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Combined Use of Cytopathological diagnosis with molecular tests performed on concentrated Fine Needle Aspirate samples to improve the lab diagnosis for Tuberculous lymphadenitis suspected patients

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List of Abbreviation

AFB – Acid Fast Bacilli

AHRI – Armauer Hansen Research Institute

ALERT – All Africa Leprosy and Rehabilitation and Training Center

AO – Auramine O

DNA – Deoxyribonucleic Acid

EPTB – Extra Pulmonary Tuberculosis

FM – Fluorescent Microscopy

FNA – Fine Needle Aspiration

FNAC - Fine Needle Aspiration Cytology

HIV – Human Immunodeficiency Virus

IS – Insertion sequence

LED - Light Emitting Diode

MDR-TB – Multi Drug Resistance Tuberculosis

MGIT – Mycobacterium tuberculosis growth indicator tube

MTB – Mycobacterium Tuberculosis

MTB/RIF - Mycobacterium Tuberculosis/Rifampin

NaOH – Sodium Hydroxide

RT PCR – Real time Polymerase Chain Reaction

PI – Principal Investigator

RNA –Ribonucleic Acid

RPM – Revolution Per Minute

TB - Tuberculosis

TBLN – Tuberculosis Lymphadenitis

WHO – World Health Organization

ZN – Ziehl Neelson

Abstract

Background: Tuberculosis lymphadenitis (TBLN) is a granulomatous infection of lymph nodes and the most frequent form of extra pulmonary Tuberculosis in Ethiopia. Issues arising in the cytopathological diagnosis of TBLN suspected patients include the inconclusive result and lack of easily accessible diagnostic tests. In both instances, considerable consequences are observed concerning clinical management, economical perspective, and time. The fate of TBLN suspected patients with an inconclusive cytopathological result will be either taking other antibiotics or be on anti-TB treatment empirically. Though both approaches are targeted towards the benefit of the patient, they may either lead to inappropriate treatment outcome or cause the patient to suffer more from the disease. This is mainly resulted in due to lack of concrete affirmative laboratory diagnostic tools that support the cytopathological diagnostic method.

Objective: To investigate the combined use of cytopathological diagnosis with molecular tests performed on concentrated FNA sample in the detection of TBLN cases.

Methods: A cross sectional study was conducted at ALERT Hospital and the Lab work was performed at AHRI, Addis Ababa from Sept 2020 – April 2021. Using consecutive sampling technique, 96 participants suspected of having TBLN were enrolled in this study. The sensitivity, specificity, positive and negative predictive values including their 95% confidence intervals (CI) was calculated by using the culture results as the "gold standard". Cohen's Kappa value was used to measure interrater variability and level of agreement. All statistical tests were considered significant when having a two-sided P-value of <0.05.

Result: Out of the 96 participant, ZN, AO, Cytopathology, Xpert, RT PCR and LJ culture prevailed 12 (12.5%), 27 (28.1%), 51 (53.1%), 43 (44.7%), 51 (53.1%), 36 (37.5%) positive result respectively. The sensitivity of Xpert, RT PCR, and FNAC were 91.7%, 97.2%, and 97.2%, and the specificity were 83.3%, 73.3%, and 68.3% respectively. GeneXpert and RT PCR when combined with Cytology detected 61 (63.5%) positive and culture being gold standard, the sensitivity reached 100% while the specificity drops to 58.3%.

Conclusion: Combination of molecular test, performed on concentrated sample, with cytopathology shown increased sensitivity, particularly geneXpert with FNAC provides an elevating alternative in case detection rate of Tubercular lymphadenitis patients with improved

specificity. It also provides affirmative results and hence aid in alleviating issues with inconclusive results.

Keywords: Tuberculous Lymphadenitis, RT PCR, Concentration technique, Cytopathology GeneXpert.

1. Introduction

1.1 Background

Tuberculosis is a disorder generally caused by mycobacterium tuberculosis complex. It remains being a scourge and a cause of death globally, and is among the factors that makes the global public health limping (1, 2). Most low income African countries are experiencing the increased burden of tuberculosis cases reported globally. The 2019 WHO report shows that African countries are having the highest prevalence of tuberculosis cases next to south East Asia region. Ethiopia was categorized among the 30 high burden countries in the world with an estimated incidence rate of 157 cases/100,000 populations (3).

Tuberculosis propagates and affects several organs of the body, but lung is the most predominantly affected organ leading to a condition known as pulmonary TB. Infection of organs other than the lung leads to a condition known as extra pulmonary TB (EPTB). Extra pulmonary tuberculosis could be possibly seen in lymph nodes, pleura, bones and joints, urogenital tract, and meninges (4). TB lymphadenitis (TBLN) is a form of tuberculosis infection of the lymph nodes and are the most frequent form of extra pulmonary TB in Ethiopia (5).

A number of laboratory diagnostic methods are available for the diagnosis of TBLN including direct smear microscopy performing Ziehl-Neelsen(ZN) and Auramine O (AO) staining, culture identification, cytopathology examination of fine needle aspirate (FNA), molecular testing performing conventional PCR, Real time polymerase chain reaction (RT PCR) tests, and geneXpert MTB/RIF assay (6, 7). In hospitals in Ethiopia where pathologists are available, cytopathological diagnosis is the commonly used method to examine the disease. Cytopathologic diagnosis of TBLN is mainly performed through characterizing the typical morphologic features i.e. caseous necrosis, Necrotizing epitheloid granuloma, non-necrotizing epitheloid granuloma, Necrosis with polymorphs, of the diseases examined in the stained smear prepared from FNA (8).

In addition to cytopathologic examination, FNA could also be analyzed using direct smear microscopy or culture. Oftentimes the culture media used is Lowenstein Jensen (LJ). It is a solid culture medium which has a lower contamination rate than MGIT tube system and is inexpensive. It is an egg based medium used for the isolation and semi quantification of the

growth of MTB complex (9). LJ medium can be supplemented with either Glycerol or Pyruvate. LJ supplemented with glycerol favors the growth of M. tuberculosis while pyruvate supplemented medium promote the growth of M. *bovis* (10).

The low sensitivity of direct smear microscopy and the time demanding nature of culture lead to the use of concentration technique. The digestion of lymph aspirates with chemical like sodium hydroxide followed by centrifugation to concentrate the bacteria has been shown by Kumar et al to increase the sensitivity of smear microscopy (11).

Real time Polymerase chain reaction (PCR) has also shown recently a considerable improvement in the diagnosis of TBLN. Siala *et. al* have shown that qPCR has a feature of higher sensitivity (100%) and specificity (92.3%) (12). A number of primers targeting multiple sites can be used in the nucleic acid amplification of MTB complex. Primers specific for IS1081with a sensitivity of 94% and rpoB gene with a sensitivity of 94.1% can be used (13). Other primer which also comes into consideration is for IS6110 which has shown a sensitivity of 70%. A study by S.Kabir *et. al* has shown a sensitivity of 87.5% when using IS6110 primer (14). MPB 64 primer with a sensitivity of 48% can also be used for the DNA amplification of MTB complex (15).

GeneXpert MTB/RIF assay is a simple, sensitive, and automated method endorsed by the WHO. The method relied on the identification of DNA dependent B sub unit of RNA Polymerase (rpoB) gene associated with RIF drug resistance (16).

In this study, qPCR using IS1081 labeled with HEX/JOE was used for the identification of mycobacterial species causing Tuberculous lymphadenitis (17). Combination of cytopathologic diagnosis on direct FNA smear and molecular tests performed on concentrated FNA was also evaluated for the diagnosis of TBLN.

1.2 Statement of the problem

Human beings have been and still are being infected with the deadly disease Tuberculosis. The infection could occur in two broad forms, both provoking the quality of having a healthy life. Pulmonary TB is one form of tuberculosis associated with lung infection. The other way of manifesting itself is by affecting organs such as abdomen, meninges, genitourinary tract, joints, bones, lymph nodes and skin and thus collectively called extra pulmonary tuberculosis. A report from WHO in 2019 has shown an estimated 1.2 million people dying from tuberculosis (3). The average prevalence of EPTB has been shown by one study to be 15% (18).

The proportion of EPTB in Ethiopia is increasing for the past decades. The Ethiopian TB control program and WHO showed an estimate of the proportion to be about 32% from total new cases of 156,928. TB lymphadenitis accounts for the majority of the proportion of EPTB (18).

The clinical management of a TBLN suspected patient with inconclusive cytopathology result put the patient either on other non-anti-TB antibiotics and wait for the outcome to decide if the patient needs anti-TB treatment, or on anti-TB treatment at the spot, empirically. Though both approaches are aimed for the benefit of the patient, they have significant drawback due to either exposing the non-TB patient for anti-TB treatment, or making the potential TB patient wait for longer time without treatment which makes the patient suffer more from the disease. Poor treatment outcomes have a direct effect on the poor and vulnerable population rendering them to experience severe clinical conditions including drug resistance and social and economic challenges. This is mainly due to lack of concrete affirmative laboratory tests. Such information are lacking in Ethiopia and most other developing nations. In order to fill this gap, this study evaluated the outcome of integrating FNA cytology, ZN, AO, culture, geneXpert, and qPCR tests performed on concentrated FNA for the improvement of the laboratory diagnosis of TBLN.

1.3 Significance of the study

The aim of this study is to look for the utility of FNA concentration technique in the detection of TBLN cases which are inconclusive with cytopathology, and improvement in the detection rate through application of molecular tests performed on concentrated FNA from suspected TBLN patients.

The use of concentration method will increase the sensitivity of detecting TBLN cases (19) and early detection will be achieved by the application of molecular methods such as RT PCR and GeneXpert MTB/RIF assay. Early detection of TBLN cases has significant role in economical perspective and in terms of time. It also enables the patients to receive the necessary treatment on time (20).

The application of molecular methods in the detection of TBLN supports the health system through making the diagnostic service easy, quick, and accessible. It also enables health care workers to correctly diagnose and treat suspected TB patients.

The findings from this study will be forwarded as a recommendation to the policy makers as it may help in improving the diagnostic algorithm of the detection of TBLN cases. This will play its role in paving the way to the better health infra-structure development and in reaching out quality assured diagnostic services to the poorest community.

Therefore, integrating molecular tests performed on concentrated FNA sample along with the cytomorphological diagnosis may increase the detection of TBLN in suspected patients.

1.4. Hypothesis

It is to test the hypothesis that integrating molecular tests performed on concentrated FNA sample along with cytology will increase the detection rate for TBLN in suspected patients.

2. Literature review

Pathologists use needles to obtain cell and tissue fragment for diagnosing the underlying pathology of cervical lymphadenitis. Fine needle aspirate cytology (FNAC) is mostly used for the diagnosis of FNA material. FNAC procedure has shown a feature of being safe, simple, decisive, and cost effective (21). A prospective study done by Khan, *et al* in Pakistan has shown a change pertaining granuloma formation that was seen in 35/85 of total cases enrolled in the study. Among the 35 cases showing granulomatous changes on FNAC smears, 34(97%) cases were in line with the histopathological diagnosis. The study also showed that there was 1 false positive and 10 false negative cases from FNAC smear indicating the limitation of the test. The sensitivity and specificity was 77% and 98% respectively (22).

A prospective study done by Aljafari et al is one of the evidence that showed combination of FNAC with PCR test could enhance the diagnostic capacity. This study was conducted on a total of 60 cases in Sudan where 17 (28.3%) had cytological features suggestive of tuberculosis lymphadenitis, 39 (65%) with cytomorphological features of reactive lymphadenopathy and four cases (6.7%) showed secondary deposits. Using PCR 26 cases (43,3%) were detected, of this 16 cases were from the FNAC tuberculosis group, 10 from the reactive and none from the malignant group showing the enhancement of diagnostic through combining FNAC with molecular tests (23).

