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Diagnostic Efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope, Spoligotyping and drug resistance patterns of *Mycobacterium tuberculosis* strains isolated from clinically suspected Tuberculous Lymphadenitis.

By: Gebeyehu Assefa

Advisors: Mr. Kassu Desta (MSc., PhD candidate, Associate professor, DMLS, AAU)
Dr. Abraham Aseffa (MD, PhD. Senior Researcher, AHRI)
Mr. Shambel Araya (MSc, DMLS, AAU)

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Research Project Submission Form

Name of PI	**Gebeyehu Assefa** Department of Medical Laboratory Sciences College of Health Sciences, Addis Ababa University
Advisors	**Mr. Kassu Desta (PhD Candidate)** Department of Medical Laboratory Sciences College of Health Sciences, Addis Ababa University **Tel:** +251 911 107 099 **Dr. Abraham Aseffa (MD, PhD)** Armauer Hansen Research Institute Tel: +251911 247 525 **Mr. Shambel Araya (MSc)** Department of Medical Laboratory Sciences College of Health Sciences, Addis Ababa University **Tel**: +251 939 459 529
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Address of the PI	**Email: gebeyehu03@yahoo.com** **Tel: +251913333530**

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Gebeyehu Assefa, entitled: **Diagnostic efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope, Spoligotyping and drug resistance patterns of *Mycobacterium tuberculosis* strains isolated from clinically suspected Tuberculous Lymphadenitis.** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner_____ Signature _____Date _____

Internal Examiner_____ Signature _____ Date _____

Advisor_____ Signature _____ Date _____

Advisor_____ Signature _____Date _____

Advisor_____Signature_____Date_____

_____

Chairman of the Department or Graduate Program Coordinator

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List of abbreviations

AFB	Acid Fast Bacilli
AHRI	Armauer Hansen Research Institute
ALERT	All Africa Leprosy Tuberculosis Rehabilitation and Training center
BA	Blood Agar
DST	Drug Resistance
EPTB	Extra Pulmonary Tuberculosis
FM	Fluorescent Microscope
FNA	Fine Needle Aspirate
FNAC	Fine Needle Aspiration Cytology
LED	Light Emitting Diode
HIV-AIDS	Human Immunodeficiency Virus-Acquired Immune Deficiency Syndrome
IUATLD	International Union Against Tuberculosis and Lung Disease
LJ	Loewensten Jensen
MDR-TB	Multi Drug Resistant Tuberculosis
MDR/RR-TB	Multi Drug Resistant/Rifampicin Resistant Tuberculosis
MTB	*Mycobacterium tuberculosis*
MTBC	*Mycobacterium tuberculosis* Complex
MTBDR	*Mycobacterium tuberculosis* Drug Resistant
PCR	Polymerase Chain Reaction
PTB	Pulmonary Tuberculosis
SNNPR	Southern Nations, Nationalities and Peoples' Region
TBLN	Tuberculosis Lymphadenitis
WT	Wild Type
ZN	Ziehl–Neelsen

Abstract

Background: Comparably efficacy of Microscopic examination for tuberculous lymphadenitis is differs from laboratory to laboratory. Emergence of drug resistant TB still a major challenge in developing countries. We therefore also investigated the drug resistant patterns and molecular epidemiology of mycobacterial isolated from tuberculous lymphadenitis patients.

Methods: A cross sectional study was conducted from clinically suspected Tuberculous lymphadenitis patients. Three smears were prepared; for cytomorphology, Auramine O and ZN staining study. The left-over sample were inoculated onto Lowenstein-Jehnsen (LJ) media. The culture isolates were tested by regions of difference (RD9) to distinguish *M. tuberculosis* from other species of *M. tuberculosis* complex. Phenotypic, genotypic first-line and genotypic second-line drugs were tested for resistance pattern of isolates by using GenoType MTBDRplus and Phenotypic DST (Drug containing 7H10 Middlebrook Media), and the Spoligotyping strain dependent polymorphism test were determined. Statistical analysis was done using STATA version 11. The sensitivity, specificity, positive and negative predictive values were calculated by considering the culture results as the gold standard.

Results: Among 211 FNA samples collected; 49.7% (105/211) were positive by Cytology, 32.7% (69/211) LEDFM, 23.69% (50/211) Culture and 13.7% (29/211). The efficacy of Ziehl-Neelsen as compared to culture, Sensitivity were 30% [95% CI: 17.9-44.6], Specificity 91.3% [95% CI: 85.8-95.2]. For LEDFM, the Sensitivity 66% [95% CI: 51.2-78.8] and the Specificity was 77.6% [95% CI: 70.4-83.8]. The Sensitivity and Specificity of Cytology was 78% [95% CI: 64-88.5], 58.8% [95% CI: 50.7-66.5] respectively. The most predominant Spoligotyping types were NEW strains following by SIT53 and the overall drug resistance patterns among 50 cultures positive isolates were 14% (7/50); out of this 8% (4/50) isolates were mono resistance for INH, whereas 6% (3/50) isolates were resistance for both INH and Rifampicin.

Conclusion: LEDFM gives a legitimate option in contrast to ZN techniques in terms of its higher sensitivity, a bit lower specificity. Three MDR-TB cases and heterogeneous strains of *M. tuberculosis* and the high extent of INH monoresistance from HIV patients were detected. The NEW and SIT53 (T) strains was the most prevailing strains in the study area.

Keywords: *M. tuberculosis*, MDR-TB, TBLN, Spoligotyping

1. Introduction

1.1. Background

Tuberculosis (TB) is a deadly bacterium as a rule caused by *Mycobacterium tuberculosis* (MTB). Tuberculosis is commonly influencing the lungs, however can likewise influence different pieces of the body (1). The continuing with difficulties of Tuberculosis control can be dispersed into 5 essential areas; lacking proper diagnostics and inadequate treatment, Directly Observed Therapy (DOTS) program, Multi Drug Resistant Tuberculosis, and HIV coinfection (2). TB keeps on being a significant worldwide medical issue and is one of the main 10 causes of death around the world. WHO revealed that in 2018, 10 million individuals became sick with TB, and 1.5 million passed on from the illness (including in average of 251,000 individuals living with HIV). There were also around 10 million new cases with resistance to rifampicin; the most effective first-line drug, of which 78% had multi drug resistance tuberculosis (MDR-TB). Among instances of MDR-TB in 2018, 6.2% were estimated to have extensively drug resistant TB (XDR-TB) (1).

To investigate the Epidemiology of Tuberculosis, there are several molecular typing tools in epidemiological investigations and validation for use of *M. tuberculosis* strain diversification and clustering: Spoligotyping, insertion s6110-based restriction-fragment-length polymorphism (IS6110-RFLP) and Mycobacterial-Interspersed-Repetitive-Units Variable Number of Tandem-Repeats (MIRU-VNTR) (3), Next-Generation-Whole-Genome-sequencing (WGS) (4). Furthermore, they give important information on genetic assorted variety and microevolution of the *M. tuberculosis* genomes available for use (5) . Whole-genome-sequencing is preferred to other typing methods because of the strength and high resolution offered by the method (3). It anyway doesn't refute the helpfulness of other typing tools because of impediments experienced in asset constrained nations. These incorporate the absence of mastery to set up libraries and to investigate sequencing information, the expense of hardware and the general running expense (4).

Extra-pulmonary tuberculosis (EPTB) accounts for 16% of the 6.3 million incident cases of TB that were notified in 2018 worldwide(6).TB accounting approximately 26% of AIDS-related deaths with human immunodeficiency virus HIV-TB co-infection(7). Despite of the fact that pulmonary tuberculosis (PTB) accounts for the majority of cases and is the main transmissible form of the disease, and also contributes to the burden of disease and does not receive specific

attention in international control strategies(8).In developing countries where the incidence of TB is high, TB lymphadenitis (TBLN) is one of the most frequent causes of lymphadenopathy (30–52%) (9).

The occurrence of Tuberculous lymphadenitis is believed to be due to reactivation of healed and growing primary tuberculosis (10). *M. tuberculosis* normally enters the human body through the respiratory tract and forms Ghon's complex in the back section of the upper projection and experiences lymphohematogenous spread. Tuberculous lymphadenitis most of the time includes the cervical lymph node followed in recurrence by the mediastinal, axillary, mesenteric, hepatic entrance, perihepatic and inguinal lymph nodes (10).

Ethiopia is among the 48 high TB burden countries in the world. The proportion of EPTB in the country is very high(1). PTB accounts for 63.4% of all TB cases and EPTB records for 36.6% of TB cases. Out of this, 80% of EPTB is TBLN(11, 12). Tb lymphadenitis is the most widely recognized type of EPTB and records for 80% of all new EPTB cases in Ethiopia (13).

The identification of infectious cases is a crucial step for TB control programs worldwide. The target of a 70% case detection rate and 85% treatment success are not likely to be achieved with the existing laboratory diagnostic methods (13).

To improve the control of tuberculosis TB control programs must approach fast and reliable laboratory diagnosis. New tools have been developed in the last decades to improve the laboratory diagnosis of TB (14). Culture takes 3-8 weeks to demonstrate the growth of the organism (15). Furthermore, in Ethiopia, TB culture facilities are unavailable in the majority of health care centers. Polymerase Chain Reaction (PCR) is a sensitive and specific method for the diagnosis of EPTB. But it is unaffordable to be used for routine diagnostic purpose. Ziehl-Neelsen (ZN) staining using Fine Needle Aspirate (FNA) has low sensitive(16). FNA cytology could be a good alternative to diagnose TBLN since obtaining FNA samples from patients is less invasive (15).

Currently, several diagnostic methods of EPTB detection are in practice in Ethiopia. Among which the Lowenstein-Jensen (LJ) culture in regional laboratories; Cyto-morphological staining and bright field microscopy are being practiced all the more much of the time. Use of Light Emitting Diode Fluorescence Microscope (LEDFM) has also been introduced currently by Federal Ministry of Health to regions with high TB burden in Ethiopia. Event though, there are a

couple of works performed at specific regions of the country to evaluate, the overall efficacy, including the sensitivity and specificity of LEDFM TBLN diagnosis using FNA samples (12,13). Well representative works are required to show the performance of the microscope concretely.

In addition to the challenges of detection of the disease, MDR-TB is an emerging difficulty for TB control in Ethiopia. World widely in 2016 there were an expected 41% of new cases and 19% of recently treated cases with MDR/RR-TB (17). Moreover, the extent and distribution of drug resistance TB in Extra-Pulmonary cases in Ethiopia is not notable. The Line Probe Assay for the detection of rifampicin resistance and the GenoType MTBDR assay for the simultaneous detection of isoniazid and rifampicin resistance are recently developed methods to assess the level of drug resistance TB from clinical specimens and culture isolates of *Mycobacterium tuberculosis* complex (MTBC) (18). The GenoTypeMTBDRPlus distinguishes resistant from isoniazid and rifampicin in culture tests based on the identification of the most widely recognized mutations in the katG and rpoB genes respectively. Genotype® MTBDRsl is recognizes explicit mutations related with resistant from fluoroquinolones flq gyra and gyrb genes and second line injectable drugs (SLIDs) (rrs and eis genes) in *Mycobacterium tuberculosis* complex (19).

1.2. Statement of the Problem

The determination of Extra-pulmonary TB is still challenging in clinical practice, especially when the clinical presentation is suggestive but bacteriological proof is lacking. Standard single best test for analysis of EPTB is not investigated at this point. EPTB is often paucibacillary and the sites of infection may not be easily accessible for the collection of specimens which could be suitable for microscopy, histology, or culture.

On the other sides, Diagnosis of TBLN still faces many challenges again, though there are many applied diagnostic tools. Direct staining for Ziehl Neelsen (ZN) using FNA has been reported as the most rapid diagnostic method (20). However, the affectability of the microscopic analysis to a great extent relies upon the sample containing an adequate number of bacilli >104/ml. Constitutional symptoms associated with EPTB, (such as fever, weakness, knight sweating and weight loss) may be infrequent and non-specific. Furthermore, in Ethiopia *Mycobacterium tuberculosis* culture facilities are unavailable in the majority of health care centers. PCR is sensitive and specific but it is unaffordable to be used for routine demonstrative purposes (21, 22).

FNAC is an active negligibly invasive and practical method for the diagnosis of TB lymphadenitis. Thus, there is a need for a rapid and cost-effective technique for reliable diagnosis of TB-L particularly in limited resource settings like Ethiopia. Fluorescence microscope is preferred to Bright Field microscope for the examination of PTB, as it is more sensitive but equally specific and requires less laboratory time per slide (15). The development of Light-emitting diode fluorescence microscope, which are far cheaper and easier to maintain than the mercury vapor lamp traditionally utilized as a light source, is likely to enable increased use of fluorescence microscope in low income settings. Therefore, this study is designed to determine the diagnostic efficacy of LEDFM from clinically suspected cases of TBLN using FNA sample.

Understanding molecular characterization of TBLN strains in the distribution of Tuberculosis in populations and typing methods for discriminating isolates of the same species for characterizing tools in infection prevention and control is still challenging. In addition to this Molecular epidemiology data from TBLN isolates is not well understood in Addis Ababa even in the whole Ethiopia. Conventional typing frameworks based on phenotypes, for example, serotype biotype

phage type or antibiogram have been utilized for a long time. However, more recent methods that examine the relatedness of isolates at a molecular level have revolutionized our ability to differentiate among very close MTB strains. So, this study is designed to characterize Mycobacterium tuberculosis strains isolated from Tuberculous lymphadenitis cases using Spoligotyping.

Drug resistant poses a serious challenge to tuberculosis control. Nevertheless, tuberculosis is still affecting human beings by resisting drugs, particularly in Africa with continues transmission, diagnosis challenge, HIV co-infection, lack of vaccine with optimal protective efficacy and also due to an increasing trend of multidrug resistant strains of MTBC. Rising levels of tuberculosis drug resistant is recording in the world and especially in high Tuberculosis burden parts of the world. Recently, there have been two kinds of identification of drug resistance strains of MTBC. These are the Genotypic (Molecular) and Phenotypic (drug containing media) to test the drug resistant profile of each MTB isolates. Conducting the DST for isolates from MTBC is essential to distinguish the strains that is causing or contributing to outbreak of MDR-TB in Ethiopia.

1.3. Significance of the study

The main aim of incorporating LEDFM in the clinical diagnostic algorithm is to establish a suitable method for the effective diagnosis of EPTB in Ethiopia. In Ethiopia, because of absence of appropriate diagnostic options in public health facilities where most of the cases are accessing the service, the majority of the cases are identified by clinical examination after repeated trails (23). Of the available antibiotic's with continuity of the sequel and sufferings. Moreover, there is a 15% over diagnosis when using clinical diagnosis alone which leads to unnecessary suffering of the cases both due to the long time improper medication and the overwhelming disease (6).

It has been shown that LED based fluorescence microscope has many advantages over Bright field microscope and in one study it has shown that the sensitivity and specificity of LED microscope was excellent from EPTB cases (23). Determining the diagnostic utility of LED based fluorescence microscope for EPTB using FNA samples has a paramount effect on TB control strategy in Ethiopia.

