Addis Ababa University

College of Health Sciences

School of Allied Health Sciences

Department of Medical Laboratory Science

Prevalence of Tuberculosis, Multidrug Resistance Tuberculosis and Associated Risk Factors among Smear Negative Presumptive Pulmonary Tuberculosis Patients in Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Waganeh Sinshaw Ayenew, entitled with: **Prevalence of Tuberculosis, Multidrug Resistance Tuberculosis and Associated Risk Factors among Smear Negative Presumptive Pulmonary Tuberculosis Patients in Addis Ababa, Ethiopia** 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.

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List of Acronyms

AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
BSC	Biosafety Cabinet
DOTS	Directly Observed Treatment Short course
DRS	Drug Resistance Surveillance or Survey
DST	Drug Susceptibility Testing Course
EPHI	Ethiopian Public Health Institute
EQA	External Quality Assurance
HIV	Human Immunodeficiency Virus
GLI	Global Laboratory Initiative
LJ	Lowenstein Jensen
LPA	Line Probe Assay
MDR	Multidrug Resistant
MTB	*Mycobacterium Tuberculosis*
NALC	N-Acetyl L-Cysteine
NaOH	Sodium Hydroxide
NPV	Negative Predictive Value
NTM	Non Tuberculosis Mycobacteria
NTRL	National Tuberculosis Reference Laboratory
PPV	Positive predictive value
PT	Proficiency Testing
PTB	Pulmonary Tuberculosis
RR	Rifampicin Resistant

SNPPTP	Smear Negative Presumptive Pulmonary Tuberculosis Patients
SNPT	Smear Negative Pulmonary Tuberculosis
SNMDR TB	Smear Negative Pulmonary Multidrug Resistance Tuberculosis
SNPP	Smear Negative Pulmonary Patients
SPPT	Smear Positive Pulmonary Tuberculosis
WHO	World Health Organization
XDR	Extensively Drug Resistant
ZN	Ziehl Neelsen

Operational Definitions

- **Smear Negative Presumptive Pulmonary Tuberculosis (SNPPT) patients:** - Are patients who have signs and symptoms (are suspected) of pulmonary tuberculosis but two of their consecutive sputum samples (spot-spot) are microscopically negative.
- **Smear Negative Pulmonary Tuberculosis (SNPT):-** Is PTB that was initially negative for two (spot-spot) consecutive sputum smear examinations and later diagnosed positive by other advanced TB diagnostic tools.

Abstract

Background: Diagnosing active smear negative PTB, which represent the majority of tuberculosis (TB) cases left difficult. Therefore, treatment is often carried out empirically, based on clinical criteria, resulting in misdiagnosis and treatment. The distribution and magnitude of smear negative PTB, smear negative MDR PTB and associated factors in the same day diagnosis strategy are not clearly known in the study area. **Objective:** The objective of the study was to determine the prevalence of *TB, MDR-TB and associated risk factors* among presumptive smear negative pulmonary tuberculosis patients by Xpert MTB/RIF Assay in Addis Ababa, Ethiopia. **Methods:** An analytic cross sectional study design was used. A total of 418 patients were consecutively enrolled and two (spot-spot) sputum samples were collected, stained with Ziehl Neelsen (ZN) method and examined for AFB in the selected health facilities from August 01 to December 30, 2017. All smear negative sputum were transported and examined by FM microscopy, Xpert MTB/RIF and Culture. Data was entered and cleared by EPI data and analyzed by SPSS Ver.20. **Results:** A total of 27/418 (6.5%) (In 95 % confidence interval (CI), 4.103-8.816) were Xpert MTB/ RIF and 26/408 were culture confirmed SNPT cases. The positivity rate among male and female was 10.2% and 3.5% (*p=0.005*) respectively. From 26 culture positive isolates 03 (11.54 %) (95 % C.I, 0.00-23.82) were MDR TB; from this 02/23 (8.7 %) were among new and 01/03 (33.3%) was among retreatment cases. All Smear negative MDR TB cases were diagnosed from men. All rifampicin resistant SNPT isolates by Xpert MTB/ RIF assay were found to be MDR TB and 07/26 (26.9%) isolates were INH mono resistant. Being health care worker and history of migration found to be a potential risk factor for developing SNPT. The odds of having SNPT is 68.7 times higher in health care workers as compared to other job category AOR 68.7 (95% C.I, 2.447-1929.15 : p<0.013). None migrants have 0.121 times less likely to have smear negative pulmonary TB in comparison with migrants, AOR 0.121(95 % C.I, 0.20-0.730, p< 0.021). **Conclusion:** Overall prevalence of SNPT in Addis Ababa was 6.5% by Xpert MTB/ RIF assay and 6.4 % by culture in Spot- Spot sample collection and diagnosis strategy. This study also demonstrated 11.54 % smear negative MDR TB prevalence. Being health care worker and history of migration were found to be a potential risk factor for developing SNPT.