A study in India done by Vimal et al conducted on 170 subjects grouped into four groups based on the cytomorphological features of tubercular lymphadenitis; epitheloid cell granulomas without necrosis, epitheloid cell granulomas with necrosis, only necrosis, and the last group necrosis with polymorphs. Based on these features, 117 cases were diagnosed as tubercular lymphadenitis. Compared with FNAC, ZN staining detected 64 cases. Though molecular method was not performed in this study, combination of FNAC with microbiological method has increase the diagnostic index and better dimension would be seen if complimented with molecular tests such as PCR (24).

Another cross sectional study undertaken in Pakistan by Ikram et al on a total of 105 cases has shown that molecular test detected MTB in 71 (67.6%) cases where as FNAC has detected 72 (68.6%) positive cases. This study also recommended the importance of combining the molecular method with FNAC indicating its quickness and helpfulness in initiating an immediate treatment (25).

A study by Tadesse et al reported that GeneXpert MTB/RIF assay will provide easy and rapid way of diagnosing tubercular lymphadenitis. The sensitivity of GeneXpert MTB/RIF assay as they reported was 87.8% [95% CI: 81.0–94.5] and specificity 91.1% [95% CI: 82.8–99.4]. When compared with FNAC

Xpert has a better Positive Likelihood Ratio of 9.8 than that of cytology which was 1.9. On the other hand, the Negative likelihood ratio of Xpert was 0.1, showing a better capacity of telling true negative result than cytology does (16).

Direct microscopy is of great use and considered as a corner stone in the diagnosis of pulmonary TB, but when it comes to extra pulmonary tuberculosis its sensitivity drops down to 20-43%. The sensitivity of direct microscopy depends to which type of staining methods used. A study from Ethiopia done by Abdissa et al showed that using Auramine O (AO) staining methods has a sensitivity of 45.8% (26). A comparative study done by Lokeshwaran et al also shown a difference in the positivity rate of the two staining methods reporting that sensitivity of ZN and AO was 44% (22/50), and 80% (40/50) respectively (27). The decreased sensitivity can be alleviated way higher than that of direct microscopy method by the use of concentration method (28).

A cross sectional study from Addis Ababa, Ethiopia done by Zewdie et al conducted on 132 subjects has shown that concentration method tend to increase the sensitivity of smear microscopy way much similar to molecular method. The study has shown out of 132 subjects, 110 cases (83.3%) showed a cytomorphological pattern consistent with tuberculous lymphadenitis. Culture was performed on LJ medium in this study and it has confirmed that 74/132 (56.1%) were TBLN cases. Direct ZN smear microscopy was compared in this study with that of smear performed on concentrated samples and reported the detection rate was 29.5% and 65.2%, respectively. Hence, the concentration method detected an additional case of 47 (35.6%) when compared with direct ZN smear (29).

Another cross sectional study done by Tadesse et al has shown how the higher sensitivity can be achieved by using concentration method in the diagnosis of tuberculous lymphadenitis. Culture performed on LJ media was isolated in 68% cases. The cytomorphological features consistent with tubercular lymphadenitis were in 79.7%. AFB detected by direct ZN staining was 25.1% (47/187) and the ZN concentration method detected 49.7% (93/187). FNAC was also compared with positive cases detected by culture and its positivity was 90.5% (115/ 127). The researchers used culture as gold standard for the sensitivity and specificity of each method. Direct smear microscopy has shown sensitivity of 34.6% and specificity of 98.2%. The concentration method was 66.0% sensitive and 87.5% specific. Sensitivity and specificity of Cytology was 90.5% and

44.6%, respectively. They concluded that using concentration method for diagnosing FNA material has a better yield and increased sensitivity can be obtained (30).

4. Study Objectives

4.1 General objective

To investigate the combined use of molecular tests with FNAC and performed on concentrated FNA sample in the detection of TBLN cases along with cytopathology

4.2 Specific objectives

- To compare the diagnostic performance of ZN, AO, RT PCR, geneXpert, and cytopathology for the diagnosis of TBLN suspected patients against the gold standard method.
- To investigate the level of agreement between methods employed in this study

5. Materials and methods

5.1 Study area and study period

Laboratory work and other general activities were done at ALERT Hospital and AHRI, Addis Ababa from Sept 2020 – April 2021.

The pathology unit of ALERT Hospital is linked with Armauer Hansen Research Institute (AHRI) Laboratory and gives service for both patients' diagnosis and research activities. The FNAC service is provided twice a week. There are 2-3 TBLN suspected patients per day, 4-6 patients per week, and 16-24 patients per months.

5.2 Study design

A cross sectional study was conducted to look for the utility of molecular tests performed on concentrated FNA sample in the detection of TBLN cases which are inconclusive with cytopathology.

5.3 Population

5.3.1 Source population

The source population was all patients attending in ALERT hospital to give FNA sample.

5.3.2 Study population

The study population were patients who were suspected for TBLN at ALERT Hospital..

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

• Willingness to participate in the study by signing a consent form

5.4.2 Exclusion criteria

- Non volunteer patient
- Critically ill patients
- Patients confirmed for malignancy in their cytology result

5.5 Variables of the Study

5.5.1 Dependent variable

- Performance of combined molecular methods for the diagnosis of TB Lymphadenitis (Auramine O, Zeihl Neelson, Cytology, RT PCR, GeneXpert, FNAC.

5.5.2 Independent variable

- Socio-demographic characteristics (Age,sex, address)
- Source of Specimen
- Host immune status (comorbidity: the coexistence of two or more related medical conditions).
- Clinical data

5.6 Sample size determination and Sampling technique

Sample size was calculated by using Buderer's formula (31).

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

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

The anticipated sensitivity (SN) that I used was Zewdie et al's sensitivity that was 94.5% (29).

For α = 5%, Z1-α/2 = 1.96

L = 0.09

Prevalence of TBLN in Ethiopia 28.8% (32)

Therefore, $n = \frac{(1.96)^2 \times 0.945 \times (1-0.945)}{(0.09)^2 \times 0.288}$

$= 86$

The estimated sample size with 10% contingency is 96.

Sampling Technique

Convenient sampling technique was used by taking consecutive participants.

5.7 Data collection procedure

Patients holding the laboratory request for TB lymphadenitis diagnosis by several physicians from anywhere in the part of the country and referred to ALERT Hospital for FNAC was traced by clinical nurses who were assigned at the study site for further communication with the patients for his/her agreement to be enrolled in the study.

The patients were informed about the consent in order to obtain their willingness. After obtaining the consent, the patient's socio-demographic characteristics, clinical data and physical examination was collected by using a structured questionnaire. After making sure that all the required information/data are obtained, the patient undergoes FNA procedure by the pathologist.

5.8 Specimen collection and processing

Before undertaking the FNA sample collection procedure, we make sure that a signed informed consent was obtained from each study participant. Information regarding the history of the patient was collected by the attending nurse in the Hospital and the physical examination was undertaken by the attending pathologist. Then using 21 Gauge needle, the pathologist collected the FNA sample using a standard procedure.

The attending pathologist began the standard procedure by cleaning the overlying area with 70% alcohol. In order to fix the enlarged node and maintain it in a stable position, the pathologist uses one hand to hold the enlarged node. The syringe was then inserted in to the node. Multiple (average six) in and out passes was made by the needle without exiting the node (approximately

100-300 micro liters was collected). On a pre-labeled clean slide a drop of sample was placed and smears were prepared after the needle has been taken out of the node. The prepared smear was then used for cytomorphological examination. The remaining part of the sample was transferred into a pre-labeled 2ml ependorff tube with patient code for further procedures of the study and transported to the TB lab of AHRI. After the decontamination process using 3% NaOH-2.9% Tri sodium citrate-0.5% NALC had been carried out, a portion of sample was inoculated on LJ media, two smears, one for Zeihl Neelsen and the other for Auramine O, were prepared. The left over sample continued to be processed using RT PCR and geneXpert analysis.

5.9 Sample flow chart and procedure

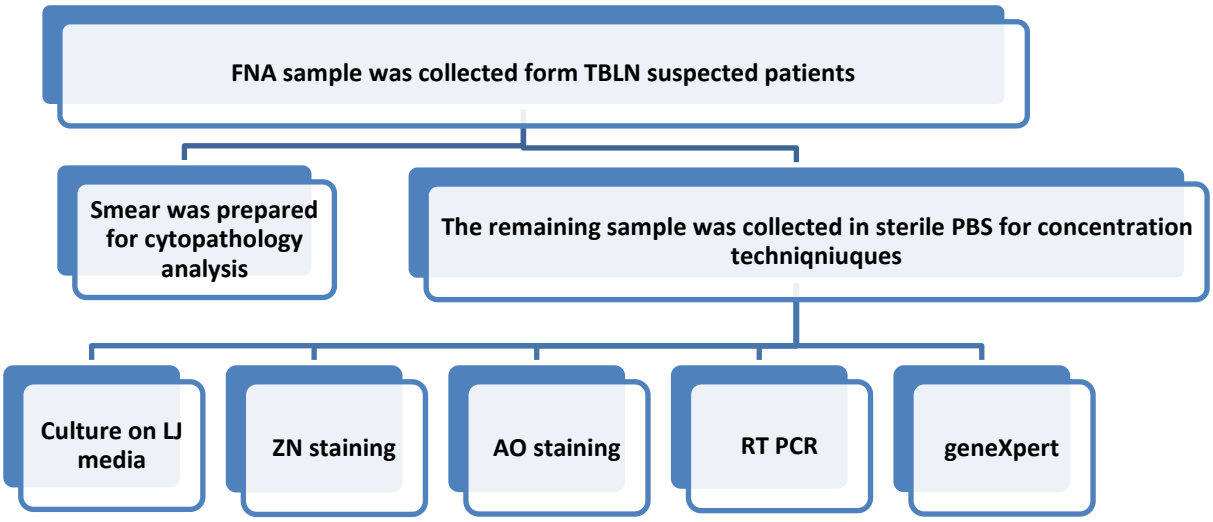

Figure 1 Sample flow diagram

5.10 Laboratory testing procedure

5.10.1 Direct smear preparation for cytology

On a clean slide a drop of aspirate was placed and smeared for cytomorphological analysis. Blood commixture was prevented, in order to make an ideal aspirate with creamy consistency with numerous cells suspended in a small amount of tissue fluid. The slide was then left to air dry. When the FNA material become diluted with blood, smear was prepared like a peripheral smear where particles tend to come to the edge of the smear (33).

5.10.2 Concentration technique

FNA sample was homogenized using 0.85% normal saline. The decontamination step was then proceeded by adding an equal volume of 3% NaOH – NALC solution into the homogenized FNA sample. After briefly vortexing, the mixture got incubated for 15 minute at room temperature. Following incubation, PBS was added to the 45ml mark of the falcon tube and the mixture was centrifuged at 3000g for 15 minute. The supernatant was then decanted and the sediment was resuspended with 1000µl of PBS. Two - four drops were used for culture. Two separate smears were prepared from the resuspended sample. One stained with ZN and the other with AO. The remaining sample were stored at 4˚c for DNA extraction and geneXpert (34) .

