to be mentioned.
3	Store in a dark bottle in a cupboard at room temperature (expiry 12 months)
4	Perform QC and record in the QA log book Note: The solution should be bright purple; if it is brick-red in colour it is oxidized discard it – rinse the bottle before refilling
Note	1% auramine: 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. 0.5 % Hydrochloric acid: Stocks and solutions should not be used over 6 months. 0.5% permanganate: Stocks and solutions should not be used over 6 months.

Annex VIII. 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 agree to accept all responsibilities for the scientific and ethical conduct of the research project. I was providing timely progress report to my advisor and seek the necessary advice and approval from my primary advisors in the course of the Research that all sources of materials used for the thesis have been duly acknowledged.

Ashebir Legesse Kitessa (M.Sc. candidate)

Signature __________________ Date of submission ___________________

This thesis has been submitted with our approval as advisors.

Advisor	Signature	Date
Kassu Desta (MSc, PhD Candidate)	____________________	________
D/r. Aster Tsegaye (MSc, PhD)	____________________	________