Drug resistance pattern and molecular characterization data of TBLN is limited, especially in high burden countries like Ethiopia. The reason behind these is believed to be difficulty in limited number of laboratories in the country having the facility to perform culture and drug susceptibility (DST) and Molecular characterization testing for *M. tuberculosis* from Tuberculous Lymphadenitis drawn specimens. The molecular epidemiology and epidemiology of DRTB from TBLN strains is not well understood in Ethiopia. In the country, there is lack of data on the rate of occurrence of DR-TB from TBLN patients. For that purpose, determining the molecular epidemiology drug resistance of TBLN among TB Lymphadenitis patients has an important significance and potential contribution of evidence base for Tuberculosis control strategies.

these study results can help to improving diagnosis of TBLN, health care organizations, patients, researchers, and governmental and non-governmental body policy makers especially for health service providers to take the appropriate action over the problem in order to get early and possible disease-causing microorganism for the patients and even for the physician. Knowing the molecular epidemiology and Drug resistant and of TBLN from this study can also put an additional knowledge to researchers and policy makers in order to know the distribution and

determinants of disease in TBLN, and to controlling antimicrobial resistant and for guiding clinicians' decisions regarding appropriate treatment of choices.

2. Literature review

In many developed countries the widely used conventional florescence microscopy for the diagnosis of Tuberculosis has several advantages over light microscope using conventional ZN staining. Among those advantages, its simpler staining procedure, greater sensitivity and the option of examination of the smear under low power objectives of the microscope makes quick and efficient report of the slides. The study anticipated that using florescent microscope decreases the reporting time of the slides by 75% than ZN stained slides. Beside great advantages of conventional florescence microscopes, their higher initial purchase price, requirement of significant maintenance and limited lifespan of the bulbs and their toxic effect if broken are the limitation of conventional FM to apply in low income developing countries easily. This study were assessed that LED florescence microscope has better sensitivity over conventional mercury lamp florescence microscope and light microscope (24). In addition to this, the molecular epidemiology and DST profile of isolates were assessed from Tuberculous Lymphadenitis suspected patients.

In 2011 in WHO policy statement report, the evidence for the efficacy of LED microscopy was assessed, on the basis of standards appropriate for evaluating both the accuracy and the effect of new TB diagnostics on patients and public health. The results showed that the accuracy of LED microscopy was equivalent to that of international reference standards, it was more sensitive than conventional Ziehl-Neelsen microscopy and it had qualitative, operational and cost effectiveness over both conventional fluorescence and Ziehl-Neelsen microscopy (25).

As a study in Bangladesh by Munshi SK. *et al.,* compared the suitable methods for the effective diagnosis of extra-pulmonary tuberculosis 390 samples had been analyzed from different extra-pulmonary specimens. They have compared Bright-Field (BF) microscopy, LED fluorescence microscopy and LJ culture methods using LJ as a gold standard. The result showed that 53, 64 and 49 positive results for LJ, LED and BF microscopes respectively. Out of positive samples which were detected by the golden standard, 12 were found to be multi-drug resistant. It was also shown that LED microscope has higher relative positivity than both LJ and BF microscopy. The diagnostic efficacy of LED and BF microscope compared with LJ was 45.28% and 33.96% respectively. Specificity of both LED and BF microscopes was also assessed and found to be 88.13% and 90.80%, respectively. They have also analyses that positivity rate of LED

microscope among Lymph node aspirates separately. Among 30 lymph node aspirate samples, the positivity rate was 36.67%, 16.67% and 10% for LJ, LED and BF microscope respectively. They have also shown that the sensitivity for LED and BF microscope compared with LJ was 45.45% and 27.2% consecutively. As LED fluorescence microscopy has detected higher fraction (16.41%) of MDR cases than the culture method (13.59%); they assumed that this method may be useful for estimation of the undetected MDR cases (26).

A study conducted in South Africa by Gupta AK. *et al.,* compared the diagnostic performance of LED (main power source) and LED (rechargeable battery-powered) Fluorescence Microscope on Papanicolaou (PAP) stained smears with the conventional Mercury Vapour Lamp (MVL) on Fine-needle aspiration biopsy (FNAB) sample taken from children suspected for mycobacterial lymphadenitis. The researchers have found 84 mycobacterial cultures positive results out 121 children which were to be 69%. The mean sensitivity with LED (mains-powered), LED (rechargeable battery-powered) and MVL was respectively 48.2%, 50.0% and 51.8%. Also, the specificity was 78.4%, 86.7% and 78.4% respectively compared to culture (9).

Marzouk M. *et al.,* in Tunis performed a study to evaluate MTBDRplus assay for rapid detection of resistance to Isoniazid and Rifampin in *Mycobacterium tuberculosis* isolates from PTB and EPTB. Among 56 selected culture positive results, 21 culture isolates were EPTB and 35 were PTB. They have taken conventional DST as golden standard and obtained a total of 20 isolates to be resistant, only 1 isolate was MDR, i.e. resistance for both rifampin and isoniazid. Indicating its slightly lower detection of rifampin-resistant strains, they have evaluated that MTBDRplus assay was quicker and more cost-effective for the detection of rifampin and isoniazid resistance, especially in reducing those several weeks of time which is required for primary isolation and conventional DST (27).

Study conducted in Jimma, Ethiopia by Abdissa K. *et al.,* which compared the diagnostic performance of LED microscopy for tuberculous lymphadenitis in a high-burden setting; a total of 144 tuberculous lymphadenitis presumptive cases were included. 66.7% (96/144) were positive for *M. tuberculosis* complex on culture. Only one isolate was identified as non-tuberculous mycobacterium. The detection rates of Ziehl–Neelsen and LED microscopy were 18.8% (27/144) and 34% (49/144), respectively. As compared to culture, sensitivity was 25.0%

[95% CI: 16.3–33.7] for Ziehl–Neelsen microscopy and 45.8% [95% CI: 35.9–55.8] for LED microscopy. The specificity was 93.8% [95% CI: 86.9–100] for Ziehl–Neelsen microscopy and 89.6% [95% CI: 80.9–98.2] sensitivity of 82.3% and specificity of 54.2%. LED microscopy detected TB bacilli in 33.3% of cases cytologically classified as suppurative abscess (15). Beyond its association with high mortality and morbidity, HIV-related tuberculosis is difficult for diagnosis. A study compared the performance of light-emitting diode (LED) Auramine fluorescent microscopy and the GeneXpert MTB/RIF assay for the diagnosis of tuberculosis in HIV infected patients in a district hospital of India. They have analyzed different type of 419 body fluid samples and obtained that GeneXpert MTB/RIF globally increased number of positive results by 16.5% especially on extra pulmonary samples i.e. CSF followed by ascetic fluid and pleural fluids. Positivity of both LED microscopy and GeneXpert MTB/RIF were found to be 30.8% (95% CI) and 47.3 % (95% CI) (28).

The molecular characterization of TBLN conducted in Uganda on 121 isolates by Dan Wamala *et al*., which assess the predominance of genotype of MTB isolates from patients with TBLN indicates that, Euro-American (T2) sub linages was the predominant with SIT420, SIT53, SIT135, SIT128 and SIT590 in descending order (29).

In Ethiopia study conducted by Biadgelegn F. *et al*., entitled Tuberculous Lymphadenitis in Ethiopia Predominantly Caused by Strains Belonging to the Delhi/CAS Lineage and Newly Identified Ethiopian Clades of the Mycobacterium tuberculosis Complex indicate that, Delhi/CAS (38.8%) lineage, followed by Ethiopia 1 (9.7%), Ethiopia 3 (8.7%), Ethiopia H37RV-like (8.2%), Ethiopia 2 and Haarlem (7.7% each), URAL (3.6%), Uganda l and LAM (2% each), S-type (1.5%), X-type (1%), and 0.5% isolates of TUR, EAI, and Beijing genotype, respectively

The drug resistant profiles from TBLN isolates were also conducted in different parts of the world, in India study entitled Drug resistance among extrapulmonary TB patients: Six years' experience from a supranational reference laboratory was determined in 58 cases with *M. tuberculosis* isolated from lymph nodes, 11 (19%) were MDR, 25 (43%) were susceptible to SM, INH, RMP, EMB and 22 (38%) were resistant to one or more drugs (2 and 3 were R and OFX mono resistant, respectively) (30).

In Ethiopia study by Biadgelegn F. et al., Drug resistance of Mycobacterium tuberculosis isolates from tuberculosis lymphadenitis patients in Ethiopia, a total of 225 isolates were assessed for drug resistant pattern. Among those 15 (6.7%) were resistant to at least one first line anti-TB drug. Three (1.3%) were MDR-TB. resistance to INH, RIF, STM, and EMB was found in 8 (3.6%), 4 (1.8%), 10 (4.4%), and 4 (1.8%) isolates, respectively. Of the 212 new TB lymphadenitis cases three (1.4%) were MDR-TB. A rifampicin resistant M. tuberculosis isolate was diagnosed from smear and culture negative newly treated cases (31).

Another study in Addis Ababa, Ethiopia by Korma W. *et al.,* entitled with Clinical, molecular and drug sensitivity pattern of mycobacterial isolates from extra-pulmonary tuberculosis cases indicate that, Resistance to rifampicin was higher (22 %) than that for INH, STM and EMB (8.1 %, 5 % and 3 % respectively). Out of the 37 isolates tested for resistance, only 2 isolates were resistant for both STM and INH and no MDR strain was found (32)

3. Study Objectives

3.1. General Objective

- To determine the diagnostic efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope, Spoligotyping and drug resistance patterns of *Mycobacterium tuberculosis* strains isolated from clinically suspected Tuberculous Lymphadenitis.

3.2. Specific objectives

- To compare the efficacy of Light-Emitting Diode (LED) Fluorescence Microscopy with routine Ziehl-Neelsen techniques, FNA smear (Cytology) and Culture technique.
- To correlate the Cytomorphological diagnosis with the Light-Emitting Diode (LED) Fluorescence Microscopy and the conventional ZN method using culture as a gold standard.
- To characterize *Mycobacterium tuberculosis* strains isolated from Tuberculous Lymphadenitis cases using Spoligotyping.
- To assess drug susceptibility pattern of *Mycobacterium tuberculosis* complex among Tuberculosis lymphadenitis isolates.

4. Material and methods

4.1. Study Area, Period and setting

This study was conducted in All African Leprosy and Tuberculosis Rehabilitation and Training Center (ALERT) and St. Peter TB Specialized Hospitals from January to April, 2020 in Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia, with counts as of 2017 are growing closer to 4 million population census conducted by the Central Statistical Agency of Ethiopia (CSA) with annual growth rate of 3.8%. Addis Ababa lies at an altitude of 2324 m (7625 ft.) above sea level (sl) and located at 8°58'N, 38°47'E and has a mean annual temperature and rainfall of 15.9 $^{O}_{C}$ and 1089 mm, respectively.

ALERT hospital is a governmental hospital under the Federal Ministry of Health and was established in 1934 E.C. It's a referral hospital, which includes dermatology, Trauma, TB/MDR-TB/TB-HIV, Gyn/Obstetrics, ophthalmology medical and surgery departments, and also an orthopedic workshop, and rehabilitation program. It gives service for patients coming for referral cases from all parts of the country. ALERT Hospital has 25-40 of TB Lymphadenitis suspected cases per week. The pathology unit is linked with Armauer Hansen Research Institute (AHRI) Laboratory and gives service for both patients' diagnosis and research activities. St. peter TB specialized hospital was established in June, 1961 EC. It is a governmental hospital under Federal Democratic Republic of Ethiopia, ministry of Health (FMOH). The hospital provides various services especially in tuberculosis diagnosis and treatment. It serves as referral TB hospital from all parts of the country.

4.2. Study Design

A cross sectional study was conducted to determine the Diagnostic utility of Light-Emitting Diode (LED) Fluorescence based Microscopy, Spoligotyping and drug resistant patterns of *Mycobacterium tuberculosis* strains isolated from clinically suspected Tuberculous Lymphadenitis. All patients who were visiting and suspected for TBLN in both Hospital TB Clinic are participated in this study.

After informed consent/assent, patient's socio-demographic characteristics, Clinical data including their HIV status physical examination and obtained from each patient with a structured questionnaire, the patient was ready for FNA procedure by the pathologist.

4.3. Population

4.3.1. Source population

All patients who were visiting ALERT and St. Peter TB specialized Hospital TB clinic during the study period.

4.3.2. Study population

The study population for this study were all TBLN suspected patients at ALERT and St. Peter TB specialized Hospital TB clinics.

4.4. Inclusion and exclusion criteria

4.4.1. Participant inclusion criteria

- ✓ Study subjects included with full conformity to the national algorithm
- ✓ Patients who were willing to give informed consent for HIV of counseling and testing was participated in this study.

4.4.2. Participant exclusion criteria

- ✓ Patients who were critically ill
- ✓ Patients with medical contra-indications for FNA
- ✓ Proven pulmonary TB and on ant-tuberculosis treatment
- ✓ Patients with insufficient FNA sample was also excluded from this study.

4.5. Variables of the study

4.5.1. Dependent variables

- ✓ Efficacy of LED, ZN and cytology examination against culture
- ✓ Drug resistant pattern
- ✓ Spoligotype of the isolates were the dependent variables.

4.5.2. Independent variables

- ✓ Socio-demographic characteristics (Age, sex, address, occupation. Marital status)
- ✓ Specimen type
- ✓ Source population
- ✓ Host immune status (comorbidity) were the independent variables of this study**.**

4.6. Sampling and sample size determination

4.6.1. Sample size determination

Sample size at the required absolute precision level for sensitivity and specificity were calculated according to Buderer's formula (33).

$$n = \frac{(Z_{1-\alpha/2})^2 \times S_N \times (1-S_N)}{L^2 \times Prevalence}$$

Where:

n = required sample size based on sensitivity

S_N = anticipated sensitivity

α= Level of significance (1 – α is the confidence level)

Z1-α/2 = standard normal deviate corresponding to the specified size of the critical region (α), and

L = absolute precision desired on either side of sensitivity or specificity

Then; by using the anticipated sensitivity (S_N) 34%from previous study by Abdissa *K. et al.*(15).

For x=0.05, $Z_{1-\alpha/2}$= 1.96

L = 0.09

Prevalence of TB lymphadenitis in Ethiopia 32% (34)

Therefore, $n = \frac{(1.96)^2 \times 0.34 \times (1-0.34)}{(0.09)^2 \times 0.32} = 333$

The estimated sample size with 10% contingency was 366. Even though, due to the pandemic (SARS-CoV-2) disease outbreak we couldn't reach the selected size of a sample. The unprecedented time were affecting our sample collection situation because patients were not come to Hospitals afraid of the Pandemic and after while the Hospitals also restrict some services including pathological examination just for the sake of mitigating the infection.

4.6.2. Sampling procedure

Consecutive sampling techniques were employed to include study participants who meet the inclusion criteria. This study was designed to collect the FNA sample according to the sample size determination. But, unfortunately, due to this the unprecedented time (Corona virus outbreak), we didn't reach to the stated sample size.

FNA sample were collected using the standardized procedure in ALERT and St. Peter TB specialized Hospitals.