5.10.3 Staining technique

5.10.3.1 Zn staining

Standard acid fast staining technique was applied. The initial step in this technique was covering the entire slide with carbol fuschin. Using Bunsen burner, heat was applied underneath the slide until vapour starts pouring out of the slide. The primary stain remains unwashed for 5 minute. Then using running water, the slide was washed and the remaining water drained off the slide. Decolorization using 3% acid alcohol was taken place for 3 minute. The slide then was washed again. Finally, the slide was counter stained by using 0.1% methylene blue for 1 minute. After air drying, the slide was examined using bright field microscopy 100x magnification for AFB organism (9).

5.10.3.2 AO staining

Dried smear was covered with 0.1% Auramine O solution for 20 minute. The slide was washed using distilled water (running water) and excess water drained off the slide. Then 0.5% acid

alcohol was added to the slide and stayed for 3 minute. Washing step proceeded. Finally, the slide was counter stained with 0.5% potassium permanganate for 1 minute. After air drying, the slide was examined using LED fluorescence microscope at 20x or 40X magnification (9).

5.10.3.3 Cytomorphological staining

Alcohol fixation was applied immediately to the air dried slide and Giemsa staining was applied.

5.10.3.4 Culture technique

The sediment of FNA sample decontaminated with 3% NAOH –NALC solution was used for culture on LJ media. Two – four drop of the sediment was added to the LJ media. The media was then placed into the incubator at 37˚C in a slant position for about one week. The media was checked two times in the first week and once a week for the remaining seven weeks (9). Result was considered positive even in those tubes with a single colony. Colony was first smeared and stained with either of the two staining methods, most often using Zheil Neelson, which were then being observed for AFB bacilli under conventional microcopy. Those LJ tubes showing no characteristic of growth after being incubated for the consecutive eight weeks were considered as negative.

5.10.3.5 GeneXpert

After the culture has been performed, GeneXpert MTB/RIF assay proceeded using the concentrated and heat inactivated sample. 500 µl of the sample was mixed with 1.5 ml of the GeneXpert MTB/RIF diluent and left undisturbed, with one single gentle mixing in the middle of incubation time, at room temperature for 15 minute. The whole mixture was then transferred to the GeneXpert MTB/RIF cartridge and was performed for the detection of MTB/RIF. The result was generated after 2 hour (7). The result was interpreted as positive with the display from the software as MTB detected as High, Medium, Low, and Very low. RIF resistance was also displayed as RIF resistance detected for those samples with RIF resistance.

5.10.4 DNA Extraction

QIAGEN kit was used for DNA extraction from FNA heat inactivated samples. Several steps were undertaken. First step was checking the kit's expiration date. All the kit's component was brought to room temperature. In a 2 ml micro centrifuge tube, 20µl of QIAGEN protinease K was added and 200µl of sample was added to the same micro centrifuge tube. 200µl of buffer AL was added to the micro centrifuge tube and vortexed for 15 sec. The mixture was incubated for

10 minute at 56°C. The mixture was then briefly centrifuged using micro centrifuge. 200µl Ethanol (96%-100%) was added to the tube and mixed by pulse vortexing. The mixture was transferred to QIAamp mini spin column (in 2 ml collection tube) without wetting the rim. After closing the lid, centrifugation proceeded at 6000xg (8000 RPM) for 1 minute. The QIAamp mini spin column was place in a new 2 ml collection tube which was provided with the kit and the first tube containing filtrate was discarded. 500µl Buffer AW1 was added to the QIAamp mini spin column without wetting the rim. And centrifuge at 6000xg (8000 rpm) for 1 min. QIAamp mini spin column was placed in a clean 2ml collection tube and the one containing the filtrate was discarded. Carefully the QIAamp mini spin column was opened, and 500µl Buffer AW2 was added without wetting the rim. The QIAamp mini spin column was centrifuged at 20000 xg (14000 rpm) for 3 minutes. The QIAamp mini spin column was placed in a clean 1.5ml micro centrifuge tube and the tube containing the filtrate was discarded. By carefully opening the QIAamp mini spin column 50µl Buffer AE or distilled water was added and incubated at room temperature (15°C - 25°C) for 1 minute and then centrifugation at 6000xg (8000 rpm) for 1 minute was followed. Labeling was strictly followed for each tube.

5.10.5 IS1081 typing by real-time PCR

Assay components:

Qiagen's HotStarTaq Master Mix Kit (Prod code: 203445)). Nuclease free water (e.g. from Qiagen) for assay and for resuspension of primers (not probe), qPCR buffer (used for re-suspending the probe; buffer is delivered together with probe synthesized by Eurofins). Primers and Hot start Taq probe: When primers and probes are dissolved in water or qPCR buffer for the first time, a master stock solution can be prepared with a concentration of 100uM. This solution can then be diluted 10x with water to the following stock solutions that can be stored frozen in aliquots:

Stock solutions of IS1081 oligonucleotide primers (designed by Roland Ashford, unpublished):

10uM IS1081_Fw 5'-GATCCTTCGAAACGACCA-3'

10uM IS1081_Rev 5'- CGGTGTCGATAAGATGAGA-3'

10uM IS1081_Probe [6FAM]-CGAAGGAAATGACGCAATGACCTC-[BHQ1]

Preparation of specimens/unknown samples

RT-PCR typing can be made directly on heat-inactivated human specimens (Fine-Needle Aspirate). Purification of the DNA can be performed using DNA extraction kits such as 'DNeasy Blood & Tissue Kits' (Qiagen, prod code 69506). To increase the DNA concentration when eluting the sample, a smaller volume than recommended (e.g. 50ul instead of 200ul) can be used. Alternatively, the sample can be eluted in 200ul but then precipitated and re-suspended in a smaller volume (e.g. 20ul) to concentrate the DNA even more.

5.10.6 Master Mix preparation

All the components of the master mix which includes, IS1081f, IS1081R, probe, molecular grade water (RNAse free water), hot start mix was brought to room temperature. Then as indicated in **Table 1** Master mix Preparation master mix was prepared in a 2ml cryo tube in a separate master mix preparation room.

Table 1 Master mix Preparation

Ingredients	**1x**
IS1081f	2.5µl
IS1081r	2.5µl
Probe Fam labeled	0.5 µl
Hot start Master Mix	12.5µl
Molecular grade water	2µl
Total	20µl

Working in the cabinet, an aliquot of 20ul of the mix was added into each RT-PCR tube. Then after Moving to a designated area in the laboratory, DNA templates (controls and unknown samples) was added to each tube containing the master mix..

Place the tubes (balanced in the RotorGene) in the RT-PCR machine and start the correct program. The RT-PCR program for respective assay is listed below:

Thermal conditions:

- 95°C – 15 min
- (95°C for 15 sec
- 58°C for 1 Min.) Repeat this for 40 cycles.

5.10.7 Interpretation of Results

Quality control was performed in each run to confirm that the assay has worked. Results from the samples were interpreted when having a control value with in expected range. Sample was considered positive if each Ct value is above the baseline, with the auto threshold set on default for the instrument (Rotor gene 3000, Germany). A sample having a Ct value with above 36 was considered as negative.

5.11 Quality Assurance

All laboratory tests in this study went through or stick with standard operating procedure. Any technical problems identified are recorded on the non-confirming event log sheet and corrective action was applied. Quality assurance in this study was cling to national guideline to conduct quality assurance in TB programme.

5.12 Data management

Log book was used to record clinical and laboratory data during the study period. Proper coding was given to each completed questionnaire and double entered into an excel spreadsheet, cleaned, verified and then transferred for statistical calculation.

5.13 Data analysis

SPSS software package (Version 26) was used for statistical analysis. The sensitivity, specificity, positive and negative predictive values including their 95% confidence intervals (CI) was calculated by using the culture results as the "gold standard". Cohen's Kappa value was used to measure inter-rater variability and level of agreement. All statistical tests was considered significant if the two-sided P-value was <0.05.

5.14 Ethical consideration

The study leads conducted after receiving an ethical approval from ALERT/AHRI ethical review committee and from the institutional ethical review board of Department of Medical Laboratory Science, College of health science, Addis Ababa University**.** Information regarding the purpose of this study was given to each study subjects. Their willingness was asked by letting them know that their personal information and FNA specimen was for the study. If the participant is children, their parent or guardian was provided with the written assent form.

5.15 Operational Definition

Likelihood ratio ratings was based on McGee, 2002 (35), and rated as follows:

0-1 – Decreased evidence for disease

1 - No diagnostic value, and

>1 – Increased evidence for disease

Cytopathological features categorization

These features were categorized into four groups:

Group 1 - Epitheloid granuloma with necrosis

Group 2 - Epitheloid granuloma without necrosis

Group 3 - Necrosis with poly mophonuclear leukocyte

Group 4 - Caseous necrosis only

Kappa values were rated (according to Landis and Koch. 1977) and calculated to observe method agreement (inter rater agreement) of the above methods with cytomorphological analysis of FNA, and was rated as follows(36).

0.0-0.20 – Slight agreement

0.21 – 0.4 – Fair agreement

0.41 – 0.6 – Good agreement

0.61 – 0.8 – Substantial agreement

0.81-1.00 – Excellent agreement

6. Results

6.1 Socio demographic characteristics

A total of 96 participants were enrolled in this study. Where 57 (59.4%) were from Addis Ababa, 31(32.3%) from Oromia region, 6 (6.3%) from Amhara region, and 2 (2.1%) were from SNNPR. Sixty six (68.8%) were urban dwellers while 30 (31.3%) were from rural areas. Among the entire participants enrolled in this study, 62 (65%) were female participants, while 34 (35%) were male. The age of the participants ranges from 6 months – 78 year with the mean age of 33 years. Many of the participants were students (42%), followed by house wives (33.3%), and government employees (22%). The socio demographic characteristics of the study participant were illustrated in **Table 2** Socio demographic characteristic of study participants, ALERT Hospital, Addis Ababa, Ethiopia, 2021.

Table 2 Socio demographic characteristic of study participants, ALERT Hospital, Addis Ababa, Ethiopia, 2021

Variable		Frequency	Percent
Sex	**Male**	**34**	**35.40%**
	Female	**62**	**64.60%**
Age	**<20**	**22**	**22.90%**
	21-40	**49**	**51%**
	41-60	**18**	**18.80%**
	61-80	**7**	**7.30%**
Employment status	**Employed**	**22**	**22.90%**
	Student	**42**	**43.70%**
	Housewife	**32**	**33.30%**
Residency	**Urban**	**66**	**68.80%**
	Rural	**30**	**31.20%**
Previous anti TB treatment	**Yes**	**6**	**6.30%**
	No	**90**	**93.70%**

6.2 Sites of lymph node and case distributions

Depending on the site of swollen lymph node, FNA samples were collected from cervical, axillary, Cervical and Axillary, and inguinal lymph node regions where the majority was from cerivical region (**Figure 2** Site of FNA collection

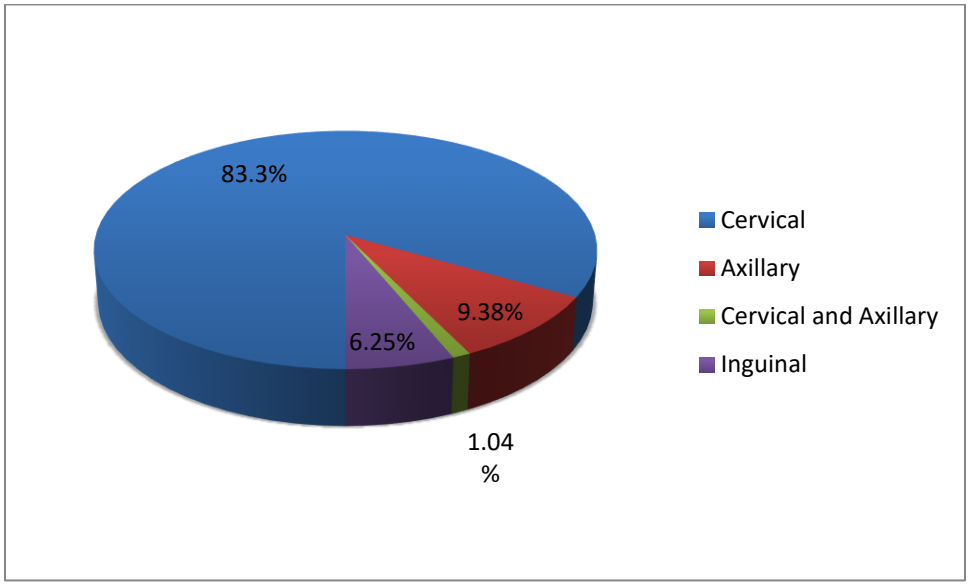

Figure 2 Site of FNA collection

Among the FNA samples, 51/96 (53.1%) were aspirates mixed with blood, 36/96 (37.5%) were purulent samples, and 9/96 (9.4%) were aspirates of cheesy like material.