Key words: Prevalence, smear negative PTB, smear negative MDR TB, Xpert MTB/RIF Assay

1. Introduction

1.1 Background

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis complex*. It typically affects the lungs but can affect other sites as well. TB is also more common among men than women, and affects mostly adults in the economically productive age groups; around two-thirds of cases are estimated to occur among people aged 15–59 years(1).Without treatment, mortality rates are high. In studies of the natural history of the disease among sputum smear-positive and HIV-negative cases of pulmonary TB, around 70% died within 10 years; among culture positive (smear-negative) cases, 20% died within 10 years(2).Mycobacterium tuberculosis may have killed more persons than any other microbial pathogen(3).

Worldwide, one person out of three is infected with *Mycobacterium tuberculosis* – two to three billion people in total, from this 5-15 % develop TB disease in their life time. TB accounts for 2.5 % of the global burden of diseases and is the commonest cause of death in young women, killing more women than all causes of maternal mortality combined. TB currently holds the first place in the global ranking of causes of death. Unless intensive efforts are made, it is likely to maintain that position through to 2020, despite a substantial projected decline in disease burden from other infectious diseases (4). Effective drugs to treat and cure the disease have been available for more than 50 years, yet every 15 seconds, someone in the world dies from TB. Even more alarming: a person is newly infected with *M. tuberculosis* every second of every day. Left untreated, a person with active TB will infect an average of 10 to 15 other people every year (5). In regions where the transmission of *M. tuberculosis* has been stable or increasing for many years, the incidence rate is highest among by recent infection or re-infection. As transmission falls, the caseload shifts to the older age groups, and a higher proportion of cases come from the reactivation of latent infection(6).While the human immunodeficiency virus (HIV) infection has clearly had a profound effect on TB epidemiology, other potentially important risk factors have been somewhat neglected(7).

There have been many different huge efforts fighting against tuberculosis in the whole world in human history and prehistory as well. World Health Organization (WHO) has declared this disease a global emergency in 1993. Though the number of TB deaths and the TB incidence rate continue to fall globally, it remains as one of the major global health problem. WHO 2016 global

report, estimates that in 2015, tuberculosis was the leading causes of death from all infectious diseases. There were 1.4 million TB deaths in 2015, and an additional 0.4 million deaths resulting from TB disease among HIV-positive people. In terms of cases, the best estimates for 2015 are that there were 10.4 million new TB cases (including 1.2 million (11%) among HIV-positive people), of which 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children. Overall, 90% of cases were adults and 10% children, and the male: female ratio was 1.6:1(8). Worldwide, the rate of decline in TB incidence remained at only 1.5% from 2014 to 2015. This needs to accelerate to a 4–5% annual decline by 2020 to reach the first milestones of the End TB Strategy (8).

Also the emergence and spread of multi-drug resistant tuberculosis (MDR-TB) is threatening to destabilize global tuberculosis control. The prevalence of MDRTB is increasing throughout the world both among new tuberculosis cases as well as among previously treated ones(9). Globally, 5% of TB cases are estimated to have MDR-TB. Among new TB cases (that account for most of the global TB burden),an estimated 3.5% have MDR-TB, higher among people previously treated for TB, i.e. 20.5% (10). About 9% of MDR-TB cases also have extensively drug-resistant TB (XDR-TB)(11). In 2015, there were an estimated 480 000 new cases of multidrug-resistant TB (MDR-TB) and an additional 100 000 people with rifampicin-resistant TB (RR-TB) who were also newly eligible for MDR-TB treatment.