4.7. Data collection procedure

4.7.1. Socio-demographic, Clinical and Physical data collection

Data collectors was identified, trained to collect the entire data as per the pre-structured questionnaire. The purpose of the study as well as any related harm and benefit were explained to the study participants accordingly. Demographic data were recorded using (Annex III)

4.8. Specimen Collection and Processing

4.8.1. Fine Needle Aspiration (FNA)

FNA sample was collected by pathologist using 21-gauge needle from enlarged node, a standard procedure was applied for each patient who are suspected for TBLN. The overlying skin were cleaned with 70% alcohol. The enlarged node was fixed and maintained in stable position by the left hand of the attending Pathologist. Then the node entered with a negative pressure applied to the syringe. Multiple (average six) in and out passes were made by the needle without exiting the node (approximately 50-60 micro liters were collected). After removing the needle, a drop of collected FNA sample were placed on clean slide. Three smears were prepared: one for Cyto-morphological examination, one for Auramine O staining and the other for AFB staining. The leftover sample were transferred into Nunc Cryo Tubes containing 1 ml of normal saline for culture on LJ. To make a smear a second slide was placed on top of the first, allowing the drop to spread then gently slides pulled apart toward opposite end with one continuous motion. Movement a perfect suction is of rich consistency with various cells suspended in a limited quantity of tissue liquid without admixture with blood. contents mixed-up with blood can be spread like a fringe smear where particles will in general go to the edge of the spread and permit the slides to air dry. For Auramine O and ZN samples were smeared on clean slides, air dried and heat fixed (35, 36).

FNA samples collected from both Hospitals were placed on an icebox and transported to AHRI laboratory for processing as soon as possible.

As the study is designed to work on fresh FNA samples, all the collected samples were processed immediately upon arrival after collection.

4.9. Flow chart of study procedure

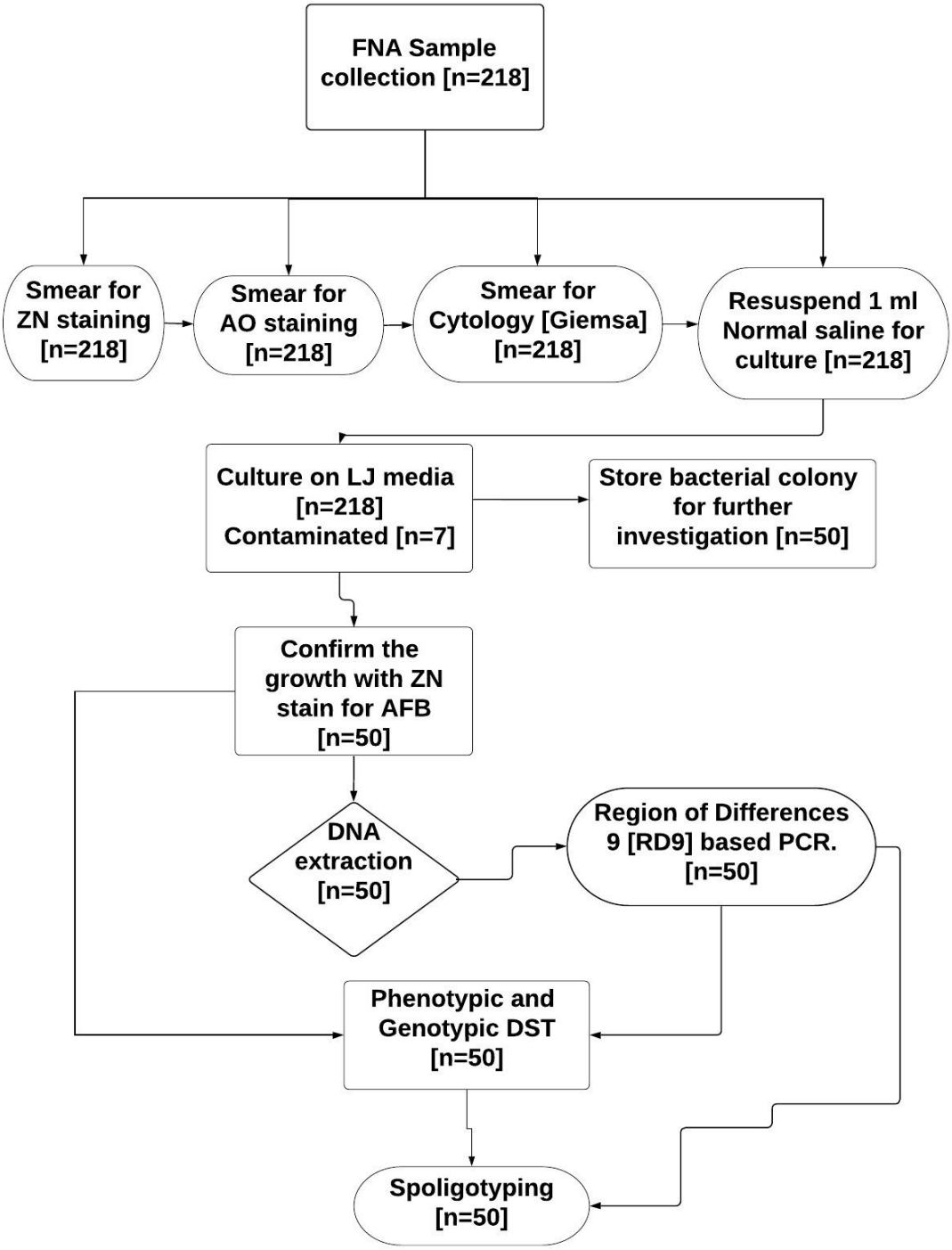


Figure 1. Flow chart of the study procedure

4.10. Laboratory Methods

4.10.1. Staining Techniques

4.10.1.1. Auramine O staining

Smears were stained with 0.1% Auramine O solution for 15 minutes, then washed with sterile water; decolorized with 0.5% Acid alcohol for 2 minutes at that point flushed with sterile water counterstained with 5% potassium permanganate for 2-4 minutes at that point washed with sterile water and permitted to air dry. After drying, smears were examined under the LED fluorescence microscope at 400X magnification (455nm) (37). The test result were interpreted as the following (38).

Table 1. Interpretation of LEDFM Quantification (Global Laboratory initiatives).

IUATLD/WHO scale (1000x field = HPF) Result	**Fluorescence Microscopy Quantification System**	
	(200–250x magnification: 1 length = 30 fields = 300 HPF)	(400x magnification: 1 length = 40 fields = 200 HPF)
Negative	Zero AFB / 1 length	Zero AFB / 1 length
Scanty	1–29 AFB / 1 length	1–19 AFB / 1 length
1+	30–299 AFB / 1 length	20–199 AFB / 1 length
2+	10–100 AFB / 1 field on average	5–50 AFB / 1 field on average
3+	>100 AFB / 1 field on average	>50 AFB / 1 field on average

4.10.1.2. Acid fast staining

Standard Ziehl-Neelsen staining procedures was applied. Carbol fuchsin stain were applied to cover the whole slide. Using a flame, the slides were slowly heated until steam arising and maintained for 5 minutes by using low or intermittent heat. After this procedure, the slides were rinsed with water and flooded with 3% acid alcohol and allowed to decolorize for 3 minutes. At the end, the slides were thoroughly rinsed with water and background stained with 0.1% methylene blue for 1 minute. Finally, the washed and dried smears were examined under the bright field microscope at 100X magnification and the test result was interpreted as the following (38).

Table 2. Interpretation of Bright-field Microscope Quantification (global laboratory initiatives)

IUATLD/WHO scale (1000x field = HPF) Result	Bright-field Microscopy Quantification system
	Bright-field (1000x magnification:1 length = 2 cm = 100 HPF)
Negative	Zero AFB / 1 length
Scanty	1–9 AFB / 1 length or 100 HPF
1+	10–99 AFB / 1 length or 100 HPF
2+	1–10 AFB / 1 HPF on average
3+	>10 AFB / 1 HPF on average

4.10.1.3. Cytomorphological staining

Air dried Smear were fixed immediately with alcohol and stained with Giemsa stain. Cytological analysis was performed by AHRI Pathologist. Epithelioid cell granulomas with or without multinucleated giant cells and caseation necrosis were the cytological criteria for diagnosis of TB-L (13).

4.10.1.4. Culture technique

From the FNA left over material, culture was performed according to standard procedure on Egg based Lowenstein-Jensen-glycerol based media following by liquefaction and by 2% N-acetyl-L-Cystine-NaOH solution. The decontaminated samples were centrifuged at 3000 rpm for 15 min. The supernatant was disposed and a drop of equal amount of Phosphate Buffer Saline solution was added into the pellet for Neutralization. The pellet was inoculated into Loewenstein-Jensen Egg based medium containing glycerol and incubated at 37ºC for at least 8 weeks, the culture was observed in weekly manner for the presence of mycobacterial colonies. All positive LJ cultures were confirmed by Ziehl-Neelsen staining and interpreted as at weeks 1 through 7. Colonies from AFB positive isolates were collected into one cryo vial and one Eppendorf tube. The Eppendorf tube was used to prepare heat killed cells for molecular testing (RD-9, ALP and Spoligotyping) at 80ºC in water bath for 45 minutes and following by 15 minutes sonication.

The other cryo-vial was stored at -80ºC in freezing medium until sub-cultured for drug sensitivity test (DST). No growth over the entire LJ media within 8 weeks were recorded as 'Negative'. Any single colony on the media was counted as positive for LJ culture. The following table is an standardized reporting scheme to report growth from the LJ culture (39).

Table 3. Interpretation of LJ Culture Quantification (World health organization)

Growth	Laboratory Report	ZN Result	Study Report
None	No growth	NA	Negative for MTB complex
1-9 colonies	Record Actual number	Positive	TB growth (1-9 colonies).
10-100 colonies	1+	Positive	TB growth (10-100 colonies).
>100-200 colonies	2+	Positive	TB growth (more than 100 colonies).
>200 colonies (too numerous to count)	3+	Positive	TB growth (innumerable or confluent).
Other Mycobacterial growth	Positive for other Mycobacteria	Positive	No MTB complex growth, but Positive for other mycobacteria.
Contamination	Contaminated	NA	Contaminated
ZN positive in presence of contamination	Positive for MTB and Contamination	Positive	Positive for MTB complex and Contaminated.

4.10.1.5. Region of difference 9 (RD-9) based polymerase chain reaction

Heat deactivated executed cells were utilized for polymerase chain reaction (PCR) based deletion typing. The nearness or nonattendance region of difference 9 was checked to distinguish *M. tuberculosis* from different types of *M. tuberculosis* complex. The primers utilized for RD9 deletion composing was: RD9flankF, 5′-GTG TAG GTC AGC CCC ATC C-3′; RD9intR, 5′-CTG GAC CTC GAT GAC CAC TC-3′; and RD9flankR, 5′-GCC CAA CAG CTC GAC ATC-3′. PCR amplification of the blends was performed utilizing a Thermal Cycler as indicated by standard methods (40). Briefly, the response blend was readied and DNA amplified with the cycling state of 10 min of compound activation at 95°C, 1 min of denaturation at 95°C, 0.5 min of annealing at 55°C, 2 min of elongation at 72°C for, including a sum of 35 cycles, and the last elongation at 72°C for 10 min. The item was electrophoresed in 1.5% agarose gel in 1× triacetate-ethylene diamine tetra acetic acid (EDTA) running buffer. Ethidium bromide at a proportion of 1:10, 100 base pair (bp) DNA ladder, and orange 6× stacking color were utilized in

gel electrophoresis and the pictured utilizing a Transilluminator (BIO RAD Laboratories Inc.). Identification of a band size of 396 bp were considered as positive for *M. tuberculosis*, while discovery of a band size of 575 bp was viewed as positive for different individuals from *M. tuberculosis* complex species *M. bovis* or *M. africanum* (40, 41).

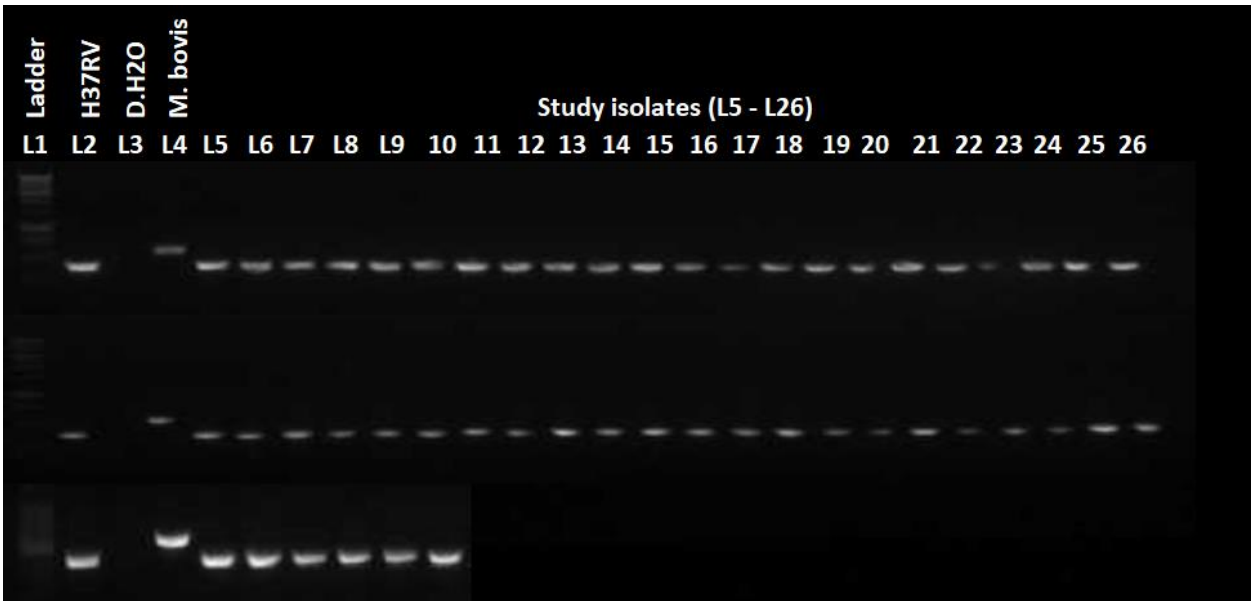


Figure 2. Region of difference 9 (RD9)-based polymerase chain reaction data drown from ALERT and St. Peter TB Specialized Hospital. Addis Ababa, Ethiopia (n =50) 2020.

4.10.1.6. Line Probe Assay (LPA) GenoType MTBDRplus test.

Bacteria grown on LJ media were used for GenoType MTBDRplus to perform according to the instructions provided by the manufacturer (Hain Lifescience GmbH, Nehren, Germany). The bacteria colony were collected with an inoculation loop and suspended in approximately 100 µL of Lysis Buffer (A-LYS) with oscillating and heated to 95^{o}C for 5 minutes in order to inactivate the vegetative bacilli in heat blocker following by spinning down briefly and adding additional 100 µL of Neutralization(A-NB). The supernatant DNA for PCR amplification were used after spinning down briefly for 5 minutes at full speed. All reagents needed for amplification such as polymerase and primers are included in the Amplification mixtures A and B (AM-A and AM-B) were optimized for this test. The solution containing 10 µl of AM-A, 35 µl of AM-B, and 5 µl of DNA template, for a final volume of 50 µl for single sample. The amplification profile comprised of 15 min of denaturation at 95°c followed by 1 cycle containing 30 sec at 95°C and 2 min at 65°C an additional 10 cycles comprehend, 25 sec at 95°C, 40 sec at 50°C, and 40 sec at 70°C, for 20 cycles and then a final extension at 70°C for 8 min in 1 cycle were used.