Based on cytological analysis, positivity rate was 51/96 (53.2%). Majority of the positive samples, 43/51 (84.3%) were FNA samples collected from cervical site followed by Axillary 5/51 (9.8%), Inguinal 2/51 (3.9%), and Cervical and Axillary 1/51 (2%) as shown in **Table 3** . In terms of proportion, Axillary site has the highest positive proportion having 5/9 (56%).

Table 3 Site wise distribution of cases.

		Cytopathology			Total
		Negative	Positive	Inconclusive	
Site of FNA	Cervical	34	43	3	80
	Axillary	4	5	0	9
	Cervical & axillary	0	1	0	1

Inguinal	4	2	0	6
Total	42	51	3	96

The case distribution by age was compared initially using the result from golden standard, culture (**Figure 3** Distribution of cases of TBLN by age as diagnosed by culture). Larger portion of the participant, 49/96 falls in age group of 21-40 years of age where 25/36 of culture positive cases belong to this group.

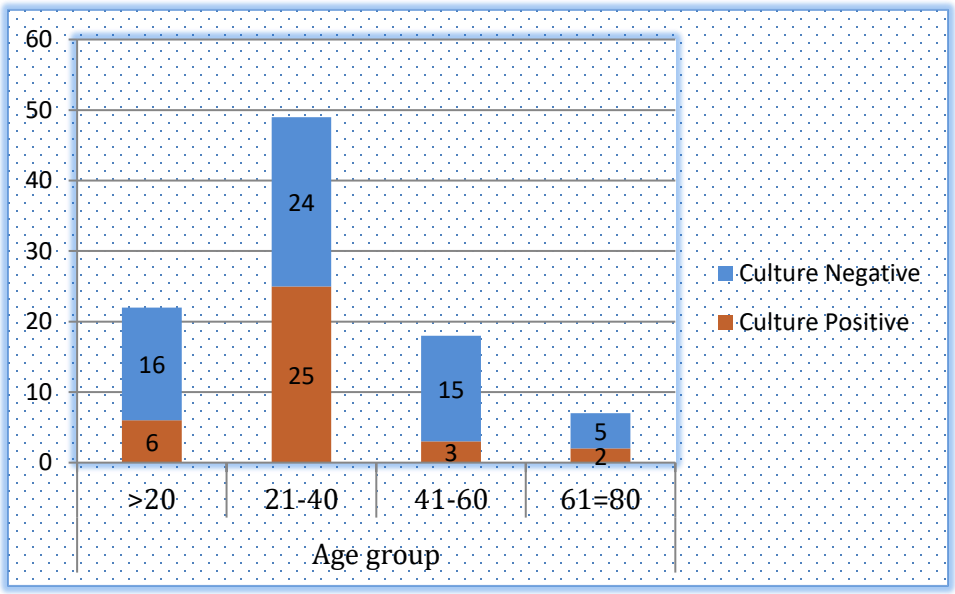

Figure 3 Distribution of cases of TBLN by age as diagnosed by culture

The case distribution in gender was also analyzed with the five parameters performed in this study. Positivity was higher in female group in all of the methods when compared with male. The molecular techniques have shown a close similarity with that of FNAC in the positive case detection rate.

Case distribution by age was also calculated as observed from the rest of the five parameters employed in this study and are shown in **Error! Reference source not found.** The positivity rate from all parameters has shown a higher proportion belonging to an age group of 21-40.

Table 4 Case distribution by age group as shown with five methods

Methods		Age group				Total
		1-20	21-40	41-60	61-80	
ZN	Positive	3	9	0	0	12
	Negative	19	40	18	7	84
AO	Positive	5	19	2	1	27
	Negative	17	30	16	6	69
FNAC	Positive	9	30	8	4	51
	Negative	12	19	8	3	42
	Inconclusive	1	0	2	0	3
GeneXpert	Positive	8	26	6	3	43
	Negative	14	23	12	4	53
RT PCR	Positive	8	31	9	3	51
	Negative	14	18	9	4	45

6.3 Detection rate and comparison of diagnostic methods

Culture was considered as a gold standard and it was positive for 36 samples out of the 96 FNA samples. Colony characteristic comprises from single colony to multiple colony as shown in **Figure 4** LJ Media showing many colonies of MTB.

Figure 4 LJ Media showing many colonies of MTB

Four samples were contaminated and were not included in the analysis of this study. The peak duration for a culture to show colony growth was 6 weeks. This was shown in two samples. Other samples showed growth within three to six weeks. Overall, 36 (37.5%) samples showed growth out of the whole 96 samples.

For all of the 96 samples, there was four different suggestive features observed from the stained smear in order to consider a smear to be positive (37).

A smear exhibiting any one of aforementioned features was considered positive or being reported as tubercular lymphadenitis or cold abscess. Of the 96 participants, 51 (53.1%) were positive with FNAC. Positivity rate was also compared with culture, GeneXpert and RT PCR assays **(Table 5** Cytomorphological pattern and positivity rate in culture, RT PCR, and GeneXpert). GeneXpert, RT PCR, and Culture have detected 5, 7, and 1 positive cases respectively that were inconsistent with TBLN.

Table 5 Cytomorphological pattern and positivity rate in culture, RT PCR, and GeneXpert

Methods		Cytomorphological pattern			
		Group 1	Group 2	Group 3	Group 4
Culture	Positive	23	2	8	2
	Negative	0	8	4	4
RT PCR	Positive	23	11	5	5

	Negative	0	5	1	1
GeneXpert	**Positive**	23	9	4	2
	Negative	0	6	3	4

Zheil Neelson and Auramine O were performed from the remaining sample left after being used for culture. Standard staining procedures were followed in both methods. Zeihl Neelson was positive in 12/96 (12.5%) while auramine O was positive in 27/96 (28.1%) samples.

GeneXpert MTB/RIF assay was performed for all of the 96 samples. The assay detected/identified 43/96 (44.5%) as positive. Previous anti TB treatment history was collected for the entire participant enrolled in this study and 6/96 had previous history of taking anti TB treatment and this was not significantly associated with MTB/RIF. None of the participants showed RIF resistance pattern with geneXpert.

Real time PCR showed a relatively better detection capacity than the Xpert assay detecting 51/96 (53.1%) as positive. Graph of RT PCR from the rotor gene was displayed in **Figure 5** Graph showing two positive samples, two positive controls, Negative control, and threshold value.

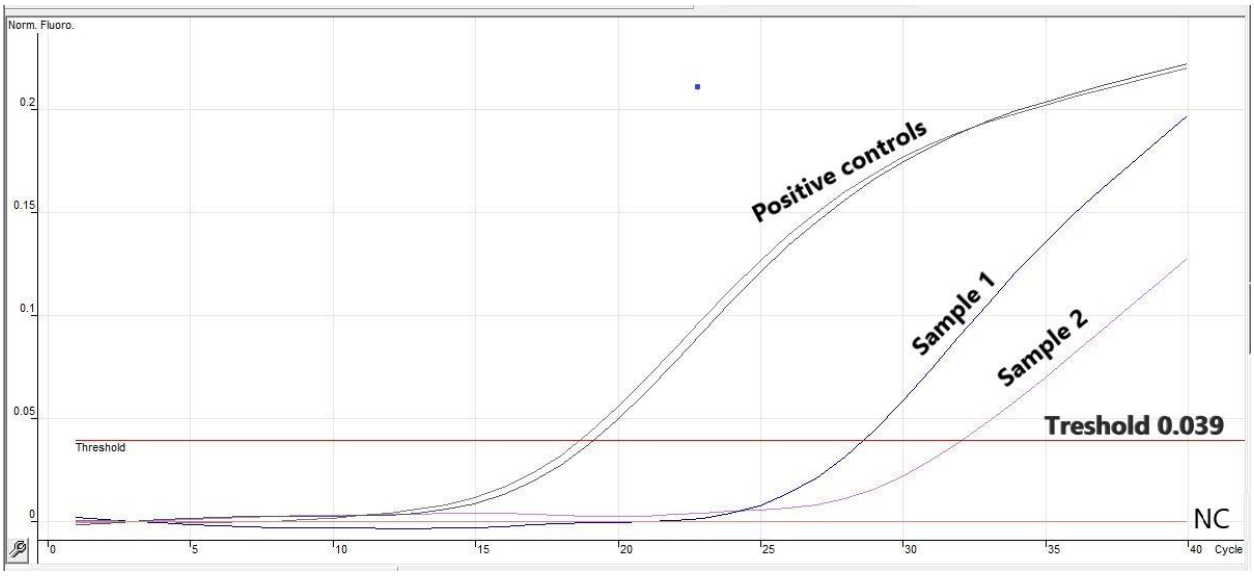

Figure 5 Graph showing two positive samples, two positive controls, Negative control, and threshold value

As shown in **Figure 5** Graph showing two positive samples, two positive controls, Negative control, and threshold value the first two lines shows signal from positive controls having strong

CT value, the second two signals were from two positive FNA samples. The line underneath the threshold line represents the negative control.

The detection rate of all parameters included in this study as compared with FNAC was summarized as shown in **Table 6** Summary of detection rate of the five methods

Table 6 Summary of detection rate of the five methods

		Cytopathology			Total	P Value
		Negative	Positive	Inconclusive		
ZN	Negative	41	40	3	84	0.004
	Positive	1	11	0	12	
AO	Negative	41	25	3	69	<0.0001
	Positive	1	26	0	27	
GeneXpert	Negative	38	13	2	53	<0.0001
	Positive	4	38	1	43	
RT PCR	Negative	36	7	2	45	<0.0001
	Positive	6	44	1	51	
Culture	Negative	41	16	3	60	<0.0001
	Positive	1	35	0	36	

ZN and AO staining have detected 1 additional positive case which was missed by FNAC. From all FNAC positives cases, 11 of them were also positive with ZN. While AO detected 26 positive cases from all FNAC positive cases.

Of the 42 cytology negative results, 4 were positive with GeneXpert MTB/RIF assay and 13 cases were negative by GeneXpert MTB/RIF from all FNAC positive cases. RT PCR detected additional 6 positive cases which were FNAC negative. Among the 3 inconclusive results, 1 was positive with both RT PCR and GeneXpert MTB/RIF assay.

Gold standard /culture result was also compared with that of FNAC where 1 of the 42 cytology negative results was culture Positive. Culture and cytology showed same positive result in 35 cases out of the 51 cytology positive results.