Although the number of TB deaths fell by 22% between 2000 and 2015, TB remained in its sovereign age (8). Ninety-five percent of all cases and 99 % of deaths occur in developing countries, with the greatest burden in sub Saharan Africa (12). Ethiopia is one of the 22 high burden countries (HBCs), the 41 high TB/HIV (among estimated all new TB cases, 13% are HIV co-infected) and MDR TB burden countries. According to the 2014 WHO report, the prevalence and incidence of all forms of TB are 211 and 224 /100,000 population, respectively. Excluding HIV related deaths, TB mortality was estimated to be 32 per 100,000 population in 2013. The national population based TB survey in Ethiopia conducted by the year 2010 / 11, revealed that the prevalence of smear positive TB among adults and all age groups was found to be 108 and 63 per 100, 000 populations, respectively. The prevalence of bacteriologically confirmed TB was found to be 156 per 100,000 populations **(13).** According to the recent national TB drug resistance surveillance report, 2.3% of new TB cases and 17.8% of previously treated TB cases were estimated to be MDR(14).

In the presence of the above challenges, globally in 2015, 4.3 million cases TB remain undiagnosed, (WHO 2016 global tuberculosis report). Smear-negative pulmonary TB is an increasing clinical problem in developing countries affected by the dual HIV/TB epidemic. In sub-Saharan Africa there is no enough data available that is required to help solve some of the problems surrounding the diagnosis of smear negative TB(15).

1.2 Statement of the problem

WHO recommends initial smear microscopy for the diagnosis of TB and detection of acid-fast bacilli, in the absence of rapid, sensitive and advanced, diagnostic tools. However, this is not a sensitive technique, and only 57% of new cases of pulmonary TB reported were smear-positive (43 % remain false negative) (16).It needs at least 5000 bacilli per ml of sputum for a positive result, sensitivity is further reduced in patients with extra pulmonary TB, children and in those who are co infected with HIV. It cannot also distinguish *Mycobacterium tuberculosis complex* from Non-Tuberculous Mycobacteria (NTM), viable from nonviable organisms and it cannot distinguish drug-susceptible strains from drug-resistant strains. Smears that have been stained with Auramine will need to be stained again if they are to be rechecked as part of an external quality assessment program(17). Sputum culture for Mycobacteria has higher diagnostic yield for smear negatives (up to 10-100 bacilli for positive culture) in comparison with smear microscopy. Nevertheless, Culture is more complex and expensive than microscopy, requiring facilities for preparing media, processing specimens and encouraging the growth of organisms, also requires specific laboratory equipment, technicians with additional skills, and appropriate bio-safety conditions,(17) more time-consuming in its results—the turn-around time is about 2–8 weeks. So it is less useful to guide the clinical decision-making process (18).Thus the treatment is often carried out empirically, based on clinical criteria, which can result in misdiagnosis of the case, unnecessary costs and toxicities(19).

Smear-negative pulmonary TB (SNPT) represents 30–60% of all pulmonary TB cases. In Brazil, 24–30% of cases of pulmonary TB in adults are SNPT. The mortality of these patients can reach 25% in populations with high prevalence of HIV infection, which may be largely related to delay in diagnosis. Furthermore, although smear-positive patients are considered to be more infectious, 10–20% of TB transmission at the population level is attributable to SNPT cases. Patients with smear-negative pulmonary TB were found to be less infectious and to have a lower mortality but a significant proportion (i.e. 50-71%) progressed to active disease justifying treatment (20-25).

In the absence of rapid, simple, and accurate TB diagnostic tools for SNPT, frequently occurring clinical features are also significant in supporting the diagnosis of SNPT, especially in areas with high TB and HIV infection like Ethiopia. But diagnosis using this clinical feature is not specific and sensitive as well that leads to misdiagnosis and empirical treatment of the patient (19).

Among TB cases notified in Ethiopia by the year 2013, 40,087(33.5%) were smear-positive cases, while more than half 41,575 (34.8%) was smear negative TB cases(26). So far the prevalence of SNPT and SNMDRTB was not well known among those smear negative pulmonary patients in Addis Ababa.

Xpert MTB/RIF is a fully automated real time hemi-nested PCR system which simultaneously detects *Mycobacterium tuberculosis* complex (MTB) genome as well as mutations that confer rifampicin resistance. It has been endorsed by WHO as the most sensitive rapid test for TB diagnosis in pauci- bacillary respiratory samples (27). This on-demand assay has advantages over microscopy; requires less than 2 hours to be performed (28), high sensitivity and specificity, low complexity, a minimum hands-on time and negligible safety concerns for persons handling the samples (29, 30). The value of the GeneXpert assay for the diagnosis of SNPT and RRTB, in spot- spot sample collection schedule was not determined.