Hybridization and detection were performed with a TwinCubator (Hain Lifescience GmbH, Nehren, Germany). The hybridization procedure included the following steps: chemical denaturation (HYB) of the amplification products at room temperature for 5 min, hybridization of the single-stranded biotin-labeled amplicons to membrane-bound probes at 45°C for 30 min, stringent washes (STR) for 15 min at 45°C in shaking TwinCubator, addition of a diluted Conjugate for 30 min on shaking platform in TwinCubator, Removing the solution by 1 ml Rinse Solution (RIN) for 1 min with approximately 1 ml of distilled water. and an AP staining reaction to detect colorimetric bands were used. To detect RIF resistance, eight wild-type bands (WT) *rpoB* probes encoding codon analyzed 505 to 533 and four probes for common mutations were utilized (42, 43).

Probes used for INH resistance detection were designed to detect a WT 315 region, with two mutant band (katG MUT1 and katG MUT2) for the highly resistant katG gene and two probes specific for WT regions, as well as four mutant probes for the inhA gene, Which shows low-level resistant when all wild type probes demonstrated positive band for an isolate and mutant probe shows no band the isolates considered as susceptible. In correlation, the isolates were viewed as resistant when either any of the wild type probe missing or any of the mutant probe was available (44).

4.10.1.7. Conventional Phenotypic drug susceptibility testing

In 7H10 (Becton Dickinson Company, USA), drugs containing medium on 24-well tissue culture plate the aberrant extent strategy was utilized. The DST convention utilized in this investigation was the standardized. As per the Armauer Hansen Research Institute (AHRI) TB lab convention, which is embraced from the rules of the World Health Organization (45) the Conventional Phenotypic DST were conducted. The known four anti-TB drugs (Isoniazid [SIGMA-I-3377], Rifampicin [SIGMA-R-3501], Ethambutol [SIGMA-E-996] and Streptomycin [SIGMA-S-6501]) were prepared with the media at the recommended concentration and dispensed into 9 wells of the 24 well tissue culture plates and two wells were used with drug free media as of control. A standardized bacterial colony suspension was prepared against 1% MacFarland Standards solution from LJ Media and 10µl of the prepared bacterial colony suspension was dispensed into each of the drug containing and one drug free media. The remaining well with drug free medium was inoculated with 10µl of 1% (1:100) bacterial suspension. The plate was incubated in an inverted position at 35°C in the presence of sufficient humidity in aerobic

condition. Bacterial growth was checked every 6 days till the 21st day of reading of the DST. Resistance was expressed as the percentage of colonies that grew on critical concentrations of the substances, i.e. 0.2µg/ml, 5µg/ml, 2µg/ml and 1µg/ml for isoniazid, ethambutol, streptomycin and rifampicin respectively. Based on the standard of interpretation the conventional DST, the result was interpreted as of, 1% for all drug (46). M. tuberculosis H37Rv strain which is sensitive to all anti-TB drugs was done alongside the isolates as internal quality control. Control strains impervious to the diverse ant-TB drugs were inoculated into the wells where the separate anti-TB drugs had been added.

4.10.1.8. Spoligotyping Techniques

The verified Isolates to be M. tuberculosis utilizing RD9 deletion typing were additionally distinguished by Spoligotyping as indicated by the standard method (47). The direct repeat (DR) region was intensified by a Thermal Cycler utilizing oligonucleotides and two biotin-labeled primers (DRa: 5'- GGT TTT GGG TCT GAC-3' and DRb: 5'- CCG AGA GGG GAC GGA AAC-3') obtained from this region. The reaction blend was intensified with PCR and the enhanced item was hybridized to a set of 43 immobilized oligonucleotides, each relating to one of the remarkable spacer DNA groupings inside the DR locus. After hybridization, the DNA was identified by the enhanced chemiluminescence and by presentation to X-ray film as indicated by the producer. The hybridization designs were changed over into binary and octal configurations and contrasted and recently announced strains in the ongoing TBinsight and SITVIT2 database (47, 48).

4.11. Quality Assurance

Standard operational procedures for all laboratory tests were employed uniformly throughout the study. Corrective actions were taken immediately for laboratory works where any technical problems are identified. National guideline was used to conduct quality assurance in TB programme.

4.11.1. Pre analytical Phase

For TBLN infection patients FNA specimens are strongly recommended for microbiological determination using sterile container because of the reduced incidence of cellular and microbial contamination Sample was taken after clinical identification of possible TBLN and before the administration of antibiotics. The sample was aseptically obtained using sterile Syringe with needle from the nodule. Before the sample collection takes place, the sample collection site was

cleaned by using 70% Ethanol. Following of these, the collected sample were transported to the Mycobacteriology Laboratory immediately by maintaining its cold chain.

4.11.2. Analytical Phase

All materials, equipment and procedures were adequately controlled. Culture media and reagents were also tested for sterility, staining and growth performance, stability and pH value**.** Standard reference strains were used as control bacteria strains. Standard operating procedures (SOPs) of the microbiology laboratory of AHRI were strictly followed and the results will be checked by the supervisors.

4.11.2.1. Quality control

4.11.2.1.1. Staining Quality control

Slide smear Quality control for ZN and Auramine O stain was done with Negative control by known *E. coli* colony and as Positive control using known bacilli (1+) from culture growth (H37Rv) of MTB colony.

4.11.2.1.2. PCR techniques Quality control

Region of differences nine tests were done using standardized procedure and controlled with Negative quality control (Molecular grade water), and two Positive control (known *M. tuberculosis* strain H37Rv and *M. bovis* AF2122/97 (SB0140), against ladder. In Spoligotyping, the membrane was tested by using 4x Molecular grade distilled water as Negative control and 4x *M. tuberculosis* H37Rv and *M. bovis* A2122/97 (SB0140) as positive control prior to using.

4.11.2.1.3. Phenotypic and genotypic Quality control

The quality of Phenotypic DST controlled with isoniazid (ATCC 35835) and rifampicin (ATCC 35838) resistant were used as of Positive control and H37Rv strain which is susceptible to all type of the drugs were also used for Negative control. The two internal control (Conjugate and amplification control) were used for Genotypic DST (LPA). The conjugate control line ought to consistently be noticeable to reflect the productivity of conjugate binding and substrate response. The amplification control was serves as references for the interpretation of Wild type (WT) and Mutant (MUT) probes.

4.11.3. Post analytical Phase

The results were recorded with the patients' identification number. In order to avoid the errors in the results of the test, the reporting was checked twice before the results given to the hospital.

4.12. Data Entry, Storage and Management

All laboratory and clinical data were recorded on a logbook during the study period. Each completed questionnaire was properly coded and double entered into an excel spreadsheet, cleaned, verified and then transferred for statistical calculation.

4.13. Data Analyses

Statistical analysis was done using STATA 11 (Stata Corporation, College Station, TX). The Socio-demographic, physical and Clinical examination data, Laboratory results, sensitivity, specificity, positive and negative predictive values including their 95% confidence intervals (CI) were calculated by using the culture results as the "gold standard". Chi-square test were used to assess whether difference between values obtained are significant. All statistical tests were considered significant if the two-sided P-value as of <0.05.

4.14. Ethical consideration

The study was commenced after obtaining ethical clearance from Addis Ababa University College of Health Science Department of Medical Laboratory Science (DRERC) and AHRI ethical review committee. Support letter were obtained from Department of Research and Ethics Review Committee of Addis Ababa University. Study subjects were clearly informed about the purpose of the research and asked to participate in the study by offering their personal information and FNA specimen. All adult participants were provided with written informed consent. The parent or guardian were also be provided with written consent paper for their children. After they agreed to participate and signed in the written consent paper, while their right to refuse or to withdraw from participation at any time will be reserved; FNA samples was taken by an experienced pathologist.

5. Results

Among those 218 collected and inoculated on LJ media FNA samples, seven study samples were contaminated and excluded from this study, the rest 211 samples were illegible.

5.1. Socio-demographic characteristics of study participants

Of the 211 eligible TBLN suspected patients, a greater part was Females 129(61.14 %). The mean age of the study participant was 29(+/- 14.45 SD) years and ranged from 0.5-76 years. The majority of the study participants 108(51.18%) were between in the age group of 21 to 40 years. About 39.1% and 30.3% of participants were Married, and Single, respectively. More than half of study participants were also coming from urban areas 67%. In this study 48(22.75%) participants identified as an Unemployed. In terms of educational status, 84(40.98%) were Primary level. On statistical analysis of culture positive findings among 50 cases, differences between marital status was found to be significant (p-value = 0.033). (Table 4).

Table 4. Socio-demographic characteristics status and culture-confirmed TB cases of study participants data drown from ALERT and St. Peter TB Specialized Hospital. Addis Ababa, Ethiopia (n =211) 2020.

Variables		**Tested on LJ (Frequency) No (%)**	**Culture Positive No (%)**	**p-value**
Age group	0.5-10	20(9.48)	0	0.128
	11-20	43(29.86)	11(22%)	
	21-30	62(29.38)	18(36%)	
	31-40	46(21.80)	12(24%)	
	41-50	25(11.85)	7(14%)	
	>51	15(7.11)	2(4%)	
Sex	Male	82(38.86)	17(38%)	0.419
	Female	129(61.14)	33(66%)	
Marital status	Single	64(30.33)	20(40%)	0.033
	Married	84(39.81)	18(36%)	
	Divorced	7(3.32)	2(4%)	
	Widowed	17(8.06)	6(12%)	
	Living with partner	2(0.95)	1(2%)	
	Separated	4(1.90)	2(4%)	
	Living with parents	33(15.64)	1(2%)	

Living Area	Urban	141(66.82)	29(58%)	0.061
	Rural	70(33.17)	21(42%)	
Occupation	House wife	48(22.75)	11(22%)	0.142
	Daily laborer	22(10.43)	10(20%)	
	Government Employed	21(9.95)	4(8%)	
	Unemployed	42(19.91)	10(20%)	
	Farmer	21(9.95)	6(12%)	
	Other	57(27.01)	9(18%)	
Education status	Primary school	84(40.98)	17(34%)	0.483
	Secondary school	66(32.20)	20(40%)	
	Higher school	12(5.85)	2(4%)	
	Illiterate	43(20.98)	11(22%)	

5.2. Clinical status of study participants

Of 211 TB lymphadenitis patients the majority had weight loss 124(58.77%) and differences between weight loss and not loss individuals were had statistically association with culture positivity (p = 0.012). The greater part of the study participants had also night sweating 115(54.5%), poor appetite 96(54.5%), generalized body weakness 143(67.77%), whereas almost 75% of them had no cough. One hundred fifty-five (73.46%) study participants had low rate in increase of neck swelling and 77(36.49%) study participants had previous anti-tuberculosis treatment exposure. Based on this previous history of treatment exposure of study participants (before enrollment), 31(79.49%) of them were finished their course of drug while, 8(20.51%) was discontinued from anti-TB drugs. Thirty-three participants (15.64%), 112(53.08%), 40(18.96%) had history of contact with known TB patients, living in the same household with livestock and history of BCG vaccination, respectively.

All 211 study participants during testing period agreed to have HIV testing. Twenty seven percent were HIV positive [57 (27.01%)]. Of the 57 confirmed HIV cases, 42 (73.68%) had a previous history of anti-HIV treatment (p-value = 0.043). (Table 5).

Table 5. Clinical status and culture-confirmed TB cases of study participants data drowns from ALERT and St. Peter TB Specialized Hospital. Addis Ababa, Ethiopia (n =211) 2020.

Variables		**Tested on LJ (Frequency) No (%)**	**Culture Positive (no (%)**	**p-value**
Weight loss	Yes	124(58.77%)	37(74%)	0.012
	No	87(41.23%)	13(26%)	
If yes	<5kg	83(67.48%)	26(70.27%)	0.887
	5-10kg	37(30.08%)	10(27.03%)	
	>10kg	3(2.44%)	1(2.70%)	
Night sweating	Yes	115(54.50%)	31(62%)	0.223
	No	96(45.50%)	19(38%)	
Poor appetite	Yes	115(54.50%)	33(66%)	0.062
	No	96(45.50%)	17(34%)	
Generalized body weakness	Yes	143(67.77%)	37(74%)	0.281
	No	68(32.23%)	13(26%)	
Cough	Yes	55(26.07%)	17(34%)	0.143
	No	156(73.93%)	33(66%)	
Neck swelling	Yes	204(96.67%)	49(98%)	0.551
	No	7(3.33%)	1(2%)	
Rate in increase of neck swelling	Slow	155(73.46%)	42(84%)	0.132
	Moderate	36(17.06%)	6(12%)	
	Fast	20(9.48%)	2(4%)	
Intake history of anti-tuberculosis treatment	Yes	77(36.49%)	19(38%)	0.800
	No	134(63.51%)	31(62%)	
If yes, course of anti-tuberculosis in take	Finished	31 (79.49%)	6(66.67%)	0.277
	Discontinued	8(20.51%)	3(33.33%)	
History in contact of known TB patient	Yes	33(15.64%)	7(14%)	0.715
	No	178(84.36%)	43(86%)	
Intake history of raw milk	Yes	112(53.08%)	28(56%)	0.636
	No	99(46.92%)	22(44%)	

Living in the same household with livestock	Yes	90(42.65%)	23(46%)	0.584
	No	121(57.35%)	27(54%)	
History of BCG vaccination	Yes	40(18.96%)	8(16%)	0.541
	No	171(81.04%)	42(84%)	
HIV status	Positive	57(27.01%)	16(32%)	0.363
	Negative	154(72.99%)	34(68%)	
Any treatment for HIV	Yes	42(73.68%)	14(43.75%)	0.043
	No	15(26.32%)	18(56.25%)	

5.3. Physical examination characteristics of study participants

Majority of lymph node aspirates were obtained from Unilateral right sided 88(41.71%) and left sided 87(41.23%) of lymph node location of lymphadenopathy of anterior and posterior cervical region, which accounted for 86(40.76%), 54(25.59%), respectively. and following by supra clavicular 23(10.90%) and axillary 20(9.48%) on gross examination, tenderness of the lymph node was 48(22.75%) while, 163(77.25%) for none tender lymph node. Number of nodes were observed for single node in 51.66% (109/211) of the cases, followed by few nodes (2-4 nodes) in 38.86% (82/211). Among those, 141(66.82%) were non mobile lymph node. Condition on the lymph nodes were also observed; most of the lymph node were firm kind 122(57.82%) and following by matted 43 (20.38%) and the list one was hard 3 (1.42%) type (Table 6).

Table 6. Physical examination status of study participants data drowns from ALERT and St. Peter TB Specialized Hospital. Addis Ababa, Ethiopia (n =211) 2020.