Positivity rate of molecular methods were compared with that of FNAC. RT PCR detected 6 positive cases which were FNAC negative and 9 GeneXpert negative cases as positive. GeneXpert also detected 4 FNAC negative cases as positive and 1 RT PCR negative case as positive. FNAC in turn detected 7 positive cases missed by RT PCR and 13 negative cases by GeneXpert were positive by FNAC. GeneXpert and RT PCR detected 6 additional positive cases which were missed by FNAC of which 5/6 were negative and 1/6 of them were inconclusive.

6.5. Level of agreement of the diagnostic methods

ZN has a poor agreement while RT PCR showed a relatively substantial agreement with FNAC. GeneXpert MTB/RIF assay also showed good agreement with FNAC **Table 7** Kappa value of all methods calculated against FNAC.

The kappa value of each method with FNAC has been illustrated in **Table 7** Kappa value of all methods calculated against FNAC

Table 7 Kappa value of all methods calculated against FNAC

Methods	Kappa Value	P value
ZN	0.168	0.004
AO	0.437	<0.001
Xpert	0.6	<0.001
RT PCR	0.675	<0.001

6.6 Diagnostic performance of ZN, AO, XPERT, RT PCR, and FNAC

The Sensitivity, specificity, positive predictive value, negative predictive value, and positive and negative likelihood ratio were calculated with 95% confidence interval with culture being gold standard and illustrated in Table 8 Sensitivity, Specificity, PPV, and NPV of the five methods with culture being gold standard..

Both ZN and AO staining showed higher specificity of 98.3% [95% CI (95.1 - 100%)]. AO has 75% sensitivity [95% CI (60.8% - 89.2%)] which is much higher than that of ZN. Both ZN and AO do have a positive predictive value of 92.3% [95% CI (77.8% - 100%)] and 96.4% [95% CI (89.5% - 100%)] while their negative predictive value showed difference being 71.1% [95% CI

(61% - 81.8%)] and 86.8% [95% CI (78% - 95%)] respectively for ZN and AO Table 8 Sensitivity, Specificity, PPV, and NPV of the five methods with culture being gold standard..

The sensitivity of GeneXpert MTB/RIF assay was slightly lower than RT PCR sensitivity which was 97.2% [95%CI (91.8% – 100%)]. On the other hand the specificity of GeneXpert MTB/RIF assay was 83.3% [95%CI (73.9% – 92.8%)] which in turn was higher when compared with that of RT PCR. The positive predictive value of both methods was 76.7% [95%CI (64.1%– 89.3%)] and 68.6% [95%CI (55.9% – 81.3%)] respectively for GeneXpert MTB/RIF assay and RT PCR. The negative predictive value was 94.3% [95%CI (88% – 100%)] and 97.8% [95%CI (93.5% – 100%)] respectively for GeneXpert MTB/RIF assay and RT PCR (Table 8 Sensitivity, Specificity, PPV, and NPV of the five methods with culture being gold standard.).

GeneXpert MTB/RIF assay had positive likelihood ratio of 5.5 [95% CI (3.1 – 9.8)], and negative likelihood ratio of 0.1 [95%CI (0.03- 0.3)]. RT PCR has a positive likelihood ratio of 3.6 [95% CI (2.4 – 5.6)], and negative likelihood ratio of 0.04 [95%CI (0.005- 0.26)].

The sensitivity, specificity, positive predictive value, and negative predictive value of cytology were 97.2% [95%CI (91.8% – 100%)], 68.3% [95%CI (60% – 82.5%)], 68.6% [95%CI (55.8% – 81.3%)], and 97.6% [95%CI (93% – 100%)] (Table 8 Sensitivity, Specificity, PPV, and NPV of the five methods with culture being gold standard.). The positive likelihood ratio was 3.46 [95%CI (2.28 – 5.27)], and negative likelihood ratio was 0.04 [95%CI (0.006 – 0.3)].

Table 8 Sensitivity, Specificity, PPV, and NPV of the five methods with culture being gold standard.

	ZN	AO	GeneXpert	RT PCR	FNAC
Sensitivity	33%	75%	91.70%	97.20%	97.20%
95% CI	17.9% - 48.7%	60.8% - 89.2%	91.8% - 100%	91.8% - 100%	91.8% - 100%
Specificity	98.3%	98.3%%	83.30%	73.30%	68.3%
95% CI	95.1% - 100%	95.1% - 100%	73.9% - 92.8%	62.1% - 84.5%	60% - 82.5%
PPV	92.3%	96.4%	76.70%	68.60%	68.6%
95% CI	77.8% - 100%	89.5% - 100%	64.1% - 89.3%	55.9% - 81.3%	55.8% - 81.3%
NPV	71.10%	86.8%	94.30%	97.80%	97.60%
95% CI	61% - 81.8%	78% - 95%	88% - 100%	93.5% - 100%	93.4% - 100%
P Value	<0.001	<0.001	<0.001	<0.001	<0.001
PLR	20	45	5.5	3.6	3.1
95% CI	2.71-147	6.4 - 317	3.1 – 9.8	2.4 – 5.6	2.1 – 4.5
NLR	0.68	0.25	0.1	0.04	0.041

95% CI	0.54 – 0.86	0.14 – 0.44	0.03 – 0.3	0.005 – 0.26	0.006 – 0.3

Legends: PPV – Positive predictive value, NPV – Negative predictive value, PLR – Positive likelihood ratio, NLR – Negative likelihood ratio,

6.7 Detection rate and level of agreement of the combination of molecular method with FNAC

Finally the molecular methods were combined together with FNAC to see how the diagnostic value can be improved. Result is shown in **Table 9** Diagnostic performance of combined methods compared against FNAC As shown in the table, the three methods when combined together detected 61 positive cases. The level of agreement with FNAC (Kappa value) was 0.772.

To see the combination effect molecular methods in separate, Xpert MTB/RIF assay with FNAC, and RT PCR with FNAC were compared with the result from FNAC **Table 9** Diagnostic performance of combined methods compared against FNAC. Kappa value was 0.856 with P value of <0.001. Similarly the kappa value was 0.815 with p value of <0.001 for combined effect of RT PCR with FNAC.

Table 9 Diagnostic performance of combined methods compared against FNAC

		Cytopathology			Total	Kappa Value	P value
		Negative	Positive	Inconclusive			
Xpert+FNAC+RT PCR	Negative	34	0	1	35	O.772	<0.001
	Positive	8	51	2	61		
XPERT+FNAC	Negative	38	0	2	40	0.856	<0.001
	Positive	4	51	1	56		
RT PCR+FNAC	Negative	36	0	2	38	0.815	<0.001
	Positive	6	51	1	58		

6.8 Diagnostic performance of combined methods

The detection rate of combined methods (Molecular methods + FNAC), Xpert + FNAC, and RT PCR + FNAC were calculated in comparison with culture **Table 10.** Detection rate of combined methods against culture

Table 10. Detection rate of combined methods against culture

	Culture		Total	Kappa	P Value
	Negative	Positive			

RT PCR+XPERT+FNAC	Negative	35	0	35	0.512	<0.001
	Positive	25	36	61		
XPERT+FNAC	Negative	40	0	40	0.60	<0.001
	Positive	20	36	56		
RT PCR+XPERT	Negative	38	0	38	0.564	<0.001
	Positive	22	36	58		

The sensitivity, specificity, PPV, NPV, level of agreement, and P value of combined method (FNAC+RT PCR+Xpert), GeneXpert + FNAC, and RT PCR + FNAC were calculated in comparison with culture (gold standard) and were shown in **Table 11.** Sensitivity, Specificity, PPV, NPV, Kappa Value, and P value of combined methods compared against cultureKappa value and P value for each methods were also shown in Table 11.

Table 11. Sensitivity, Specificity, PPV, NPV, Kappa Value, and P value of combined methods compared against culture

	Xpert+FNAC	RT PCR+FNAC	XPRT+RT PCR+FNAC
Sensitivity	100%	100%	100%
95% CI	100%	100%	100%
Specificity	66.70%	63.30%	58.30%
95% CI	54.7% - 78.6%	51.1% - 75.5%	45.8% - 70.8%
PPV	64.30%	62.10%	59.00%
95% CI	51.7% - 76.8%	49.5% - 74.5%	46.7% - 71.3%
NPV	100%	100%	100%
95% CI	100%	100%	100.00%
Kappa value	0.6	0.564	0.512
P value	<0.001	<0.001	<0.001

7. Discussion

Diagnosis of TBLN in Ethiopia mainly relies on FNAC. The method was proven to be simple, sensitive, and inexpensive but often times, it is based mainly on suggestive features of tuberculosis such as epithelioid granuloma and caseous necrosis rather than depending on the

direct detection of bacteria which might occur due to factors other than Tuberculosis and may produces inconclusive results in some instances (24). Some reports have shown that combining FNAC with other diagnostic methods such as bacteriological method and with molecular methods such as PCR may alleviate the problem associated with the non-specific nature of FNAC and improve the diagnostic index (24, 38).

The youngest age among the entire participant enrolled in the present study was 6 months, while the oldest was 78 years of age. Similar reports have been shown by Jadhav *et al* (39) and Mitra *et al* (40). Jadhav *et al* reported the youngest age of 7 months and the oldest being 77 years of age. Mitra *et al* on the other side have shown a youngest participant being 6 months old and oldest age of 69 years.

The number of female participant in this study dominates over male with a male to female ratio of 1:2. Same report has been shown by Vimal *et al* (24). Positivity rate was also high in females when compared with male. The same pattern of an increase of positive cases in female population has also been reported by Muluye *et al* (41).

Beside an increased proportion of female participants, another observation in this study was the higher proportion of the younger population with the age group of 21-40. Similar observation was seen in study from Aljafari *et al* (38). Lesser number of participants was seen in older population with an age group of 61-80. Positivity rate of cervical lypmphadenitis were seem to be higher in the younger population of age group 21-40. Similar observation was reported by Kumar *et al* (42). Approximately 70% of all positive cases detected by culture method in this study were in this group.

In this study, FNA was collected from four sites, of which FNA collected from cervical region dominates over other sites reaching 83.3% of the 96 enrolled participants. This finding was in line with Mitra, *et al.* and Hemalatha, *et al.* which states that cervical region was the most dominant site of the overall three sites (40)**.**

Based on the result seen in FNAC method, 85.1% of all FNAC positive cases were seen in samples from cervical region, followed by axillary (8.5%), inguinal (4.2%), and cervical and axillary in combination (2.1%). Similar observation has been reported by Ketema *et al* (43). Almobarak *et al* also reported cervical site domination over other sites (44).

The distribution of positivity rate of the Cytomorphological pattern, which was categorized into four groups, was compared with the corresponding appearance in RT PCR, GeneXpert, and Culture. Many of the positive cases were seen in group 1 in all of the three assays consistently. The same increased number of positive cases in Epitheloid granuloma with necrosis was reported by Vivek Gupta and Arvind Bhake (45).

Molecular methods showed similar findings of positivity rate in group 2, but positivity rate with culture methods was far lower than the molecular assays. Positive cases of group 3 and 4 seen with GeneXpert and culture method were low as compared with RT PCR which showed a relatively higher number of positive cases in both groups.

Anti-TB treatment status was assessed among the participants enrolled in this study where 6.3% have had a previous history of taking anti TB treatment, but none of them were positive by any one of the methods included in this study neither they showed drug resistance pattern with GeneXpert MTB/RIF assay. The enlarged lymph node in those participants with history of anti TB treatment and visiting once again with the same complaint might be due to misdiagnosis in the initial diagnosis, as sometimes there is an empirical treatment given to patients with inconclusive FNAC result. Their current complaint could also be caused by factors other than tuberculosis. This finding was consistent with reports by Asma *et al* (46). Inconsistent with the finding from this study has been shown by Raja *et al* which showed a RIF resistance pattern of 2 cases from 42 GeneXpert cases (47). Also inconsistent with this study was the finding from Tadesse *et al* which states 4 samples showing rifampin resistance among 86 Xpert positive samples (16). Sarfaraz *et al* have shown 6 samples with rifampin resistance among 76 Xpert positive samples (48).