1.3 Significance of the study

This study come up with prevalence of *Mycobacterium tuberculosis*(M.TB) and *Multidrug resistance tuberculosis (MDR TB)* and associated risk factors on the new national tuberculosis sample collection and diagnosis strategy, (same day diagnosis) on smear negative presumptive pulmonary Tuberculosis patients that can help for decision makers for interventions and control strategy of TB and MDR TB. It can be used as an initial reference data for other studies. The diagnostic yield and performance of Xpert MTB/RIF assay for the diagnosis of SNPT and SNMDR TB was also determined.

2. Literature Review

The diagnosis of TB still offers big diagnostic challenges related to the detection limit of smear microscopy, long time to culture-confirmation and variable sensitivity of molecular tests. In particular, diagnosing active smear-negative pulmonary TB (PTB), which represent the majority of TB cases is a major concern. According to the 2015 European Centre for Disease Prevention (ECDC) report, out of all PTB cases diagnosed in Italy in 2014, 68.1% were smear negative (31). Diagnostic delays and poor microbiological accuracy within these cases lead to a late response to clinicians and consequently to a delayed optimal treatment and poorer treatment response (32).

A study conducted in Brazil found a very high prevalence of SNPT among patients with TB in a setting with high TB and HIV prevalence. The absence of cough in the presence of other symptoms suggestive of TB, and having no radiographic pattern typical of TB where independent predictors of SNPT(19). In the study 198 patients met the inclusion criteria [positive culture for *Mycobacterium tuberculosis* in spontaneous sputum (n = 49), induced sputum (n = 54), or broncho-alveolar lavage (n = 95)] and were included in the analysis. Out of these patients, 69 (34.8%) were smear positive (SPPT) and 129 (65.2%) were smear negative (SNPT). Among SNPT there were 53 (41.1%) HIV positive patients, and among SPPT there were 25 (36.2%) HIV positive patients (p = 0.608).Cough was the most common symptom in both groups, although it was more frequent in SPPT (54 [78.3%]) than in SNPT (71 [55.0%]) (p = 0.002). Dyspnea and hemoptysis were also more frequent in patients with SPPT (32 [46.4%] and 9 [13.0%], respectively) than in patients with SNPT (35 [27.1%] and 4 [3.1%], respectively) (p = 0.010 and p = 0.013, respectively). As expected, the median length of hospital stay was higher in SNPT patients (20 days [10–49 days]) than in SPPT patients (14.5 days [7–26 days]) (p = 0.010)(19).

Improving the diagnostic accuracy and reducing diagnostic delay in smear-negative PTB is critical. A study conducted in Belarus (eastern Europe) to determine the proportion of MDR-TB among SN-PTB patients registered (Retrospective cohort study using countrywide data from the national electronic TB register) in 2012 and associated clinical and demographic factors shows that the need to use rapid diagnostic test for the early diagnosis of SN-PTB and SP-PTB.

Of 5377 registered TB patients (after excluding transfer-in cases), 4868 (91%) had pulmonary TB; 2960(55%) of these were SN-PTB: 2115 (70%) were men, the median age was 43 years (interquartile range [IQR]33–53) and 1813 (61%) came from urban areas. Nearly half (1347, 46%) were unemployed. HIV status was reported in only 551 patients (19%) and 111/551 (20%) patients tested were HIV-positive. Of 2960 (55%) SN-PTB, 1639 (55%) were culture-positive, of whom 768 (47%) had MDR-TB: 33% (363/1084) were new and 73% (405/555) previously treated patients. Previous history of treatment, age, region, urban residence, human immunodeficiency virus (HIV) status and being a pensioner were independently associated with MDR-TB. Generally, about half of culture-positive SN-PTB patients have MDR-TB and this rises to over 7/10 for retreatment cases which needs a national policy decision to extend rapid molecular diagnostics universally to all PTB patients, including SN-PTB, and Steps need to be taken to ensure implementation of this urgent priority, given the patient and public health implications of delayed diagnosis(33).