Variables		Tested on LJ (Frequency) No (%)	Culture Positive No (%)	p-value
Location of lymph node				0.413
	Unilateral right sided	88(41.71%)	19(38)	
	Unilateral left sided	87(41.23%)	19(38)	
	Bilateral	35(16.59%)	12(24)	
	Generalized	1(0.47%)	0	
Position of lymph node				0.445
	Anterior cervical	86(40.76%)	21(42)	
	Posterior cervical	54(25.59%)	14(28)	
	Supra clavicular	23(10.90%)	6(12)	

	Axillary	20(9.48%)	7(14)	
	Mandibular	8(3.79%)	2(4)	
	Inguinal	16(7.58%)	0	
	Occipital	2(0.95%)	0	
	Femoral	1(0.47%)	0	
	Chest	1(0.47%)	0	
Tenderness of the lymph node				0.809
	Tender	48(22.75%)	12(24)	
	Non tender	163(77.25%)	38(76)	
Number of nodes				0.157
	Single node	109(51.66%)	22(44)	
	Few nodes (2-4)	82(38.86%)	20(40)	
	Multiple nodes (>5)	20(9.48%)	8(16)	
Mobility of the nodes				0.585
	Mobile	70(33.18%)	15(30)	
	Non mobile	141(66.82%)	35(70)	
Condition of the nodes				0.059
	Discrete	14(6.64 %)	5(10)	
	Matted	43(20.38 %)	16(32)	
	Firm	122(57.82 %)	21(42)	
	Soft	5(2.37 %)	0	
	Hard	3(1.42 %)	0	
	Fluctuant	17(8.06 %)	6(12)	
	Draining sinus	7(3.32 %)	2(4)	

5.4. Diagnostic accuracy of conventional ZN microscopy, LEDFM and cytology against culture result.

Diagnostic accuracy of ZN microscopy, LEDFM and Cytology was done against LJ culture. Mycobacterial culture was seen as positive in 23.7% (50/211). Among those culture positives, 30% (15/50) were detected by ZN microscopy, 78% (39/50) by cytology and 66% (33/50) by LEDFM. (Table 7).

Table 7. Diagnostic accuracy of ZN microscopy against culture result data drown from ALERT and St. Peter TB Specialized Hospital, Addis Ababa, Ethiopia. (n=211) (2020)

ZN Microscopy	**LJ culture at 8h week**		
	Positive	Negative	Total
Positive	15	14	29
Negative	35	147	182
total	50	161	211
	Percentage	[95% CI]	
Sensitivity	30%	[17.9-44.6]	
Specificity	91.3%	[85.8-95.2]	
Positive predictive value	35.5%	[22.2-51.4]	
Negative predictive value	89.1%	[87.2-90.8]	
likelihood ratio (+)	3.45%	[1.79-6.65]	
likelihood ratio (-)	0.767%	[0.636-0.925]	

Among culture negative samples, 77.6% (125/161) were correctly identified as negative by LED microscopy, 58.3% (94/161) by Cytology and 91.3% (147/161) by ZN microscopy. The sensitivity of ZN, Cytology and LED microscopy was 30%, 78%, and 66%, respectively against culture. The specificity of ZN microscopy was 91.3% and Cytology 58.8%, whereas 77.6% for conventional LED microscopy (P=0.001). Cytological morphology was indicative for TB by 49.7% (105/211) of total TB Lymphadenitis suspected cases. among those, 62.8% (66/104) were culture negatives. In general, a total of 78% (39/50) of culture-positive cases were classified as TB on cyto-morphological study.

Table 8. Diagnostic accuracy of LEDFM against culture result data drown from ALERT and St. Peter TB Specialized Hospital, Addis Ababa, Ethiopia. (n=211) (2020)

LEDFM	**LJ culture at 8th week**		
	Positive	Negative	total
Positive	33	36	69
Negative	17	125	142
total	50	161	211
	Percentage	[95% CI]	
Sensitivity	66%	[51.2-78.8]	
Specificity	77.6	[70.4-83.8]	
Positive predictive value	58.9%	[50.3-67.1]	
Negative predictive value	82.5%	[76-87.5]	
likelihood ratio (+)	2.95	[2.08-4.19]	
likelihood ratio (-)	0.438	[0.295-0.65]	

Cytological examination has a highest sensitivity and detection rate. At the same time a few false negatives were observed from cytology. However, cytology has the lowest specificity 58.8%. LEDFM has a greater sensitivity (66%) and detection rate next to cytology with (77.6%) Specificity. 13.7% (29/211) samples were detected for AFB by conventional ZN microscopy; 32.7% (69/211) were positive by LED microscopy and 49.7% (105/211) by cytology.

Table 9. Diagnostic accuracy of cytology against culture result data drown from ALERT and St. Peter TB Specialized Hospital, Addis Ababa, Ethiopia. (n=211) (2020)

Cytology	**LJ culture at 8th week**		
	Positive	Negative	total
Positive	39	66	105
Negative	11	95	106
total	50	161	211
	Percentage	[95% CI]	
Sensitivity	78%	[64-88.5]	
Specificity	58.8%	[50.7-66.5]	
Positive predictive value	65%	[59.4-70.1]	
Negative predictive value	73.1	[61.4-82.3]	
likelihood ratio (+)	1.89%	[1.49-2.4]	
likelihood ratio (-)	0.374%	[0.219-0.641]	

After all, just one case recognized positive by conventional ZN microscopy yet not distinguished by LED microscopy and cytology.

Table 10. Over all Diagnostic accuracy of conventional ZN microscopy, LEDFM and cytology against culture result data drowns from ALERT and St. Peter TB Specialized Hospital. Addis Ababa, Ethiopia

	ZN microscopy	**LEDFM**	**Cytology**
Sensitivity [95% CI]	30% [17.9 -44.6]	67% [51.2-78.8]	78% [64- 88.5]
Specificity [95% CI]	91.3% [85.8-95.2]	77.6% [70.4-83.8]	58.8% [50.7-66.5]
NPV [95% CI]	89.1% [87.2-90.8]	82.5% [76-87.5]	73.1 [61.4-82.3]
PPV [95% CI]	35.5% [22.2-51.4]	58.9% [50.3-67.1]	65 [59.4-70.1]
Likelihood ratio (+) [95% CI]	3.45% [1.79-6.65]	2.95 [2.08-4.19]	1.89 [1.49-2.4]
Likelihood ratio (-) [95% CI]	0.767 [0.936-0.925]	0.438 [0.295-0.65]	0.374 [0.219-0.641]
PPV=Positive predictive value. NPV=Negative predictive value.			

Based on cytomorphological examination, TB Lymphadenitis was screened in 49.7% (105/211), none TB in 12.8% (27/211), reactive lymphadenitis in 6.6% (14/211), malignancy in 5.7% (12/211) of the condition were observed. Among the conditions with suggestive cyto-morphological examination of TBL, 52.4% (55/105) were screened positive by LEDFM and 27.6% (29/105) by ZN microscopy. Tuberculous Lymphadenitis were likely to be positive by LED microscopy than to non-TB cytomorphological features [P-value = 0.005].

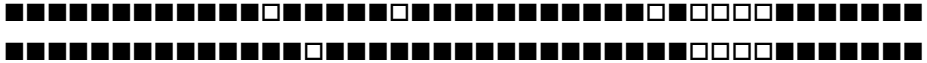
Table 11. Condition of the node and cytomorphological result comparison against LEDFM detection data drowns from ALERT and St. Peter Specialized Hospital, Addis Ababa, Ethiopia (n =211). (2020)

LED Fluorescent Microscopy				
		Positive	**Negative**	**P-value**
Condition of node	Discrete (n=14)	8(57.14%)	6(42.86%)	0.016
	Matted (n=43)	18(41.86%)	25(58.14%)	
	Firm (n=122)	33(27.05%)	89(72.95 %)	
	Soft (n=5)	1(20.00%)	4(80.00%)	
	Hard (n=3)	0	3(100.0%)	
	Fluctuant (n=17)	9(52.94%)	8(47.06%)	
	Draining sinus (n=7)	0	7(100.0%)	
Cytomorphology	TB Lymphadenitis (n=105)	55(52.38%)	50(47.61%)	0.005
	Reactive lymphadenitis (n=14)	1(7.14%)	13(92.85%)	
	Lymphoid hyperplasia (n=6)	0	1(100%)	
	Abscess (n=7)	1(14.24%)	6 (85.71%)	
	Malignancy (n=12)	1(8.33%)	11(91.67%)	
	Necrosis (n=1)	0	1(100%)	
	Granulomatous lymphadenitis (n=3)	1(33.33%)	2(66.67%)	
	Lymphoma (n=5)	0	5(100%)	
	Anaplastic plasmacytoma (n=1)	1(100%)	0	
	Subcutaneous lymphoma (n=1)	0	1(100%)	
	None TB (n=27)	6(22.22%)	21(77.78%)	
	Nodular colloid (n=5)	0	5(100%)	
	Inconclusive (n=7)	1(14.29%)	6(85.71%)	
	Other (n=8)	1(12.5%)	7(87.50%)	

5.5. Genetic diversity and family pattern of the bacterial isolates

About 86 Auramine O positive samples had *Mycobacterial* culture performed; 50 (58.13%) culture positives isolates were confirmed by polymerase chain reaction (PCR)-based deletion typing as of *M. tuberculosis*. Seven (8.1%) were contaminated and 29 (33.72%) failed to grow on culture. Spoligotyping found to be 50 different patterns of which, 31 (62%) were previously known in the international database and 19 (38%) were newly found patterns. The distribution of lineages family indicated that 15 (30%) isolates belongs to Euro-American (T), 10 (20%) to Euro-American (T3), 6 (12%) to Indo-Oceanic (CAS1-Delhi) and Euro-American (T3-ETH), 3 (6%) to Euro-American (H3) and Indo-Oceanic (CAS1-Kili), 2 (4%) to Indo-Oceanic (H1) and the rest are; Indo-Oceanic (T5-RUS1), Euro-American (H3-Ural-1), Euro-American (X1), Indo-Oceanic (Manu2), Euro-American (T2) had a 1 (2%) of each. The predominant spoligotype were the NEW strains 19(38%), SIT53 11(22%), SIT37 8(16%), SIT149 5(10%) and following by 1(2%) each for SIT118, SIT119, SIT777, SIT463, SIT52, SIT47 and SIT498.

In general, using of TBinsight and SITVIT2 website the linages show that 76% (38/50) of the isolates were belongs to the Euro-American, 22% (11/50) Indo-Oceanic and 2% (1/50) to East-African-Indian lineage.

SN	1st Spoligotyping Pattern	SIT No	Octal Number	Family	Lineage	Frequency (%)
1		New	777737377720771	H3	EA	19(38)
2		118	777767777760771	T	EA	1(2)
3		New	713777760003571	CAS1-Delhi	IO	19(38)
4		New	771760401762571	T5-RUS1	IO	19(38)
5		777	777777777420771	H3-Ural-1	EA	1(2)
6		149	777000377760771	T3-ETH	EA	5(10)
7		New	713777760003571	CAS1-Delhi	IO	19(38)
8		53	777777777760771	T	EA	11(22)
9		463	777777777720571	H3	EA	1(2)
10.		119	777776777760771	X1	EA	1(2)
11.		New	713777760003771	CAS1-Delhi	IO	19(38)
12.		New	713777760763771	Manu2	IO	19(38)
13.		52	777777777760731	T2	EA	1(2)
14.		53	777777777760771	T	EA	11(22)
15.		53	777777777760771	T	EA	11(22)
16.		47	777777774020771	H1	EA	1(2)
17.		New	777000777760771	T3-ETH	EA	19(38)
18.		37	777737777760771	T3	EA	7(14)
19.		New	713377420001771	CAS1-Kili	IO	19(38)
20.		New	713777761003571	CAS1-Delhi	IO	19(38)
21.		New	713777764003571	CAS1-Delhi	IO	19(38)
22.		53	777777777760771	T	EA	11(22)
23.		New	777774377760771	T	EA	19(38)
24.		498	777677777760771	T	EA	1(2)
25.		37	777737777760771	T3	EA	7(14)
26.		149	777000377760771	T3-ETH	EA	5(10)
27.		New	777377420001771	H1	IO	19(38)

EA = Euro-American, IO= Indo-Oceanic

Figure 3. Spoligotype pattern of M. tuberculosis strains isolated from TBLN patients from ALERT and St. Peter Specialized Hospital Addis Ababa, Ethiopia. (2020)

SN	2nd Spoligotyping Pattern	SIT No.	Octal Number	Family	Lineage	Frequency (%)
28.	■■■■■■■■□□□□□□□□□□■■■■■■■■■■■■■□□□□■■■■■■■	149	777000377760771	T3-ETH	EA	5(10)
29.	■■■■■■■■□□□□□□□□□□■■■■■■■■■■■■■□□□□■■■■■■■	149	777000377760771	T3-ETH	EA	5(10)
30.	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	53	777777777760771	T	EA	11(22)
31.	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	53	777777777760771	T	EA	11(22)
32.	■■■□□■□■■■■■■■■■■■■■■□□□□□□□□□□□□■■□□■■■■■■	New	713777740003171	CAS1-Delhi	EAI	19(38)
33.	■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	37	777737777760771	T3	EA	7(14)
34.	■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	37	777737777760771	T3	EA	7(14)
35.	□□□□□□■■□■■■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	New	006737777760771	T3	EA	19(38)
36.	■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	37	777737777760771	T3	EA	7(14)
37.	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	53	777777777760771	T	EA	11(22)
38.	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	53	777777777760771	T	EA	11(22)
39.	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	53	777777777760771	T	EA	11(22)
40.	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	53	777777777760771	T	EA	11(22)
41.	■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	37	777737777760771	T3	EA	7(14)
42.	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	53	777777577760771	T	EA	11(22)
43.	■■■■■■■■□□□□□□□□■■■■■■■■■■■■■■■□□□□■■■■■■■	New	777003777760771	T	EA	19(38)
44.	■■■■■■■■□□■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	New	777337777760771	T3	EA	19(38)
45.	■■■■■■■■■■■□■■■■■□■■■■■■■■■■■□■□□□□■■■■■■■	New	777737377720771	H3	EA	19(38)
46.	■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■□□□■■■■■■■■	37	777737777760771	T3	EA	7(14)
47.	■■■□□■□■■□■■■■■■■■■□□□■□■□■□■■□□□□□□■■■■■■■	New	713377425601771	CAS1-Kili	IO	19(38)
48.	■■■■■■■■□□□□□□□□□□■■■■■■■■■■■■■□□□□■■■■■■■	149	777000377760771	T3-ETH	EA	5(10)
49.	■■■□□■□■■□■■■■■■■■■□□□■□□□□□□□□□□□□■■■■■■■	New	713377420001771	CAS1-Kili	IO	19(38)
50.	■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	37	777737777760771	T3	EA	7(14)

EA = Euro-American, IO= Indo-Oceanic, EAI = East-African-Indian

Figure 3. Spoligotype pattern of M. tuberculosis strains isolated from TBLN patients from ALERT and St. Peter Specialized Hospital Addis Ababa, Ethiopia. (2020)

The black squares represent positive hybridization signals and the white one is representing a lack of hybridization

5.6. Drug susceptibility profile of the isolates

First-line anti-tuberculosis drugs (RIF, INH, EMB and STM) was tested for drug susceptibility for 50 isolates followed by second-line genotypic DST for those are exhibit resistant to first-line phenotypic and genotypic DST. Genotypic and phenotypic DST were used to compliment the result for first-line anti-tuberculosis drugs; a total of 11 (22%) isolates were found to be resistant to any single drug evaluated. Any resistant to any single drug were mostly identified for INH 7 (14%) followed by RIF 3 (6%) and only 1 (2%) STM in both phenotypic and genotypic DST.