The time consuming nature of culture was noted well with the current study. The earliest growth noticed in this study was three weeks and the longest period being six weeks. In this study, Culture has shown a positive detection rate in 37.5% of the total participants. Among FNAC positive cases 70.5% of them were also positive with culture. The remaining positive cases missed by culture from all FNAC positive cases might reflect the non-specific nature of FNAC. Same finding has been shown by Kant *et al* (49). Same proportion of detection rate that is 70.6% was produced by culture method from all positive cases detected by RT PCR. RT PCR specificity was lower than GeneXpert MTB/RIF assay but higher than those observed in FNAC, and the reason why many culture missed positive cases by RT PCR could be due to the low sensitivity of culture or may be due to the high diagnostic yield nature of RT PCR. Among the GeneXpert positive cases, 76.6% were also positive by culture. In contrast, Tamana *et al* has shown

GeneXpert and Culture intersecting at 95.9% in detecting positive cases (50). Based on kappa statistics analyzed in this study, GeneXpert MTB/RIF assay relatively has better agreement with culture having a kappa value of 0.72 when compared with level of agreement of culture with RT PCR and FNAC. The kappa value reported in this study was much higher than that of the report made by Tamana *et al* (50). Tamana *et al* reported a kappa value of 0.39 showing a relatively poor agreement between GeneXpert and culture when compared with the current study. This might be due to the fact that in this study Culture, Xpert MTB/RIF assay and other methods were performed on the same sample after decontamination process has been carried out.

Culture method also detected positives cases of 28.6% from all ZN negative cases. Zewdie et al observed an inconsistent finding from this study showing 8.7% positivity of culture method from all ZN negative cases (29). In both studies, concentration method has been performed, but the sensitivity of ZN is much lower in this study when compared with the finding from Zewdie et al. The lower bacterial load might have contributed for the lower sensitivity of ZN method in this study. Among the AO negative cases, 13% were positive with culture. These two staining methods provide results in a simple, quick and inexpensive way as compared to culture. The problem especially when it comes to diagnosing FNA samples is the low sensitivity of the two staining methods although the sensitivity of AO is higher than ZN. AO is better not only in sensitivity but the level of agreement with culture method is higher than ZN. Their kappa value was 0.38 for ZN and 0.79 for AO.

This study aimed at integrating the molecular methods such as the GeneXpert MTB/RIF assay and RT PCR with FNAC to improve the laboratory diagnosis of TBLN patients. GeneXpert MTB/RIF assay is a simple, sensitive, and automated method endorsed by the WHO. The method relied on the identification of rpoB gene associated with RIF drug resistance. RT PCR assay in this study targets IS1081 gene present in all MTBC and in a stable copy number of 5-7 repeats per genome (51, 52). The finding from this study showed the prevalence of positivity of 44.5% and 53.1% for GeneXpert and RT PCR respectively. Report from Tadesse *et al* showed increased positivity of GeneXpert, that is 60.1%, in comparison with the present study (16). In this study, many FNA sample was those that are mixed with blood which might have contributed for the sample to be of lower quality and for the geneXpert positivity to be lower.

When compared with culture, GeneXpert has detected an additional 10 (16.67%) positive cases which were culture negative. This finding showed a slight similarity with Samreen *et al* who reported 38 (19.6%) additional positive cases by GeneXpert from culture negative cases (48). RT PCR on the other hand has detected 26.67% additional positive cases which were missed by culture. The kappa value of the two molecular methods has been analyzed against culture, and GeneXpert assay has a better agreement with culture having a kappa value of 0.72. RT PCR still has a good agreement with culture having a

kappa value of 0.65, but seems to have a little lower agreement when compared with GeneXpert. The two molecular methods have a kappa value of 0.79, a substantial agreement, when compared with each other.

Each method was compared with findings from FNAC and to observe their agreement, kappa value was calculated. ZN and AO each has detected 1 positive case which was missed by FNAC. This was statistically significant having a p value of <0.05. ZN has 23.5% detection rate of positive cases among all FNAC positives while AO has 52.9% detection rate of positive cases from all FNAC positive samples. ZN has poor or slight agreement with a kappa value of 0.185, and AO relatively has fair agreement with a kappa value of 0.455.

GeneXert and RT PCR have detected 4 (7.8%) and 6 (11.7%) additional positive cases which were negative with FNAC. Tadesse *et al* reported 15.6% additional positive cases which didn't have suggestive feature of TBLN by FNAC (53). Among all FNAC positive cases, 74.5% were also positive with GeneXpert MTB/RIF assay and 86.2% were also positive with RT PCR assay. Both molecular methods showed moderate agreement with FNAC, but RT PCR showed a slightly higher agreement with FNAC. Their kappa value was 0.6 and 0.675 for GeneXpert and RT PCR respectively.

Culture showed a detection rate of 68.6% of positive cases from all FNAC positive cases, and 1 additional positive case was detected with culture which has a negative FNAC result. Kappa value was 0.6, moderate agreement with FNAC as that of molecular methods.

The sensitivity of ZN was way far less from the sensitivity of AO staining technique. Goswami et al reported a sensitivity of 70.27% for ZN staining (54). Similarly Mohana et al reported a sensitivity of 71.4% for ZN staining (55). This finding was in contrast with the present study which showed a sensitivity of 33.3% for ZN staining. This might be due to the presence of low bacterial load in many of the FNA samples collected in this study. Ghulam et al have shown that there should be 5000–10,000 bacilli per ml of sample so to be detected by ZN staining (56). A report from Mengistu et al showed a lower sensitivity of 39% for ZN staining (57). This finding was similar to the present study but controversially the sensitivity of 47% for AO that Mengistu et al reported was much less than what was reported in this study which was 75%. The specificity that was seen in both methods was 100%. Similarly, they both have a positive predictive value of 100% and negative predictive value of 71.4% and 87% respectively for ZN and AO. In this case, AO became a better method in showing the real negative value when the test is negative.

In contrast to the finding from Mengistu et al, which reported a sensitivity and specificity of 78% and 74% for Xpert MTB/RIF assay (57), the present study showed a higher sensitivity and specificity of 91.7% and 83.3%. The increased sensitivity and specificity observed in this study may have resulted from the application of concentration method. Xpert MTB/RIF assay and all other parameters included in this study were performed after concentration method has applied. The sensitivity in the present study was

still higher when compared with what has been reported by Mulualem et al. In their study Xpert MTB/RIF assay was performed from a concentrated FNA sample but having a sensitivity of 87.8% which was closer but still lower to what has been shown in this study (58).

The sensitivity and specificity of RT PCR in this study was found to be 97.2% and 73.3%. This finding is not consistent with Babafemi, E.O., Cherian, B.P., Banting, L. *et al,* which showed a sensitivity of 70% and higher specificity of 99% (59). A very low sensitivity of 17.1% was reported by Linasmita *et al* when compared with this study and a specificity of 100% (60) which happens to be higher than that of the specificity observed in the present study. The reason for the big difference seen in the two studies might be due to the difference of primer set used in both studies. The positive predictive value of both GeneXpert and RT PCR in the present study was 76.7% and 68.6% respectively. GeneXpert has a better PPV value than RT PCR because among all culture negative cases, GeneXpert detected smaller number of positive cases in comparison with that of RT PCR. The negative predictive value was 94.3% and 97.8% respectively for GeneXpert MTB/RIF assay and RT PCR.

The positive likelihood ratio of GeneXpert was better than that of RT PCR, which showed that GeneXpert MTB/RIF assay has a better capacity of narrating the true positive result than RT PCR does, with a value of 5.5 compared with 3.6. The negative likelihood ratio of RT PCR on the other hand was better showing a value of 0.04 compared with 0.1 of that of Xpert MTB/RIF assay. In this case, RT PCR happened to be a better narrator of true negative result that Xpert MTB/RIF assay.

FNAC has a sensitivity of 97.2% and a specificity of 68.3%. Tamana E-Nur also reported the same pattern of FNAC having a higher sensitivity and lower specificity. The finding from Tamana E-Nur showed the sensitivity of 79.7% and specificity of 48.1% (61). This seems to be lower than the finding from this study. Gunjan P. Upadhyay, and Rameshchandra M. Thakker also reported a sensitivity of 90.9% and specificity of 67.2% (62) showing a similar decrease in specificity with the present study. FNAC in this study has a PPV and NPV of 68.6% and 97.6%. The PPV reported by Tadesse et al was higher than the finding from this study. Tadesse *et al* showed a PPV of 79/1% and NPV of 59.1% (58) which happened to be a smaller value in comparison with the NPV from this study. Report from Fantahun *et al* showed a PPV and NPV of 62.1% and 91.8% (57) which is relatively closer result with this study. PLR and NLR were 3.46 and 0.04 respectively. This finding is higher than the one reported by Tadesse *et al* which showed PPV and NPV of 1.9 and 0.3 (16).

A study by Manitchotpisist *et al* have shown that the combined use of PCR with FNAC was helpful in improving the diagnosis of TBLN. They have shown how the sensitivity and sensitivity of FNAC can be improved from 48% and 87.5% to 84% and 100% (63). The molecular techniques have been combined with FNAC in this study to see how they will overcome the diagnostic limitation of FNAC observed so

far. Initially, they were combined with FNAC and the level of agreement was assessed with FNAC. Sensitivity, Specificity, PPV, and NPV were calculated using culture as gold standard. FNAC when combined with Xpert MTB/RIF assay (FNAC+Xpert MTB/RIF assay), it has a substantial agreement with a kappa value of 0.856. This finding was very close with the kappa value calculated for the combination of FNAC with RT PCR (FNAC+RT PCR) which showed a kappa value of 0.815. GeneXpert+FNAC showed a slightly better capacity in terms of accuracy and precision. The sensitivity, specificity, PPV, and NPV showed a slight increment when compared with what was observed in FNAC+RT PCR.

Up on the combination of two molecular methods with FNAC, the diagnostic performance observed was similar as that seen in the combination of FNAC+RT PCR. The sensitivity, Specificity, PPV, and NPV of Xpert + RT PCR + FNAC showed slight difference with that observed in the combination of FNAC+RT PCR. Even the Kappa value of Xpert + RT PCR +FNAC was relatively lower than that of FNAC+RT PCR.

8. Limitation of the study

In this study, the RT PCR analysis relied on using only a single primer, which is IS1081 which can be found in all MTB complexes. Therefore, to avoid any possible false positive signal that might be generated due to the amplification of bacteria other than MTB, other set of primers such as RD9 and RD4 should have been used to distinguish between the MTBC.

Inadequate FNA sample was frequent in this study, which could have contributed for the lower sensitivity of ZN and AO

9. Conclusion

The Xpert MTB/RIF assay, when solely combined with FNAC, level of agreement was higher than what was observed in the combination of FNAC+ RT PCR, and even with the combination of FNAC+Xpert MTb/RIF+RT PCR. The diagnostic performance when compared with culture method as a gold standard was also higher than the result obtained from the combination of FNAC+RT PCR and FNAC+Xpert MTb/RIF+RT PCR.

Therefore the combination of molecular methods with FNAC observed in this study clearly showed how the diagnostic value could be enhanced to an improved state than what was observed in FNAC alone. Especially combining FNAC with Xpert MTB/RIF assay generated a very useful outcome.