A study conducted in Adama, Ethiopia in 2013, indicates that of 232 sputum smear negative pulmonary tuberculosis suspects, 28(12.1%) were culture confirmed smear negative pulmonary tuberculosis and 204(87.9) smear negative pulmonary tuberculosis cases were still etiologically unexplained by culture. However among 28 culture confirmed smear negative pulmonary tuberculosis 22(78.6%) correctly treated as SNPTB and 6(21.4%) were under diagnosed or not treated following the national Tuberculosis guidelines. Among 204 (87.9 %) smear negative pulmonary tuberculosis suspects, 14(6.9%) over treated as smear negative pulmonary tuberculosis which still etiologically unexplained by culture. The binary- logistic regression identified that Productive cough(OR, 95% CI= 0.82; 0.81-0.91), hemoptysis (OR, (95% CI= 2.43; 1.04-5.68), night sweats (OR,95% CI)= 6.85; 2.00-23.39), Unexplained weight loss (OR,95% CI)= 3.44; 1.53-7.72), and being HIV positive (OR ,95% CI)=4.92; 2.1-11.5) were independently associated with confirmed SNPTB. But sex, Fatigue/tiredness, Shortness of breath, Fever and Contact history had no significant association with confirmed SNPTB ($p > 0.05$). A multivariate regression model was developed from significant and clinically important covariates. Multivariate regression analysis found that Productive cough (AOR; 95 % CI)= 0.82;0.81-0.91), night sweats (AOR .95% CI)= 8.27; 2.27-30.1), and being HIV positive (AOR;95 %CI)= (4.16; 1.45-11.96) were significantly associated with confirmed SNPTB(34).

A cross-sectional study and retrospective data analysis on TB undertaken in Northwest Shewa, Ethiopia, shows that from a total of 92 suspected TB cases, 27.17% (25/92) were positive for microscopic examination and 51% (47/92) for culture. The sensitivity and specificity of microscopic examination with 95% CI were 48.94% (34.08% to 63.93%) and 95.56% (84.82 to 99.33%), respectively. The positive and negative predictive values were 92% (73.93% to 98.78%) and 64.18% (51.53% to 75.53%), respectively. Of 8150 pulmonary TB cases in the retrospective study, 58.9% was smear negative. The proportion of TB-HIV co-infection was 28.66% (96/335). As it is indicated from the study poor sensitivity of smear microscopy together with the advent of HIV/AIDS elevated the prevalence of smear negative pulmonary TB, this in turn increased the risk of TB transmission(35).

MDR TB prevalence among SNPPTPs is also not clearly known in Addis Ababa. A cross sectional study conducted in St. Peter Tuberculosis specialized hospital, Addis Ababa, Ethiopia between November 2004 and October 2005, including 297 pulmonary tuberculosis suspected patients shows that 36 were found to be positive by smear microscopy and 37 (12.46%) pulmonary TB isolated from smear negative pulmonary patients. Of this 37 *M. Tuberculosis* isolates, 21/37 (29.8%) showed resistance to any of the drugs tested. No MDR-TB strains (resistant to INH and Rifampicin) were observed in this study. No statistically significant differences appeared in the frequency and pattern of resistance between isolates from smear positive and negative cases. The study shows that value of culture in the diagnosis of smear-negative cases to certain extent in untreated newly diagnosed PTB patients. SNPP have a probability of harboring DR TB strains (36).

The diagnosis of Pulmonary TB in adults in most African countries is based on simple techniques such as clinical assessment, sputum smear microscopy and chest radiography. Although specificity is high for smear microscopy, major concerns include low sensitivity (37) and delayed diagnosis of smear-negative disease (38). The accuracy of both microscopy and radiography is reduced by HIV, and so assessment of diagnostic approaches with existing methods and continuing research into new diagnostics are necessary (39).

A diagnostic accuracy study of Xpert MTB/RIF in HIV positive patients with high clinical suspicion of pulmonary tuberculosis in Lima, Peru was done from April 2010 to May 2011 HIV-positive patients with high clinical suspicion of TB were enrolled from two tertiary hospitals in

Lima, Peru. Detection of TB by MTB/RIF was compared to a composite reference standard Lowenstein-Jensen (LJ) and liquid culture. Detection of rifampicin resistance was compared to the LJ proportion method. We included 131 patients, the median CD4 cell count was 154.5 cells/mm3 and 45 (34.4%) had TB. For TB detection among HIV patients, sensitivity of MTB/RIF was 97.8% (95% CI 88.4–99.6) (44/45); specificity was 97.7% (95% CI 91.9–99.4) (84/86); the positive predictive value was 95.7% (95% CI 85.5–98.8) (44/46); and the negative predictive value, 98.8% (95% CI 93.6–99.8) (84/85). MTB/RIF detected 13/14 smear-negative TB cases, outperforming smear microscopy [97.8% (44/45) vs. 68.9% (31/45); p = 0.0002]. For rifampicin resistance detection, sensitivity of MTB/RIF was 100% (95% CI 61.0–100.0) (6/6); specificity was 91.0% (95% CI 76.4–96.9) (30/33); the positive predictive value was 66.7% (95% CI 35.4–87.9) (6/9); and the negative predictive value was 100% (95% CI 88.7 –100.0) (30/30)(40). In HIV patients in Peru with a high clinical suspicion of TB, MTB/RIF performed well for TB diagnosis and outperformed smear microscopy.