The highest proportion of only single drug resistant was identified for only INH, 4 (8%). There was a combined drug resistant observed; only two drugs for INH and RIF 3 (6%) were identified by phenotypic and genotypic DST followed by RIF, INH and STM 1 (2%) by only phenotypic DST. There were no significant differences in susceptibility ability between first line phenotypic and genotypic DST. But, only one drug (STM) was found to be resistant by phenotypic DST which were not tested by genotypic DST. Three (6%) MDR/RR-TB was found in this study. for those of first-line MDR resistant to phenotypic and genotypic drugs (INH and RIF resistant) isolates, genotypic second-line [FLQ, KAN/AMK/CAP, KAN/CAP/VIO, KAN.AMK/CAP/VIO, Low-level KAN] DST were done. fortunately, no resistant was detected for second line genotypic DST. Two MDR-TB was detected in an HIV positive study participant.

5.7. Genotypic strains Vs drug resistant

Genotypic strains variance of the multi-drug resistant indicated that 18.2% (2/11) CAS1-Delhi and 9% (1/11) were T3-ETH strains.

Table 12. Comparison and status of genotypic resistance pattern of Second-line anti-TB drugs among first-line resistant strains data drown from ALERT and St. Peter TB Specialized Hospital. Addis Ababa, Ethiopia (n=50) (2020)

Variables				
Resistant to any single drug. case=7 (n=50)				
		By Phenotypic DST	By Genotypic DST	Percentage (%)
		1st-line	1st-line	
	RIF	3	3	6%
	INH	7	7	14%
	STM	1	-	2%
Resistant to only single drug. case=4 (n=50)				
		By Phenotypic DST	By Genotypic DST	Percentage (%)
		1st-line	1st-line	
	INH only	4	4	8%
Resistant to only two drugs. case=3 (n=50)				
		By Phenotypic DST	By Genotypic DST	
		1st-line	1st-line	
	INH + RIF	3	3	6%
Resistant to multiple drugs. case=1 (n=50)				
		By Phenotypic DST	By Genotypic DST	Percentage (%)
		1st-line	1st-line	
	RIF+INH+STM	1	0	2%

6. Discussion

This investigation identified and portrayed *M. tuberculosis* from TBLN patients at ALERT and St. Peter TB Specialized Hospitals. In association, diverse risk factors were assessed to see their relationship with the TBLN. The aftereffects of this examination demonstrated that there was no statistically noteworthy association of age, sex, occupation, night sweating, poor appetite, cough, previous contact with TB patients, history of raw milk utilization, living with the same household intake history of any anti-tuberculosis treatment, HIV status and BCG immunization with the event of TBLN. This finding is in concurrence with past discoveries (49-51).

Diagnosis of TB Lymphadenitis is very challenging due to the feature of a low bacterial load in specimen by routine laboratory methods. In spite of this, the invasive examination might be a necessary like FNA and Biopsy. However, FNA sample has diverse sensitivity for the determination of LNTB in various techniques. Sensitivity of cytological examination is the highest especially in the invasive FNA sample comparing to other methods (45).

According to WHO recommendations, LED microscopy is an option in contrast to conventional ZN microscopy (25). in these days, studies have evaluating the performance of LED microscopy for the direct detection of TB lymphadenitis specially in high incidence countries like Ethiopia. In this study, although differences were statistically insignificant, highest sensitivity was achieved with LEDFM with a good overall agreement of 66% [52.9-79.7] 95% CI.

This is an important finding and provoke previous studies that demonstrated the diagnostic utility of fluorescence microscopy, compared with conventional ZN microscopy (15, 52).

Lower specificity of LED microscopy contrasted with conventional ZN microscopy has been accounted for beforehand (15, 35, 53) Most investigations (54, 55) found that the specificity of LED microscopy was practically relative with that of conventional ZN microscopy. In our study, the specificity of LED microscopy was marginally lower than that of ZN microscopy, yet the thing that matters was not factually noteworthy. We found that scanty AFB and volume of the samples results on driven microscopy was less inclined to be related to a positive culture result. All things considered, mycobacteria from paucibacillary samples were killed during FNA sample processing procedure and fail to grow in culture (15, 56).

Time saving with LEDFM can be attributed to speedier filtering of each field in view of expanded perceivability of mycobacteria. Slides can be analyzed at a lower 40x magnifications, consequently permitting the assessment of extensive field for per unit of time. The decreased magnification utilized with LEDFM compared with light microscopy (400x Vs 1000x

objectives) may have contributed towards the slight affectability contrasts noted as has as of now been referenced in different investigations (57-59). The presentation of driven LEDFM in a high TBLN incidence setting would hence fundamentally minimize lab workload and perhaps permit better quality microscopy to be cultivated with a similar human resource contrasted with ZN microscopy (59).

In terms of sensitivity and specificity LEDFM and cytological examination offer different advantages over each other and also, another agreement with (15) LED microscopy, TB bacilli stood apart as splendid articles against a dull background of the microscope, which makes them effectively recognizable thus causing less eye strain and quicker evaluating of the bacillus. in fact, cytology has generally high sensitivity in any case with lower specificity in overall agreement (15, 53-55, 59, 60). This is because of the way that the cytomorphological examination includes in FNA cytology need specificity and, patients could likewise be over diagnosed.

Considering the utility of LEDFM among TBLN analyzed cases as non-TBL can advance the treating of the patient. Additionally, as a cytological examination is less specific and ZN microscope is less sensitive as studied by our this and past study (15, 54, 59), it is recommended to supplement the demonstrative algorithm with LED microscopy with the existing and future laboratories. Also, especially in the pastoral areas of the countries where FNA cytology laboratories are unavailable, LED based microscopy can be an option.

Taking everything into account, our investigation affirms that LED microscopy has higher sensitivity and bit lower specificity in examining of TB bacilli in FNA sample when contrasted with ZN microscopy. Enhancing cytology with LED microscopy can build specificity and improves the executives of patients associated with having TBLN. Besides, the more drawn out life expectancy of the LED frameworks, the quicker perusing time of smears and convenience would make this instrument best for fringe research facilities in resource-poor settings to improve proof-based finding and treatment.

In these studies, fifty MTBC species were briefly isolated from TBLN cases and further distinguished by RD9 deletion-based PCR followed by Spoligotyping, independently. In accordance with prior investigation, 100% of TBLN cases were *M. tuberculosis* (49, 56). No *M. bovis* were isolated in our study. This finding was in agreement with the discoveries that were

accounted for in various parts of Ethiopia (49, 51, 61). and the absence of *M. bovis* as a causative agent of TBLN in patients could recommend the trivial role of bovine TB in human beings.

In the current study, the majority of the isolates (76%) belonged to the Euro-American lineage followed by Indo-Oceanic 22% and only one case of East-Africa-Indian, 2%. A recent study in a capital city of Ethiopia (which was geographically similar to our study) reported that 63.3% of the isolates were Euro-America and 58.3% of them were Indo-Oceanic (56). Studies from Northern Ethiopia (Dessie) reported that 57.1% Euro-American, 28.6% Indo-Oceanic and 14.3 East-African-Indian (49). Euro-American lineages is more widely distributed and more predominant than all other lineages combined.

As per to the SITVIT2 and TB insight database the Spoligo International Typing (SIT) numbers the most prevalent shared types in the present study were NEW strains (38%), SIT53 (26%), SIT37 (16%), and followed by SIT149 (10%). These findings have a bit similarity and difference with previous reports from Ethiopia. O. Zewdie *et al*. reported the dominant strain from SIT149 followed by SIT53 and SIT26 (56) and Tadesse B. *et al*. also reported the NEW strains in higher rate following by different SIT numbers (49). Furthermore, SIT53 and NEW strains were reported to be the main strain in Addis Ababa, Ethiopia (49, 56).

The perception of a high level of clustering and recurrence of this M. tuberculosis Spoligotyping in the study area could propose the epidemiological significance of these strains. In general, it was noted that the geographic distribution and proportion of lineages in TBLN has not well studied in Addis Ababa even in wider in Ethiopia.

In the current study 86 percent 43/50 of the *M. tuberculosis* isolates were sensitive to the entirety of the first and second line TB drugs attempted. 14% of the analyzed TBLN patients were resistant to in any event one first-line anti-tuberculosis treatment. Regarding the degree of the issue, this pervasiveness could be considered as significant. Nonetheless, compared to the same study result, is relatively higher to the prevalence rates revealed in TB lymphadenitis drug-resistant in Ethiopia (62) and lower to the rate reported in India (30) .

MDR/RR-TB was found in three (6%) TB lymphadenitis patients as it was. a recent report directed in Ethiopia by Biadgelign F. *et al.* (31) indicated that 1.4% MDR-TB in new and previously treated TBLN patients, and 0 percent by Workneh K. *et al*. (32) from Addis Ababa,

Azger D. *et al*. (30) reported 1.6% from India . INH only resistant was 8% (4/50) in the present investigation and it is higher with reports from Ethiopia, 3.6% (8/225) (31).

In the current study, Among the three MDR/RR cases two of them are Positive for HIV and also, INH mono resistant was more associated with HIV patients. Since the study sites was a referral Hospitals, especially St. Peter for TB cases, the patients come from different areas of the countries to be treated after repeated treatment trial with different anti-biotics might be a reason behind to be failed to react to first-line ant-TB drugs.

INH monoresistance is the initial move towards anti-tuberculosis drug-resistant to tranquilize opposition and it is the regular pathway for the advancement of MDR-TB (36). We have also identified that there were no meaningful contrasts in susceptibility ability between first-line phenotypic and genotypic DST. But, only one drug (STM) was seen as resistant by phenotypic DST which were not tested by genotypic DST. There is a limited resource regarding to drug resistance pattern of EPTB, especially in high burden countries like Ethiopia. The reason behind these is believed to be difficulty in limited number of laboratories in the country having the facility to perform culture and drug susceptibility testing for M. tuberculosis from extra pulmonary specimens and even the epidemiology of DRTB is not well understood in Ethiopia (63). Because of these issues we highly recommend to further detail studies in these regards.

7. Conclusion and Recommendations

In conclusion, our study shows that ,the LED fluorescence microscopy gives a legitimate option in contrast to conventional ZN techniques in terms of its higher sensitivity, a bit lower specificity, time saving and minimal effort. LED-based fluorescent microscopy offers the most practical alternative for improved case finding in resource-restricted settings; enormous scope field analysis is required to evaluate the benefit and attainability of replacing or aligning conventional ZN microscopy with LED-based fluorescence microscopy in current algorithm as a first-line lab testing.

we have also identified three MDR/RR-TB cases and heterogeneous strains of M. tuberculosis from TBLN in a capital city of Ethiopia within both Hospitals (ALERT and St. Peter TB Specialized Hospital). nonetheless, the high extent of INH monoresistance from HIV patients is a critical risk for the potential development of MDR-TB as INH monoresistance is the underlying advance towards anti-TB resistant opposition and a typical pathway to the occurrence of MDR-TB. The NEW and SIT53 (T) strains was the most prevailing strains in the study area.

Thus, in general further similar studies should be conducted in this and different areas of the country in order to fulfill the existing research gap on drug resistance pattern and molecular characterizations from TBLN.

8. Dissemination of results

The study report will be submitted to College of Health Science, School of Allied Health Science, Department of Medical Laboratory Science of Addis Ababa University. It will also be submitted to Armauer Hansen Research Institute. A scientific paper on the results of the study will be submitted to an international or national peer reviewed journal for publication.

9. Facilities available for the study (major facilities)

The following facilities are available at AHRI laboratory for this study; TB culture, Molecular and Pathology laboratory. Laboratory equipment's like bio-safety cabinet, thermo cycler (PCR machine), LED-FM, autoclave, heat block, pipettes, incubator, water baths and all necessary reagents that are important for this particular study. The data management facilities available at AHRI will be used for data entry, cleaning and analysis.

10. References

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Annex I: Information sheet for study subjects (English version)

Principal Investigator: Gebeyehu Assefa

Armauer Hansen Research Institute

You are requested to participate in this study for which we would like to use the Fine Needle Aspirate (FNA) specimens. FNA sample means a sample which the pathologist will take from you using syringe and needle for your routine diagnostic purpose. We are going to use no additional sample but the leftover of your sample for a research purpose. So, we kindly request to give some of your time to read under understand the prepared explanation and ask any kind of question which is not clear for you. You have a full right not to be involved in this study or ever to terminate the participation even in between the study and this will never affect your routine diagnosis.

Purpose:

The purpose of this study is to determine the diagnostic efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope for the diagnosis of Tuberculous Lymphadenitis from Fine Needle Aspirate (FNA). Determining the diagnostic performance of LED microscope will help to improve the diagnosis of Tuberculosis Lymphadenitis and early diagnosis will help the patient to be treated with the appropriate treatment timely.

Procedures to be carried on:

you are invited to participate in the study after giving your consent by giving FNA sample for Cyto-morphological, bacteriological analysis and drug resistance pattern of the organisms as the doctors find best for you and you will give 50-60 micro-liter (≈ 3-4 drops) of FNA samples. We will do an investigation for *Mycobacterium Tuberculosis* at Armauer Hansen Research Institute TB laboratory.

Risks associated with the study:

The most common complication is swelling or bruising due to collection of blood under the skin (hematoma) at the needle insertion site. This is harmless and will resolve on its own, but may cause some discomfort and slight, temporary difficulty swallowing.

Serious complications such as bleeding (hemorrhage), infection, or inadvertent puncture of other structures in the neck are extremely rare. Bleeding risk is higher in patients who take aspirin, ibuprofen or blood thinners. The well experienced pathologist together with the clinical nurses in

the FNA sample collection room is able to control all these rarely happening risks immediately. If the participant senses any sign of infection at the site of needle insertion for the sample collection, he/she should communicate the focal person with the attached telephone number for appropriate treatment.

Benefits of the study:

There will be no financial or other direct benefit to you. But the result of the study will play a role in the TB control program. Confirmed positive results for MTB and MDRTB will be reported immediately to the physicians to follow every mandatory measure for the proper management of the patients and also the result will be used as an input for further epidemiological studies.

Compensations:

Study participants will not have any financial incentives or other inducements from participating on this study. However, based on the diagnosis result your child will be treated accordingly. Most importantly, the result of the study will be beneficial to design effective prevention and control measure of Tuberculous Lymphadenitis. Hence, you/your child are indirectly benefiting other patients and the society in this respect.

Confidentiality of your information:

The results of the lab 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. Separate registration form will be preparing to give a code number for individual participants and this registration code will kept very confidentially. To keep the confidentiality of the participants we will use the necessary codes given to the participants while analyzing results. All collected raw data will be stored at AHRI data management unit in secured manner.

Termination of the study:

We will respect your decision if you later on change your mind. Your withdrawal of consent will not affect your right to receive medication. The collected sample will be used only for your routine diagnosis and treatment. We are not using your sample for our study if you have decided to withdraw yourself from the study.