This have an implication in the handling of the tubercular lymphadenitis patient treatment by providing affirmative result due to the application of molecular testing in addition to FNAC.

Finally, this study concluded that combining FNAC with molecular testing like that of Xpert MTB/RIF assay and RT PCR have a very useful implication in many aspects such as initiating early and appropriate treatment, and in providing better health care service.

10. Recommendation

From the points observed in this study finding, we would like to forward the following recommendation:

- Easily accessible, simple and rapid diagnostic methods such as Xpert MTB/RIF assay and RT PCR should be provided in order to overcome the diagnostic challenge of tubercular lymphadenitis.

- In order to provide early treatment, tubercular lymphadenitis patient should be supplemented with affirmative diagnostic method such as Xpert MTB/RIF assay and RT PCR so to alleviate an inconclusive result.
- In the light of providing diagnostic methods with better diagnostic performance, the application of molecular methods in combination with FNAC could be very helpful.
- Finally, since this study was conducted in the capital city of Ethiopia, we recommend country wide studies should be performed which would give a clearer picture regarding the diagnosis of Tubercular lymphadenitis.

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

Principal Investigator: Abay Atnafu

Addis Ababa University

Our kindly inquiry here in this study is your participation which involves taking Fine Needle Aspirate (FNA) samples. To divulge, FNA specimen collection involves the pathologist who is going to undertake the procedure using syringe and needle for a routine diagnostic use. This

sample is the one that the pathologist draws for your diagnosis. When it comes to this study, no additional specimen is required or you will not be subjected to give additional sample for this study, rather we are going to use the left over sample from the one that you gave for your diagnosis purpose. Hence, we hereby, kindly requesting you to take a portion of your precious time to read and understand the intent of this consent form and to forward us any sort of inconvenience that might appear in this course of time. And we would like to let you know that you hold a full right of not participating in this study or ever to halt your participation even in between the course of this study and your decision has nothing to do with your routine diagnosis.

Purpose:

The purpose of this study is to look for the utility of PCR performed on concentrated FNA sample in the detection of TBLN cases which are inconclusive with cytopathology and to look for an increase in the detection rate due to the application of PCR technique on concentrated FNA sample. Integrating the cyto-morphological method with PCR tests performed on concentrated FNA sample will help increase the detection rate of TBLN in a suspected patient.

Procedures to be carried on:

After collecting the signed consent form from you, you will be giving the FNA specimen which will pass through cyto-morphological analysis, bacteriological analysis, concentration techniques, and also PCR analysis. This whole test will be performed from the first specimen that you gave for routine diagnosis and the volume will be an average of 60 μl (≈ 3-4 drops) of the FNA sample. Armauer Hansen Research Institute Pathology Laboratory is the place where we will do an investigation for Mycobacterium tuberculosis.

Risks associated with the study:

At the site where the needle will be inserted, there will be an accumulation of blood which is referred as hematoma and there will be a swelling due to the accumulation of blood. It should be noted here that this manifestation is harmless and have a high tendency to self heal. But it may cause some discomfort and slight and temporary difficulty swallowing.

Bleeding (hemorrhage), infection, or inadvertent puncture of other structures in the neck is among the rarest complication. Patients with a history of drug such as aspirin, ibuprofen or blood thinners might have a bleeding risk. The courtesy of the pathologist and the clinical nurses in the

FNA collection room enables for the handling of such rare cases immediately. If the participant is experiencing any sign of inconvenience or noticing a sign of infection at the site of needle insertion for sample collection, the focal person's telephone number is attached herewith so that the participant will be able to make a communication in order to get him/her an appropriate treatment.

Benefits of the study:

Monetary compensation or other benefit will not be due here in this study rather you will acquire an indirect gain from the fact that a confirmed positive result for MTB will be reported to the ordering physician as soon as possible in order to offer you an appropriate treatment. Another point where a benefit from this study will glow arises on the result obtained from this study that will bid its own role in the TB control program.

Confidentiality of your information:

No person other than the researcher and the responsible physician will be able to have an access to the result obtained from the laboratory. No personal information pertaining the participant will be included in this study. What data from the participant happens to be included here is only the code we will assign to each participant and each code will be registered in a separate registration book to which the confidentiality will be assured. This code will go through all the process till the final result. The storage of collected raw data will be at AHRI data management in a secured manner.

Termination of the study:

Any sort of turning away or your decision to halt your participation sometime in the course of this study will be respected. And your decision will have no consequences in your routine diagnosis. No left over sample will be enrolled in this study without your agreement.

For any information regarding this study please contact the down listed personnel:

Abay Atnafu (principal investigator**)**

Cell phone: +251-912-20-5822

AAUERC

Phone:

AAERC

Phone: +251-11-8962183

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 Integration of cytopathology with PCR tests performed on concentrated FNA sample to improve the lab diagnosis of TBLN suspected patients 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 look for the utility of to look for the utility of FNA concentration technique in the detection of TBLN cases which are inconclusive with cytopathology.
- I understand that if I am enrolled in this study, an average of 60 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 Integration of cytopathology with PCR tests performed on concentrated FNA sample to improve the lab diagnosis of TBLN suspected patients 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 look for the utility of FNA concentration technique in the detection of TBLN cases which are inconclusive with cytopathology.
- I understand that if I am enrolled in this study, an average of 60 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_____

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 Integration of cytopathology with PCR tests performed on concentrated FNA sample to improve the lab diagnosis of TBLN suspected patients 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 look for the utility of FNA concentration technique in the detection of TBLN cases which are inconclusive with cytopathology select a simple, cost effective, and applicable method at peripheral health facilities.
- I understand that if I am enrolled in this study, an average of 60 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 look for the utility of Integration of cytopathology with PCR tests performed on concentrated FNA sample to improve the lab diagnosis of TBLN suspected patients in ALERT 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._____

    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. Adequate number of samples collected? YES NO

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

4. Concentration Done? YES NO

5. Left over ample stored for PCR? YES NO

1.2 Laboratory Results

1.2.1 ZN staining **1** NEG

2 Scanty 1+ 2+ 3+ 4+

1.2.2 Auramine O staining (LED FM)

NEG

1.

Scanty 1+ 2+ 3+ 4+

1.2.3 Culture result after eight week

NEG

POS

1,2.4 **PCR** result POS NEG

1.2.5 GeneXpert result DET MND

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)

የመረጃቅጽ

ለጥናቱ ተሳታፊወች መረጃ መስጫ ቅፅ

ማብራሪያ፡

ይህ ጥናት የቲቢ ባክቴሪያ ከሳምባ ውጭ የሚያስከትለውን በሽታ ምርመራ ማሻሻል እና ማሳደግ ላይ ያተኩራል። በዚህም ጥናት ላይ እርስዎ እንዲሳተፉ ተጋብዘዋል። ስለሆነም ከውድ ጊዜዎት ትንሽ አፍታ ወስደው ቀጥሎ የተፃፈውን የጥናቱን ማብራሪያ እንዲያነቡና ግልጽ ያልሆነልዎትን ማንኛውም ጥያቄ በመጠየቅ በቂ ግንዛቤ እንዲያገኙ በትህትና እንጠይቅዎታለን።

ለመደበኛ ምርመራዎ የሚሆነውን ናሙና ባለሙያው (ፓቶሎጂስቱ) ስሪንጅ በመጠቀም ከወሰደልዎት በኋላ እና ለመደበኛ ምርመራዎ የሚሆን በቂ ናሙና ጥቅም ላይ ከዋለ በኋላ የሚተርፈው ናሙና ለዚህ ጥናት ስራ ይውላል። ይህም የሚሆነው የእርሶ በጥናቱ ላይ የመሳተፍ ስምምነት ከተረጋገጠ በኋላ ነው። በዚህ ጥናት ላይ ያሎትን ተሳትፎ በማንኛውም ጊዜ የማቋረጥ መብትዎት የተረጋገጠ ነው። ተሳትፍዎትን በማቋረጥዎትም በመደበኛ ምርመራዎት ላይ የሚያስከትለው ምንም አይነት ተፅዕኖ አይኖርም።

ዓላማ፡

የዚህ ጥናት ዓላማ የፒሲአር ዘዴ ከሳይቶፓቶሎጂ ዘዴ ጋር በማቀናጀት የቲቢ ምርመራ ማሳደግና ማቀላጠፍ እንዲሁም በሽታው በተሻለ ሁኔታ የመገኘት እድሉን ከፍ ማድረግ ነው። ይህም ከሆነና በሽታው ቶሎ ከተገኘ በኋላ አስፈላጊው ህክምና ታማሚው እንዲያገኝ ይረዳል።

ቅደምተከተል:

በዚህ ጥናት ላይ ለመሳተፍ ፈቃደኛነዎትን ካረጋገጥን በኋላ እርስዎ ናሙናውን እንዲሰጡ ይደረጋል። የሚወሰደው የናሙና መጠን በአማካይ ከ3-4 ጠብታ ያክል ይሆናል። በቅድሚያ የእርስዎ መደበኛ ምርመራ ከተወሰደው ናሙና ላይ ከተሰራ በኋላ የሚተርፈውን ናሙና ለዚህ ጥናት ስራ እንጠቀምበታለን። የእርስዎም መደበኛ ምርመራ ሆነ የዚህ ጥናት የላቦራቶሪ ስራ የሚከናወነው በአህሪ የምርምር ማዕከል ውስጥ ነው።

ተያያዥ ስጋቶችና ጉዳት

ናሙናውን የሚወስዱት ባለሙያወች በሙያው ላይ በቂ እውቀት ያላቸውና በስነምግባርም የታነፁ ናቸው፡ ፡ ሆኖም ግን ናሙናው የተወሰደበት እባጭ ላይ መጠነኛ የሆነ የደም መጠራቀም ሊኖር ይችላል። በዚህም

ምክንያት መጠነኛ የሆነ እብጠት ሊከሰት ይችላል። እብጠቱንም ተከትሎ መጠነኛ ምቾት ማጣት ሊከሰት ይችላል። ይህ እብጠት ግን ጊዜያዊ እና በራሱ ጊዜ የሚድን ነው።

አንዳንድ ከታማሚው የቀደመ መድሃኒት የመጠቀም ታሪክ ጋር ተያይዞ ሊከሰቱ የሚችሉ ሁኔታዎች ሊኖሩ ይችላሉ። ለምሳሌ የደም ማቅጠኛ መድሃኒት የሚወስዱ ላይ የደም መፍሰስ አለመቆም ሊፈጠር ይችላል። ይህንና ሌሎችንም ተያያዥ ችግሮች ቢፈጠሩ በናሙና መቀበያው ክፍል ውስጥ የሚገኙት ፓቶሎጂስቱ እና ነርሶች ሁኔታውን ለመቆጣጠርና መፍትሄ ለመስጠት ይችላሉ። ከዚህም በተጨማሪ ከጥናቱ ጋር ተያይዞ ለሚኖረዎት ማንኛውም አይነት ጥያቄ አድራሻው ከዚህ ፅሁፍ በታች የተጠቀሰውን ግለሰብ መጠየቅ ይችላሉ።

ማበረታቻ ጥቅሞች

በዚህ ጥናት በመሳተፍዎት ምንም አይነት ገንዘብም ሆነ ገንዘብ ነክ ጥቅማጥቅሞች አይኖሩም። ነገር ግን ይህ ጥናት የሚያስገኘውን የተሻሻለ ውጤት እና ተፋጥኖ የሚደርስልዎት እና ለሀኪምዎ በጊዜው የሚላክልዎት የምርመራ ውጤት ለእርስዎ ቀጥተኛ ያልሆነ ጥቅም ተደርጎ ይቆጠራል።