A study conducted in Kenya on Comparison of performance between ZN microscopy and GeneXpert in MTB detection in sputum specimens at KEMRI/CDC TB laboratory. From October 2010 to December 2010, a total of 262 specimens were tested with ZN microscopy and Xpert MTB/RIF. Of those, 34 of 262 (13%) were positive by Xpert MTB/RIF but only 8 of 262 (3%) were also positive by ZN microscopy, 26/34(76%) TB diagnoses would have been missed by ZN microscopy alone(41).

In South Africa a study conducted to evaluate Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting from July 2006 to June 2007, 1267 specimens (763) from routine mine health services and 504 from the Thibela TB study) had culture results available from both LJ and MGIT. Among the 1267 participants, 99.4% (*n* = 1260) were male, reflecting the sex distribution of this workforce, with a median age of 43 years (range 19–67, *n* = 1256); 51 (4.0%) were taking IPT at the time of enrolment and 35 (2.8%) had previously taken IPT; 1105 of the 1267 (87.2%) sputum specimens were smear-negative. Among smear-negative samples, the yield of Mycobacteria was higher for MGIT (225/1105, 20.4%) compared to LJ (145/1105, 13.1%. MGIT alone detected an additional 109 cultures positive for Mycobacteria where LJ was either negative or contaminated, compared to the additional 29 positive cultures detected by LJ alone. Among 88 Mycobacteria isolates from smear-negative

specimens ‘gained’ on MGIT (i.e., specimens that were culture-positive on MGIT but negative or contaminated on LJ), only 22.7% were *M. tuberculosis*, the majority being NTM(42).

Different risk factors have different impacts on the occurrence and diagnosis of SNPT. A study conducted in Mwanza, Tanzania to determine factors associated with smear negative and culture positive results among Pulmonary Tuberculosis Patients. Pulmonary tuberculosis patients were consecutively recruited for 12 months from five health facilities. Sputum examinations were done at the recruitment center and at the TB reference laboratory using Auramine O technique. Culture was done at the TB reference laboratory using Lowenstein Jensen solid media. A post-hoc analysis compared patients who had a smear negative culture positive result (case) with patients who had not (controls). A total of 655 pulmonary TB patients were recruited, 18 had no culture results and were excluded from the analysis. Of the remaining 637 patients, 127 (19.9%) had three negative smears at the recruitment Centre and 34 patients were a case. Current smoking was strongly associated with being a case, especially in women. Of the 127 patients who had three negative smears at the recruitment center, 104 (81.9%) also had a negative smear at the reference laboratory. Of these, 13 (12.5%) were still culture positive. In general the frequencies of smear-negative culture-positive results differ between health facilities, indicating possible difference in quality of laboratory procedures(42).

Another study conducted in, Ethiopia from October 2009 to January2011. Results indicates that a total of 459 TB suspects were enrolled during the study period; 336 (73.2%) were HIV positive. Acid fast bacilli sputum smear microscopy was done for 74.7% (251/336) HIV positive TB suspects; 94.4% (237/251) were smear negative. A Mycobacterium tuberculosis culture was performed in 63.7% (151/237). The median TB diagnostic delay for smear negative cases was 3 days (interquartile range 3–4 days). Of the 75 patients diagnosed with smear negative pulmonary TB, 89. 4% (67/75) were diagnosed by chest X-ray, 9.4% (7/75) by culture and 1.3% (1/75) by clinical suspicion only(43).

3. Hypothesis

Null hypothesis

- Smear negative pulmonary TB prevalence among presumptive smear negative pulmonary tuberculosis patients was different from other previous studies in Ethiopia.
- The prevalence of MDR TB in smear negative presumptive pulmonary TB patients was different from other studies conducted previously in Ethiopia. Therefore, the null hypothesis was rejected and alternative one was accepted

4. Objectives of the Study

4.1 General objective

- To determine the prevalence of tuberculosis, multidrug resistance tuberculosis and associated risk factors among smear negative presumptive pulmonary tuberculosis patients in Addis Ababa, Ethiopia.