If you have any question you can ask the following individuals

Gebeyehu Assefa (principal investigator**)**

Cell phone: +251-913-333-530

AAERC

Phone: +251-011-8962183

Annex II: Consent form (English version)

Consent form for adults (≥ 18 years) participants

- I, the undersigned person, understand that this study is going to be conducted on Diagnostic efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope for the diagnosis of Tuberculous Lymphadenitis from Fine Needle Aspirate at Armauer Hansen Research Institute. I understand that the result from this study will help the control of Tuberculosis by improving early detection and management of the disease.
- It is explained for me that the objective of the study is to determine the diagnostic efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope for the diagnosis of Tuberculous Lymphadenitis from Fine Needle Aspirate and select a simple, cost effective, and applicable method at peripheral health facilities.
- I understand that if I am enrolled in this study, 50-100 µL of fine needle aspirate will be collected from the enlarged lymph node by the pathologist.
- I understand that all the results will be explained to me and all the data obtained will be kept strictly confidential by using only code numbers. If my result is positive, I will be treated according to the national guideline.
- I understand being enrolled in this study is fully dependent on my agreement.
- I am clear for all explanation and agree to participate in the study. I put my signature for my agreement.

Participant Name_____

Signature_____

Date_____

Consent form for participants between 12 and 17 years' old

- I, the undersigned person, understand that this study is going to be conducted on Diagnostic efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope for the diagnosis of Tuberculous Lymphadenitis from Fine Needle Aspirate at Armauer Hansen Research Institute. I understand that the result from this study will help the control of Tuberculosis by improving early detection and management of the disease.
- It is explained for me that the objective of the study is to determine the diagnostic efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope for the diagnosis of Tuberculous Lymphadenitis from Fine Needle Aspirate and select a simple, cost effective, and applicable method at peripheral health facilities.
- I understand that if I am enrolled in this study, 50-100 µL of fine needle aspirate will be collected from the enlarged lymph node by the pathologist.
- I understand that all the results will be explained to me and all the data obtained will be kept strictly confidential by using only code numbers. If my result is positive, I will be treated according to the national guideline.
- I understand being enrolled in this study is fully dependent on my agreement. I am also informed that my participation in this study also needs my parent's agreement.
- I am clear for all explanation and agree to participate in the study. I put my signature for my agreement.

Participant Name_____

Signature _____

Date_____

Name of Parents/guardian_____

Signature of Parents/guardian_____

Date_____

Consent form for parents of participants below 12 years' old

- I, the undersigned person, understand that this study is going to be conducted on Diagnostic efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope for the diagnosis of Tuberculous Lymphadenitis from Fine Needle Aspirate at Armauer Hansen Research Institute. I understand that the result from this study will help the control of Tuberculosis by improving early detection and management of the disease.
- It is explained for me that the objective of the study is to determine the diagnostic efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope for the diagnosis of Tuberculous Lymphadenitis from Fine Needle Aspirate and select a simple, cost effective, and applicable method at peripheral health facilities.
- I understand that if I am enrolled in this study, 50-100 µL of fine needle aspirate will be collected from the enlarged lymph node by the pathologist.
- I understand that all the results will be explained to me and all the data obtained will be kept strictly confidential by using only code numbers. If my result is positive, I will be treated according to the national guideline.
- I understand the enrolled of my child in this particular study id fully dependent on my agreement.
- I am clear for all explanation and agree the participation of my child in the study. I put my signature for my agreement.

Name of Parents/guardian_____

Signature of Parents/guardian_____

Date_____

Annex III-Questionnaire

This questionnaire form is intended to determine the diagnostic efficacy of Light-Emitting Diode (LED) Fluorescence based Microscope for the diagnosis of Tuberculous Lymphadenitis from Fine Needle Aspirate (FNA) in ALERT and St. Peter TB Specialized Hospital. The study will be conducted through analysis of FNA samples from patients who are suspected of having Tuberculosis-Lymphadenitis (TB-L).

I. Socio-demographic Data

I. Patient Identification Date____/____/_____

1. Study code number _____
2. Hospital/health centre number. _____
3. Region _____ Zone _____ kebele _____ (Tele)_____
4. Age_____
5. Sex_____
6. Living Area: Urban_____ Rural_____
7. Marital Status 1. Single_____ 2. Married_____ 3. Divorced_____ 4. Widowed_____5. Living with partner_____
8. Occupation 1. House wife_____ 2. Daily labourer_____ 3. Government employ_____ 4. Unemployed_____ 5. Farmer_____ 6. Others_____
9. Educational Status (year of Schooling) 1. 1-6 yrs. ____ 2>7 yrs._____ 3. Illiterate_____

II. Clinical Data

1. Temperature (in 0^0). _____
2. Weight loss 1. Yes _____2. No _____
3. If yes 3.1. weight loss < 5kg___ 3.2. weight loss 5-10 kg____3.3 weight loss > 10kg____
4. Night sweating 1. Yes _____2. No_____
5. Poor appetite 1. Yes _____2. No_____

6. Generalized body weakness 1. Yes _____2. No_____

7. Cough 1. Yes_____2. No_____

8. The duration of the neck swelling or cough (in weeks) _____

9. Rate of increase of the swelling 1. Slow _____2. Moderate_____3. Fast_____

10. Intake of antibiotic for the swelling 1. Yes _____2. No_____

    If yes what kind (if they know the name or describe the colour, size, shape)

11. History of anti-tuberculosis treatment previously 1. Yes _____2. No_____

    If yes, when_____

    1. Finished the course_____ 2. Discontinued_____

12. History of contact with known TB patient

    1. Yes _____2. No._____

13. History of intake of raw milk 1. Yes _____2. No_____

14. Living in same house hold with livestock (cattle, calves, sheep, goats, etc.)

    1. Yes _____2. No_____

15. Regular direct contact with livestock

    1. Yes _____2. No_____

16. History of BCG vaccination (BCG scar on right arm)

    1. Yes _____2. No_____

17. Do you know your HIV status? 1. Yes _____2. No_____

18. Time since diagnosis of HIV _____

19. Any treatment for the HIV 1. Yes _____2. No_____

    If yes, what type_____

III. Physical examination

1. Lymph node description_____
   _____
   _____
2. Location.1.1 Unilateral right sided_____1.2. Unilateral left sided_____2. Bilateral_____
3. Position 1. Anterior cervical_____2. Posterior cervical_____3. Supra clavicular_____
4. Tenderness 1. Tender _____2. Non-tender_____
5. Number of nodes 1. Single node_____2. Few nodes (2-4) _____3. Multiple nodes (>5) _____
6. Size (~) _____cm
7. Mobility 1. Mobile_____2. Non mobile_____
8. Conditions of the nodes
   1. Discrete_____2. Matted_____3. Firm_____4. Soft_____
      5. Hard_____6. Fluctuant_____7. Draining sinus_____

9. Clinical diagnosis _____

_____

_____

IV. laboratory Examination Form

1. Information of Aspirated Sample and Laboratory Results

1.1 Aspirated Sample information

Date of sample collection _____day_____ Month _____year. Time of sample collection _____

Total no of sample received

1. Smear on Slides 1 2 3

2. FNA suspended in Normal saline YES NO

3. Adequate number of samples collected YES NO

4. If NO, why? A. Withdrawal B. Insufficient amount of aspirate

1.2 Laboratory Results

1.2.1 ZN staining **1** NEG

2 Scanty 1+ 2+ 3+

1.2.2 Auramine O staining (LED FM)

1. NEG

2. Scanty 1+ 2+ 3+ 4+

1.2.3 LJ culture result at 8th week

1 NEG

2 POS

1.2.4. GenoType MTBDR plus test result

Method used:□Phenotypic DST □1st line drugs□2nd line drugs □ Molecular DST [LPA] □1st line drugs□2nd line drugs

DST	1st line drugs					2nd line drugs				
	INH	RMP	STM	EMB	PZA	FLQ	KAN/AMK/ CAP	KAN/CAP/ VIO	KAN/AMK/ CAP/VIO	Low-level KAN

NOTE: INH=Isoniazid RMP=Rifampicin STM=Streptomycin EMB=Ethambutol PZA=Pyrazinamide.FLQ=Floquinolones. KAN=kanamycin. CAP=Capreomycin. VIO=Viomycin. AMK=amikacin. S= Sensitive; R = Resistant; C = Contaminated; ND = Not done.

1.2.4. Cyto-morphology result

1. Clinical information: _____
2. Gross appearance of FNA Sample _____

_____

3. Report (Microscopy) _____
4. Conclusion

_____

_____

_____

_____

_____

_____

Annex IV: Information sheet for study subjects (Amharic version)

የመረጃ ቅጽ

የሊድ ፎሎረሰንት የማይክሮስኮኘ ከሳንባ ውጪ የቲቢ በሽታ ምርመራን ለማሻሻል ያለውን አስተዋጽዎ ለማወቅ የሚደረግ ጥናት ማብራሪያ

የዋና ተመራመሪው ስም: ገበየሁ አሰፋ

የድርጅቱ ስም: አርማወር ሐንሰን የምርምር ኢንስቲቱዮት

ማብራሪያ:

ከሳንባ ውጪ ያለ በሽታ/ቲቢ/ምርመራ ለማሻሻል በሚደረገው ጥናት እርስዎ እንዲሳተፉ ተጋብዘዋል:: ለዚህ ጥናት የሚሆነዉን ናሙና የምንጠቀመዉ ፓቶሎጂስቱ ለእርሶ መደበኛ ምርመራ የሚሆን ናሙና በስሪንጅ ከወሰደ በሁዋላ የሚተርፈዉን ነዉ። በመሆኑም ጥቂት ጊዜ ወስደው ስለጥናቱ የተዘጋጀውን ይህን የማብሪረያ ጽሁፍ እንዲያነቡና ግልጽ ያልሆነለዎትን ማንኛውንም ጥያቄ በመጠየቅ ስለጥናቱ በቂ ግንዛቤ እንዲኖሮዎት ያስፈልጋል:: በዚህ ጥናት ለመሳተፍ ካልፈለጉ እይገደዱም እንዲሁም መሳተፍ ከጀመሩ በኋላ በማንኛውም ግዜ ከጥናቱ አቋርጠው መውጣትይ ችላሉ:: ይህንን በማድረግዎ በማህከሉ የሚደረግልዎትን ህክምና በምንም መልኩ አያስተጓጉልም::

አላማው:

የዚህ ጥናት ዓላማ ተሻሽሎ የተሰራዉን ፍሎሮሰንት ማይክሮስኮፕ ከሳንባ ዉጪ የሚከሰተዉን የቲቢ በሽታ የመመርመር ብቃት መፈተሽ ነዉ። ይህም በመደረጉ ከሳንባ ዉጪ የሚከሰተዉን የቲቢ በሽታ በተገቢዉ ጊዜና ፍጥነት ለመመርመርና ህክምናዉን ቶሎ ለመጀመር ይረዳል።

ቅደምተከተል:

በተደረገልዎት አጠቃላይ ምርመራ በዚህጥናት ለመሳተፍ ሃኪሙ ብቁ መሆንዎት ካረጋገጠ ስለ ጥናቱ አላማ እና ስለ ሚጠበቀው ውጤት በቂ መረጃ ይሰጠዎትና የስምምነት ቅጽ እንዲፈርሙ ይደረጋል:: በዚህ ጥናት ሲሳተፉ በትክክል የቲቢ ታማሚ መሆንዎን ለማወቅ የሚያስችል በጤና ባለሙያ የህክምና ምርመራ ይደረግልዎታል:: ከዚህ በተጨማሪ ለመደበኛ ለላቦራቶሪ ምርመራ አገልግሎት የሚውል ከእብጠቱ ቦታ ላይ በመርፊ የሚወሰድ ናሙና ይሰጣሉ። የተረፈዉ ናሙና ለምርምሩ ይዉላል። ይህም በግምት ከሶስት እስከ አራት ጠብታ ይሆናል። ሁሉም የምርምር አይነቶች አለርት ሆስቲታል በሚገኘዉ

በአርማወር ሃንሰሀ የምርምር ኢንስቲቲዉት ይኪያሄዳሉ። የምርመራ አይነቶቹም የኤችአይቪ፣ ፓቶሎጂ፣ እና ቲቢ ባክቴሪያ ከተገኘ መድሀኒቱን መላመድ አለመላመዱ ይመረመራል።

ስጋት እና ጉዳት:

በዚህ ጥናት ሊሳተፍ ናሙናው የሚወሰደው የሙያው ስነ-ምግባር በሚያሟላ እና በቂ እዉቀት ባላዉ ባለሙያ እና አዳዲስ እቃዎች ይከናወናል:: አልፎ አልፎ በእብጠቱ አካባቢ የተጠራቀመ ደም ካለ እና በተለይ መድማትን የሚያባብሱ መድሀኒቶችን ከወሰዱ ናሙናዉ በሚወሰድበት ጊዜ የመድማት ምልክት ሊያሳ ይይችላል። ነገር ግን ናሙናዉን የሚወስደዉ ሀኪም እና ነርሶች አስፈላጊዉን ህክምና ያደርጉሎታል። ናሙናዉን ከሰጡበት በኁላ የኢንፌክሽን ምልክት ካዩ ከታች በተጠቀሱት ስልክቁጥሮች በመደወል አስፈላጊዉን እርዳታ ያገኛሉ።

ጥቅሞች:

በዚህ ጥናት በመሳተፍዎ የሚያገኙት ቀጥተኛ የሆነ ልዩ ጥቅም የለም:: ነገር ግን የጥናቱ ውጤቶች ወደ ፊት የቲቢ በሽታን ሒደት ለማወቅና ለመቆጣጠር ለሚደረገው ጥረት ከፍተኛ ጥቅም ይሰጣል:: በተለይ መድሀኒቱን የተላመደ የቲቢ ባክቴሪያ ከተገኘ ተገቢዉን የህክምና አይነት አንዲጀምሩ ወድያዉኑ ለሀኪሙ ሪፖርት ይደረጋል።

ምስጢራዊነት:

ከዚህ ምርምር የምንሰበስበው መረጃ በምስጢ ርይያዛል:: ከእርስዎ የሚገኘው መረጃ የተሳታፊውን ስም በማይጠቅስ መልኩ በቁጥር ወይም በኮድ መልክ ይመዘገባል:: የቱ ቁጥር ወይም ኮድ የየትኛው ተሳታፊ ግለሰብ እንደሆነ በማይታወቅበት ሰነድ በተቆለፈ ቦታ ይቀመጣል:: ይህም ምስጢር ለዋናው ተመራማሪ እና ለሃኪሙ ብቻ ካልሆነ በስተቀር ለሌላ ለማንም ሰው አይሰጥም::

በጥናቱ ያለመሳተፍ ወይም አቋርጦ የመውጣት መብት:

በዚህ ጥናት ለመሳተፍ ካልፈለጉ አይገደዱም:: እንዲሁም መሳተፍ ከጀመሩ በኋላ በማንኛውም ጊዜ ከጥናቱ አቋርጠው መውጣት ይችላሉ:: ይህንን በማድረግዎ በማህከሉ የሚደረግልዎትን ህክምና በምንም መልኩ አይስተጓጉልም::