የመረጃ ምስጢር ተጠባቂነት

ከሀኪምዎ እና ከተመራማሪው በስተቀር የላቦራቶሪ ውጤትዎን ማንም ሰው ሊያየው አይችልም። በዚህ ጥናት ላይ የእርስዎን ምንም አይነት ግላዊ መረጃ የማንጠቀም መሆኑ የተረጋገጠ ነው። ምስጢራዊ ልዩ መለያ ብቻ ለእያንዳንዱ ተሳታፊ የሚሰጥ ይሆንና ይህም መለያ ውጤቱ እስኪጠናቀቅ እና ከዚያም በኋላ በሚኖረው የመረጃ መጠናቀር ሂደት ድረስ እንጠቀምበታለን። የላቦራቶሪ መዝገባችንም ላይ የምጠቀመው ይህንኑ ልዩ ምስጢራዊ መለያ ይሆናል። በጥናቱ የሚገኙትም መረጃዎች በአህሪ የመረጃ ቋት ደህነታቸው ተጠብቆ የሚቀመጡ ይሆናል።

መብቶች

ተሳታፊው/ዋ በዚህ ጥናት ላይ ያለመሳተፍ ሙሉ መብቱ/ቷ የተጠበቀ ነው። እንዲሁም የተሳታፊነት ስምምነት ከሰጠ/ች በኋላ በማንኛውም ጊዜ ተሳትፎን የማቋረጥ መብቱ/ቷ የተጠበቀ ነው።

አሁንም ሆነ በማንኛውም ጊዜ ለሚኖረዎት ማንኛውም ጥያቄ አድራሻቸው ከታች የተገለፁትን ግለሰቦች ማነጋገር ይችላሉ።

አባይ አጥናፉ

ዋናተመራማሪ

ስልክቁጥር+251-912-20-5822

አህሪ/አለርትየምርምርስነምግባርኮሚቴ

ስልክቁጥር: +251-118-962-183

Annex V. Consent form (Amharic version)

<u>ለቲቢሊንፋዲናይትስ /ከሳንባውጭየቲቢበሽታ/ ምርምርየአዋቂዎችፈቃደኝነትመጠየቂያቅፅ</u>

- ✓ እኔ ስሜ ከዚህ በታች የተገለጸዉ ይህ ጥናት በአርማወር ሐንሰን የምርምር ተቋም ተመራማሪዎች ከሳንባ ዉጪ ላለ የቲቢ በሽታን ለመመርመር የሚያስችልን የመመርመሪያ ዘዴ (ፒሲአር) 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 በአማካይ ከአራት እስከ አምስት ጠብታ ፈሳሽ ከእባጩ ላይ በሰለጠነ ባለሙያ በመርፌ እንደሚወሰድ ተነግሮኛል።
- ✓ ከሳንባ ውጭ የቲቢ በሽታ ምርመራ እንደሚደረግልኝና የቲቢ ጥገኛ ህዋስ በተወሰደው ናሙና ውስጥ ካለ አስፈላጊውን ህክምና እንደማገኝ ተረድቻለሁ።
- ✓ የምርመራ ውጤቴም እንደሚነገረኝና በሚስጥርም እንደሚያዝ ተረድቻለሁ።

- ✓ በጥናቱ ብሳተፍም ባልሳተፍም አስፈላጊውን ለማንኛውም ህሙማን የሚሰጠውን አገልግሎት በአቅራቢያዬ ካለው ሆስፒታል ወይንም ጤና ጣቢያ እንደማገኝና በዚህ ጥናት መሳተፍ ወይንም ያለመሳተፍ እንዲሁም በፈለኩት ጊዜ ከጥናቱ የመውጣት መብት እንዳለኝ ግልጽ ተደርጎልኛል።
- ✓ ከላይ የተደረገልኝን ማብራሪያ በሚግባ ተረድቻለዉ በጥናቱም ዉስጥ እንደምሳተፍ በፊርማዬ አረጋግጣለዉ።

የተሳታፊውስም_____

ፊርማ_____

ቀን _____

<u>ለቲቢሊንፋዲናይትስ /ከሳንባውጭየቲቢበሽታ/ ምርምርዕድሜያቸዉከ12 እስከ 17 ለሆኑተሳታፊታዳጊወጣቶችፈቃደኝነትመጠየቂያቅፅ</u>

- ✓ እኔ ስሜ ከዚህ በታች የተገለጸዉ ይህ ጥናት በአርማወር ሐንሰን የምርምር ተቋም ተመራማሪዎች ከሳንባ ዉጪ ላለ የቲቢ በሽታን ለመመርመር የሚያስችልን የመመርመሪያ ዘዴ (ፒሲአር) 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 በአማካይ ከአራት እስከ አምስት ጠብታ ፈሳሽ ከእባጩ ላይ በሰለጠነ ባለሙያ በመርፌ እንደሚወሰድ ተነግሮኛል።
- ✓ ከሳንባ ውጭ የቲቢ በሽታ ምርመራ እንደሚደረግልኝና የቲቢ ጥገኛ ህዋስ በተወሰደው ናሙና ውስጥ ካለ አስፈላጊውን ህክምና እንደማገኝ ተረድቻለሁ።
- ✓ የምርመራ ውጤቴም እንደሚነገረኝና በሚስጥርም እንደሚያዝ ተረድቻለሁ።
- ✓ በጥናቱ ብሳተፍም ባልሳተፍም አስፈላጊውን ለማንኛውም ህሙማን የሚሰጠውን አገልግሎት በአቅራቢያዬ ካለው ሆስፒታል ወይንም ጤና ጣቢያ እንደማገኝና በዚህ ጥናት መሳተፍ ወይንም ያለመሳተፍ እንዲሁም በፈለኩት ጊዜ ከጥናቱ የመውጣት መብት እንዳለኝ ግልጽ ተደርጎልኛል።
- ✓ ከላይ የተደረገልኝን ማብራሪያ በሚግባ ተረድቻለዉ በጥናቱም ዉስጥ እንደምሳተፍ በፊርማዬ አረጋግጣለዉ።

የተሳታፊውስም_____የተሳታፊውወላጅ/አሳዳጊስም

_____

ፊርማ_____ ፊርማ _____

ቀን_____

ቀን_____

<u>ለቲቢሊንፋዲናይትስ /ከሳንባውጭየቲቢበሽታ/ ምርምርለሚሳተፉከ 12 አመትበታችለሆኑህጻናትወላጆችፈቃደኝነትመጠየቂያቅፅ</u>

- ✓ እኔ ስሜ ከዚህ በታች የተገለጸዉ ይህ ጥናት በአርማወር ሐንሰን የምርምር ተቋም ተመራማሪዎች ከሳንባ ዉጪ ላለ የቲቢ በሽታን ለመመርመር የሚያስችልን የመመርመሪያ ዘዴ (ፒሲአር) ያለውን የመመርመር ብቃት ለማጥናት እንደሚካሄድ ተገልፆልኛል።
- ✓ ከዚህ ጥናት የሚገኘው ውጤት በሽታውን ለመቆጣጠር የሚደረገውን ጥረት ሊደግፍ አንደሚችል ተረድቻለሁ።
- ✓ ልጄበዚህጥናትውስጥእንዲሳተፍ/እንድትሳተፍፍቃደኛከሆንኩለጥናቱየሚያስፈልገውን በአማካይ ከአራትእስከአምስትጠብታ ፈሳሽከአበጠውቦታላይበሰለጠነባለሙያበመርፌእንደሚወሰድተነግሮኛል።
- ✓ ለልጄከሳንባውጭየቲቢበሽታምርመራእንደሚደረግለት/ላትናየቲቢጥገኛህዋስበተወሰደውናሙና ውስጥካለአስፈላጊውንህክምናእንደሚያገኝ/ታገኝተረድቻለሁ።
- ✓ የምርመራውጤቱምእንደሚነገረኝናበሚስጥርምእንደሚያዝተረድቻለዉ።
- ✓ ልጄበጥናቱቢ/ብትሳተፍምቢ/ባትልሳተፍምአስፈላጊውንለማንኛውምህሙማንየሚሰጠውንአገልግ ሎትበአቅራቢያዬካለውሆስፒታልወይንምጤናጣቢያለልጄእንደማገኝናልጄንበዚህጥናትየማሳተፍ ወይንምያለማሳተፍእንዲሁምበፈለኩትጊዜከጥናቱየማስወጣትመብትእንዳለኝግልጽተደርጎልኛል።
- ✓ ከላይየተደረገልኝንማብራሪያበሚግባተረድቻለዉልጄበጥናቱምዉስጥእንዲሳተፍ/ድትሳተፍበፊር ማዬአረጋግጣለዉ።

የተሳታፊውወላጅ/አሳዳጊስም _____

ፊርማ_____

ቀን _____

Annex VI: Questionnaire (Amaharic Version)

መጠይቅ

ይህ መጠይቅ የተዘጋጀዉ የፒሲአር ዘዴ ከእብጠት ላይ በመርፌ ከተወሰደ ናሙና ዉስጥ ከሳንባ ዉጪ የቲቢ በሽታ ምርመራን ለማሻሻል ያለዉን አስተዋጽዖ ለማወቅ በአለርትና በቅዱስ ጴጥሮስ ሆስፒታል የሚደረገዉን ጥናት ለማገዝ ነዉ።ጥናቱ የሚካትተዉ’ የሊንፍ ኖድ ቲቢ አለባቸዉ ተብለዉ የተጠረጠሩ ተሳታፊዎችን ነዉ።

ቀን:_____ /_____/_____

I. አጠቃላይየተሳታፊዉመረጃ

1. የተሳታፊዉ ልዩ ኮድ: _____
2. የተሳታፊዉ የሆስፒታል ካርድ ቁጥር: _____
3. ክልል: _____ ዞን: _____ ቀበሌ: _____
   ስልክቁጥር:_____
4. እድሜ: _____
5. ጾታ: _____
6. የመኖርያቦታ 6.1.ከተማ:_____ 6.2.ገጠር: _____
7. የጋብቻሁኔታ 7.1.ያላገባ: 7.2.ያገባ: 7.3.አግብቶየፈታ: 7.4.የሞተበት:
   7.5.ከእጮኛጋርየሚኖር:
8. 8. ስራ: 8.1.የቤትእመቤት:____ 8.2.የቀንሰራተኛ: ___ 8.3.የመንግስትሰራ: ____
   8.4.ስራየሌለዉ: ____ 8.5.ገበሬ: ____ 8.6.ሌላ(ግለጽ): _____
9. የትምህርትሁኔታ (በዓመት): _____

II. ክሊኒካልመረጃ

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: _____ 5.2የለም: _____
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 ህክምና አለ?: 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 ይግለጹ:____ 11.4.መድሀኒቱን ጨርሰዋል: _____ 11.5.መድሀኒቱን አቋርጠዋል: _____
12. ተሳታፊዉ ከቲቢ በሽታ ታማሚ ሰዉ ጋር ቅርበት ነበራቸዉ? 12.1.ነበራቸዉ: _____

    12.2.አልነበራቸዉም: _____
13. ከዚህ በፊት ለቲቢ ተከትበዉ ያዉቃሉ (በክንድ ላይ ያለ የቲቢ ክትባት ጠባሳ)? 16.1.አዎ:

    _____ 16.2.የለም: _____

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: **Abay Atnafu (BSc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

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Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Kassu Desta (PhD cabdidate)

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Place: Addis Ababa, Ethiopia.

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