4.2 Specific objectives

- To determine smear negative pulmonary tuberculosis prevalence among smear negative presumptive PTB patients (SNPPTPs)
- To determine prevalence of smear negative MDR TB among SNPPTPs
- To identify associated risk factors for smear negative pulmonary tuberculosis and SNMDRTB
- To determine the SNPT positivity rate in different sputum qualities by Xpert MTB/ RIF assay
- To determine performance characteristics of Xpert MTB/RIF assay, for the diagnosis of SNPT in comparison with culture

5. Materials and methods

5.1 Study design and study period

An analytic cross sectional study was conducted among smear negative presumptive pulmonary tuberculosis patients in Addis Ababa, Ethiopia from August 01 to December 30/ 2017.

5.2 Study site and setting

Addis Ababa is the Federal Capital of Ethiopia and a Chartered City; having three layers of Government: City Government at the top, 10 Sub City Administrations in the Middle, and 99 Kebeles Administrations at the bottom.

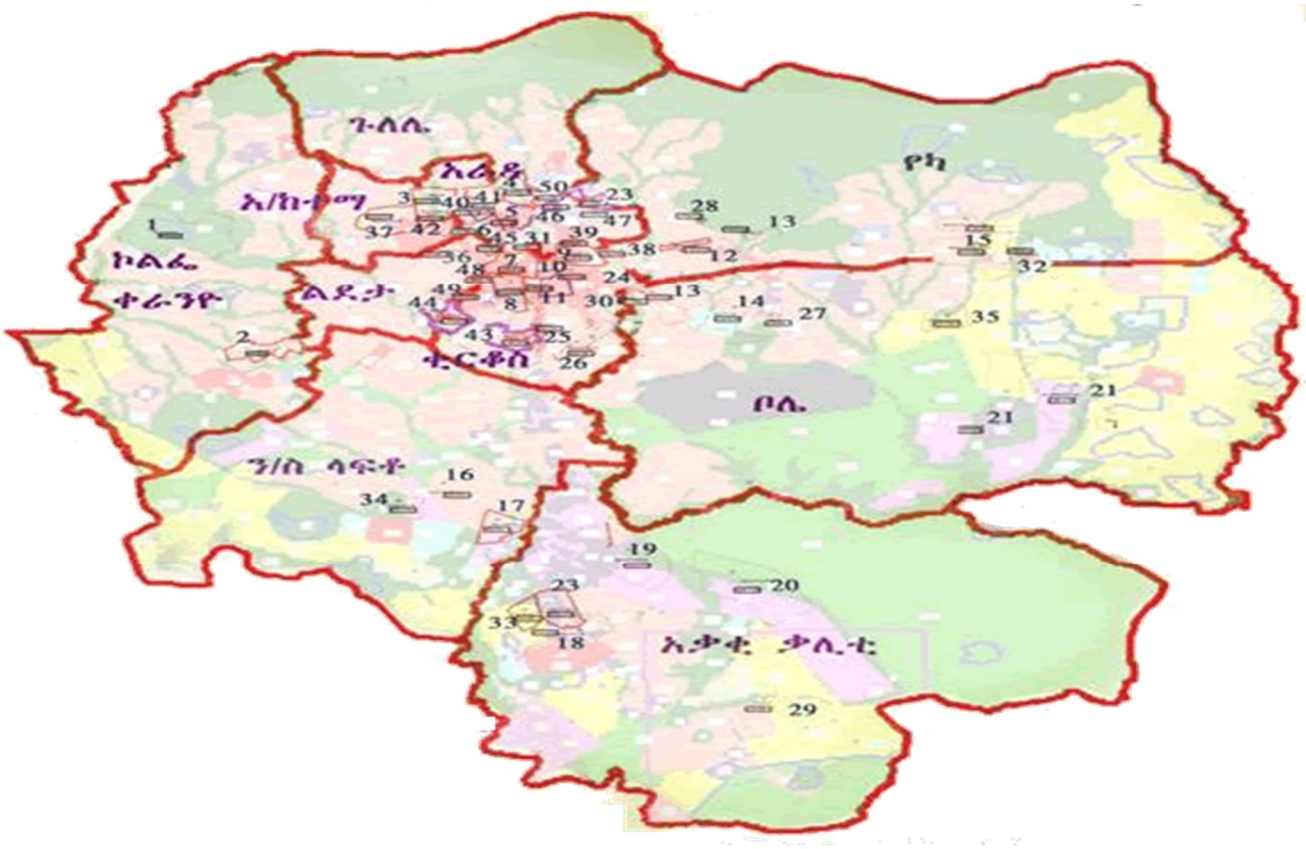


Figure 1: Map of Addis Ababa, Ethiopia

The location of Addis Ababa is between 8055' and 9005' North Latitude and between 38040' and 38050' East Longitude. Total land area is 54,000 hectares. Date of Establishment: November, 1887. (by Emperor Menelik II and Empress Taitu). The Population is more than 3 million and its average elevation is 2,500 meters above sea level, and hence has a fairly favorable climate and