ጥያቄ ካለዎት አሁን ወይም ሌላ ግዜ ሊጠይቁ ይችላሉ::

ሌላ ጊዜ ለመጠየቅ ቢፈልጉ ከዚህ በታች የተጠቀሱትን ግለሰቦች ማነጋገር ይችላሉ::

ገበየሁ አሰፋ

ዋና ተመራማሪ

ስልክ ቁጥር +251-913-333-530

አህሪ/አለርት የምርምር ስነምግባር ኮሚቴ

ስልክ ቁጥር: +251-118-962-183

Annex V. Consent form (Amharic version)

<u>ለቲቢ ሊንፋዲናይትስ /ከሳንባ ውጭ የቲቢ በሽታ/ ምርምር የአዋቂዎች ፈቃደኝነት መጠየቂያ ቅፅ</u>

- ✓ እኔ ስሜ ከዚህ በታች የተገለጸዉ ይህ ጥናት በአርማወር ሐንሰን የምርምር ተቋም ተመራማሪዎች ከሳንባ ዉጪ ላለ የቲቢ በሽታን ለመመርመር የሚያስችልን የመመርመሪያ ዘዴ (ፍሎሮሰንት ማይክሮስኮፕ) ያለውን የመመርመር ብቃት ለማጥናት እንደሚካሄድ ተገልፆልኛል።
- ✓ ከዚህ ጥናት የሚገኘው ውጤት በሽታውን ለመቆጣጠር የሚደረገውን ጥረት ሊደግፍ አንደሚችል ተረድቻለሁ።
- ✓ በዚህ ጥናት ውስጥ ለመሳተፍ ፍቃደኛ ከሆንኩ ለጥናቱ የሚያስፈልገውን ከ50-100 ማይክሮሊትር (ከአራት እስከ አምስት ጠብታ) ፈሳሽ ከአበጠው ቦታ ላይ በሰለጠነ ባለሙያ በመርፌ እንደሚወሰድ ተነግሮኛል።
- ✓ ከሳንባ ውጭ የቲቢ በሽታ ምርመራ እንደሚደረግልኝ እና የቲቢ ጥገኛ ህዋስ በተወሰደው ናሙና ውስጥ ካለ አስፈላጊውን ህክምና እንደማገኝ ተረድቻለሁ።
- ✓ የምርመራ ውጤቴም እንደሚነገረኝና በሚስጥርም እንደሚያዝ ተረድቻለሁ።

- ✓ በጥናቱ ብሳተፍም ባልሳተፍም አስፈላጊውን ለማንኛውም ህሙማንየ ሚሰጠውን አገልግሎት በአቅራቢያዬ ካለው ሆስፒታል ወይንም ጤና ጣቢያ እንደማገኝ እና በዚህ ጥናት መሳተፍ ወይንም ያለመሳተፍ እንዲሁም በፈለኩት ጊዜ ከጥናቱ የመውጣት መብት እንዳለኝ ግልጽተ ደርጎልኛል።
- ✓ ከላይ የተደረገልኝን ማብራሪያ በሚግባ ተረድቻለዉ በጥናቱም ዉስጥ እንደምሳተፍ በፊርማዬ አረጋግጣለዉ።

የተሳታፊው ስም_____

ፊርማ_____

ቀን _____

<u>ለቲቢ ሊንፋዲናይትስ/ከሳንባ ውጭ የቲቢ በሽታ/ ምርምር ዕድሜያቸዉ ከ12 እስከ 17 ለሆኑ ተሳታፊ ታዳጊ ወጣቶች ፈቃደኝነት መጠየቂያ ቅፅ</u>

- ✓ እኔ ስሜ ከዚህ በታች የተገለጸዉ ይህ ጥናት በአርማወ ሐንሰን የምርምር ተቋም ተመራማሪዎች ከሳንባ ዉጪ ላለ የቲቢ በሽታን ለመመርመር የሚያስችልን የመመርመሪያ ዘዴ (ፍሎሮሰንት ማይክሮስኮፕ) ያለውን የመመርመር ብቃት ለማጥናት እንደሚካሄድ ተገልፆልኛል።
- ✓ ከዚህጥናትየሚገኝውውጤትበሽታውንለመቆጣጠርየሚደረገውንጥረትሊደግፍአንደሚችልተረድቻለሁ።

- ✓ በዚህ ጥናት ውስጥ ለመሳተፍ ፍቃደኛ ከሆንኩ ለጥናቱ የሚያስፈልገውን ከ50-100 ማይክሮ ሊትር (ከአራት እስከ አምስት ጠብታ) ፈሳሽ ከአበጠው ቦታ ላይ በሰለጠነ ባለሙያ በመርፌ እንደሚወሰድ ተነግሮኛል።
- ✓ ከሳንባ ውጭ የቲቢ በሽታ ምርመራ እንደሚደረግልኝ እና የቲቢ ጥገኛ ህዋስ በተወሰደው ናሙና ውስጥ ካለ አስፈላጊውን ህክምና እንደማገኝ ተረድቻለሁ።
- ✓ የምርመራውጤቴምእንደሚነገረኝእናበሚስጥርምእንደሚያዝተረድቻለሁ።
- ✓ በጥናቱ ብሳተፍም ባልሳተፍም አስፈላጊውን ለማንኛውም ህሙማን የሚሰጠውን አገልግሎት በአቅራቢያዬ ካለው ሆስፒታል ወይንም ጤና ጣቢያ እንደማገኝ እና በዚህ ጥናት መሳተፍ ወይንም ያለመሳተፍ እንዲሁም በፈለኩት ጊዜ ከጥናቱ የመውጣት መብት እንዳለኝ ግልጽ ተደርጎልኛል።
- ✓ ከዚህም ባሻገር የኔ በጥናቱ ዉስጥ መካተት የወላጆቼ ወይም የአሳዳጊዎቼ ፈቃድ እንደሚያስፈልግ ተረድቻለዉ።
- ✓ ከላይ የተደረገልኝን ማብራሪያ በሚግባ ተረድቻለዉ በጥናቱም ዉስጥ እንደምሳተፍ በፊርማዬ አረጋግጣለዉ።

የተሳታፊው ስም_____ የተሳታፊው ወላጅ/አሳዳጊ ስም _____

ፊርማ_____ ፊርማ _____

ቀን_____ ቀን_____

<u>ለቲቢ ሊንፋዲናይትስ/ከሳንባ ውጭ የቲቢ በሽታ/ ምርምር ለሚሳተፉ ዕድሜያቸዉ ከ 12 አመት በታች ለሆኑ ህጻናት ወላጆች ፈቃደኝነት መጠየቂያ ቅፅ</u>

- ✓ እኔ ስሜ ከዚህ በታች የተገለጸዉ ይህ ጥናት በአርማወር ሐንሰን የምርምር ተቋም ተመራማሪዎች ከሳንባ ዉጪ ላለ የቲቢ በሽታን ለመመርመር የሚያስችልን የመመርመሪያ ዘዴ (ፍሎሮሰንት ማይክሮስከፕ) ያለውን የመመርመር ብቃት ለማጥናት እንደሚካሄድ ተገልፆልኛል።
- ✓ ከዚህ ጥናት የሚገኘው ውጤት በሽታውን ለመቆጣጠር የሚደረገውን ጥረትሊ ደግፍ አንደሚችል ተረድቻለሁ።
- ✓ ልጄ በዚህ ጥናት ውስጥ እንዲሳተፍ/እንድትሳተፍ ፍቃደኛ ከሆንኩ ለጥናቱ የሚያስፈልገውን ከ50-100 ማይክሮ ሊትር (ከአራት እስከ አምስት ጠብታ) ፈሳሽ ከአበጠው ቦታ ላይ በሰለጠነ ባለሙያ በመርፌ እንደሚወሰድ ተነግሮኛል።
- ✓ ለልጄ ከሳንባ ውጭ የቲቢ በሽታ ምርመራ እንደሚደረግለት/ላት እና የቲቢ ጥገኛ ህዋስ በተወሰደው ናሙና ውስጥ ካለ አስፈላጊውን ህክምና እንደሚያገኝ/ታገኝ ተረድቻለሁ።
- ✓ የምርመራውጤቱምእንደሚነገረኝእናበሚስጥርምእንደሚያዝተረድቻለዉ።
- ✓ ልጄ በጥናቱ ቢ/ብትሳተፍም ቢ/ባትልሳተፍም አስፈላጊውን ለማንኛውም ህሙማን የሚሰጠውን አገልግሎት በአቅራቢያዬ ካለው ሆስፒታል ወይንም ጤና ጣቢያ ለልጄ እንደማገኝ እና ልጄን በዚህ ጥናት የማሳተፍ ወይንም ያለማሳተፍ እንዲሁም በፈለኩት ጊዜ ከጥናቱ የማስወጣት መብት እንዳለኝ ግልጽ ተደርጎልኛል።
- ✓ ከላይ የተደረገልኝን ማብራሪያ በሚግባ ተረድቻለዉ ልጄ በጥናቱም ዉስጥ እንዲሳተፍ/ድትሳተፍ በፊርማዬ አረጋግጣለዉ።

የተሳታፊው ወላጅ/አሳዳጊ ስም _____

ፊርማ_____

ቀን _____

Annex VI: Questionnaire (Amharic Version)

መጠይቅ

ይህ መጠይቅ የተዘጋጀዉ ‹‹ የኤል. ኢ. ዲ. ፍሎሮሰንት ማይክሮስኮፕ ‹‹ ከእብጠት ላይ በመርፌ ከተወሰደ ናሙና ዉስጥ ከሳንባ ዉጪ የቲቢ በሽታ ምርመራን ለማሻሻል ያለዉን አስተዋጽዖ ለማወቅ በአለርት ሆስፒታል የሚደረገዉን ጥናት ለማገዝ ነዉ።ጥናቱ የሚካትተዉ ’‹‹ የሊንፍኖድ ቲቢ ‹‹ አለባቸዉ ተብለዉ የተጠረጠሩ ተሳታፊዎችን ነዉ።

ቀን:_____ /_____/_____

I. አጠቃላይ የተሳታፊዉ መረጃ

1. የተሳታፊዉ ልዩኮድ: _____

2. የተሳታፊዉ የሆስፒታል ካርድ ቁጥር: _____

3. ክልል: _____ ዞን: _____ ቀበሌ: _____ ስልክ ቁጥር:_____

4. እድሜ: _____

5. ጾታ: _____

6. የመኖርያ ቦታ 6.1. ከተማ: _____ 6.2. ገጠር: _____

7. የጋብቻ ሁኔታ 7.1. ያላገባ: 7.2. ያገባ: 7.3. አግብቶየፈታ: 7.4. የሞተበት: 7.5. ከእጮኛ ጋር የሚኖር:

8. 8. ስራ: 8.1. የቤት እመቤት:____ 8.2.የቀንሰራተኛ: ___ 8.3.የመንግስትሰራ: ____
8.4. የሌለዉ: ____ 8.5. ገበሬ: ____ 8.6. ሌላ (ግለጽ): _____

9. የትምህርትሁኔታ (በዓመት): _____

II. ክሊኒካል መረጃ

1. የሰዉነት ሙቀት መጠን(በ0^0): _____
2. ክብደት መቀነስ: 2.1. የለም: _____ 2.2. አለ: _____
3. ክብደት መቀነስ ካለ: 3.1.ከ5 ኪሎ በታች: ___ 3.2.ከ5- 10 ኪሎ: ___ 3.3 ከ 10 ኪሎ በላይ: _
4. በምሽት ጊዜ ማላብ:4.1. አለ: _____ 4.2. የለም: _____
5. የምግብ ፍላጎት መቀነስ: 5.1 አለ: _____ 5.2. የለም: _____
6. አጠቃላይ የሰዉነት ድካም: 6.1. አለ: _____ 6.2. የለም: _____
7. ተደጋጋሚ ሳል:7.1. አለ: _____ 7.2. የለም: _____
8. እብጠቱ ከወጣ ያስቆጠረዉ ጊዜ (በሳምንት ሲለካ):_____
9. የእብጠቱ መጠን የመጨመር ሁኔታ: 9.1. ዝቅተኛ:___ 9.2.መካከለኛ:___ 9.3.ፈጣን: ___
10. ለእብጠቱ የተወሰደ ህክምናአለ?: 10.1.አለ: _____ 10.2.የለም: _____

    ካለ በዝርዝር ግለጽ:_____
11. ከዚህ በፊት ለቲቢ የተወሰደ የቲቢ መድሃኒት አለ? 11.1. አዎ: ____ 11.2. የለም: ____ 11.3. ካለ

    ጊዜዉን ግለጹ:____ 11.4.መድሀኒቱን ጨርሰዋል: _____ 11.5. መድሀኒቱን አቋርጠዋል: _____
12. ተሳታፊዉ ከቲቢ በሽታ ታማሚ ሰዉ ጋር ቅርበት ነበራቸዉ? 12.1. ነበራቸዉ: _____

    12.2. አልነበራቸዉም: _____
13. ያልተፈላ ወተት ይጠቀማሉ? 13.1. እጠቀማለሁ:_____ 13.2.አልጠቀምም: _____
14. በመኖርያ ግቢ ዉስጥ የቀንድ ከብቶች አሉ? (ላም፣ በሬ፣ፍየል፣በግ): 14.1. አሉ: __ 14.2. የሉም:

    _

15. ተደጋጋሚ የሄነ ከቀንድ ከብቶች ጋር ንክኪ አለ? 15.1. አለ: _____ 15.2. የለም: ____

16. ከዚህ በፊት ለቲቢ ተከትበዉ የዉቃሉ (በክንድ ላይ ያለ የቲቢ ክትባት ጠባሳ)? 16.1. አዎ: _____

    16.2. የለም: _____

17. ለኤች. አይ. ቪ ቫይረስተመርምረዉያዉቃሉ? 17.1. አዎ: _____ 17.2. አላዉቅም: _____

18. ከተመረመሩመቼ? _____

19. ለኤች. አይ. ቪ. ቫይረስተመርምረዉፖዘቲቭከሆኑመድሃኒቱነመዉሰድጀምረዋል?

    19.1. ጀምረያለዉ: _____ 19.2. አልጀመርኩም: _____

20. ከጀመሩ የመድሀኒቱን አይነት: _____

Declaration

I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

M.Sc. candidate:	**Gebeyehu Assefa (BSc.)**
Signature:	_____
Date of submission:	_____

This thesis has been submitted with our approval as advisors.

Advisor:	**Dr. Abraham Aseffa (MD, PhD)**
Signature:	_____
Date:	_____
Place:	Addis Ababa, Ethiopia.

Advisor:	**Ass. Pro. Kassu Desta (MSc, PhD candidate)**
Signature:	_____
Date:	_____

Place: Addis Ababa, Ethiopia.

Advisor: **Mr. Shambel Araya (BSc. MSc,)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Declaration

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.

M.Sc. candidate: Gebeyehu Assefa (B.Sc.)

Signature: _____

Date of submission: _____

Place: Addis Ababa, Ethiopia

This thesis has been submitted with our approval as advisors.

Advisor: Ass. Pro. Kassu Desta (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mr. Shambel Araya (BSc. MSc,)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Dr. Abraham Aseffa (MD, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.