moderate weather conditions; neither cooling in summer nor heating in winter and no vector borne disease. Addis Ababa is the seat of the African Union (AU) and the United Nations Economic Commissions for Africa (UNECA) (44).

All governmental and non-governmental health facilities that are currently incorporated in DOTS program in Addis Ababa region are listed (there are 137 DOTS sites) and first we had selected where to start from the list by lottery method then by using simple random sampling technique we have selected 20% out of them (Annex 1).

5.3 Population

5.3.1 Target population

All smear negative presumptive pulmonary TB patients in Addis Ababa.

5.3.2 Sampling population

All smear negative presumptive pulmonary TB patients visiting the selected health facilities during study period.

5.3.3 Study subjects

Sputum Smear Negative Presumptive Pulmonary Tuberculosis Patients who are above 1 (one) year old visiting the selected health facilities during the study period.

5.4 Inclusion and Exclusion Criteria

5.4.1 Inclusion Criteria

- All smear negative presumptive pulmonary TB patients visiting selected health facilities during the study period whose age were greater than two years.

5.4.2 Exclusion criteria

- Those who were taking anti-TB drugs for greater than one week.
- Those patients incapable of producing sputum

5.5 Sample size & sampling technique

5.5.1 Sampling technique

The study was conducted in 16 governmental and private health facilities that provide TB diagnostic and treatment service in Addis Ababa. Simple random sampling method was used to select study health facilities. A list of all health facilities that provide TB services was prepared on excel sheet and rand command was used to select health facilities from the list. The number of study participants for each health facility was determined proportionally by using August to October 2016 report. All consecutive smear negative presumptive pulmonary tuberculosis patients visiting selected sites during the study period was included in the study until the allocated sample size for each site fulfills (Annex 1).

5.5.2 Sample size determination

Sample size calculation was performed for both objectives (prevalence of MTB and MDR TB) by using single population proportion formula with corrected. Then the larger sample size was taken to represent the other.

For prevalence of smear negative pulmonary tuberculosis:

n = N*(1-P)/ (d^2 /$z^2\alpha$ /2* (N-1) +P*(1-P) (we have used this formula because our population was less than 10, 000.

N=624, P= 50, d = 5% (N was taken from Addis Ababa region 2016 quarterly report

Where n = sample size,

Z = Z statistic for a level of confidence (95% level of confidence; z=1.96),

N = general population (there were 624 smear negative pulmonary patients)

P = expected prevalence or proportion

(*P*= 0.5 (50%) taken because there is no literature found that was done by Xpert MTB/RIF assay on the same day diagnosis strategy, and *d* = precision (in proportion of one; if 5%, *d* = 0.05).

n = N*(1-P)/ (d^2 /z^2 α /2* (N-1) +P*(1-P)

n= **240**, considering 10% non-response rate, that was 24 contingency, the total number of participants was **264.**

For prevalence of SNMDR TB cases:

$n = N*(1-P)/ (d^2 /z^2 \alpha /2* (N-1) +P*(1-P)$

N=624, P= 50, d = 5% (N was taken from Addis Ababa region 2016 quarterly report

Where n = sample size,

N = general population (there were 624 smear negative pulmonary patients)

Z = Z statistic for a level of confidence (95% level of confidence; z=1.96),

P = expected prevalence or proportion

(*P*= 0.03 (3%), taking maximum MDR TB prevalence (46), and

d = precision, 1%, d= 0.01), d has to be less than P by 25 %

$n = N*(1-P)/ (d^2 /z^2 \alpha /2* (N-1) +P*(1-P)$

$n = 624*(1-50)/(0.01)^2 / (1.96)^2 (624-1) +0.01(1-0.01)$

n= 375

Taking 10% nonresponse rate that was 37.5, the total sample size was = 412.5 =**413**

Therefore, the **413** was the total sample size for this study.

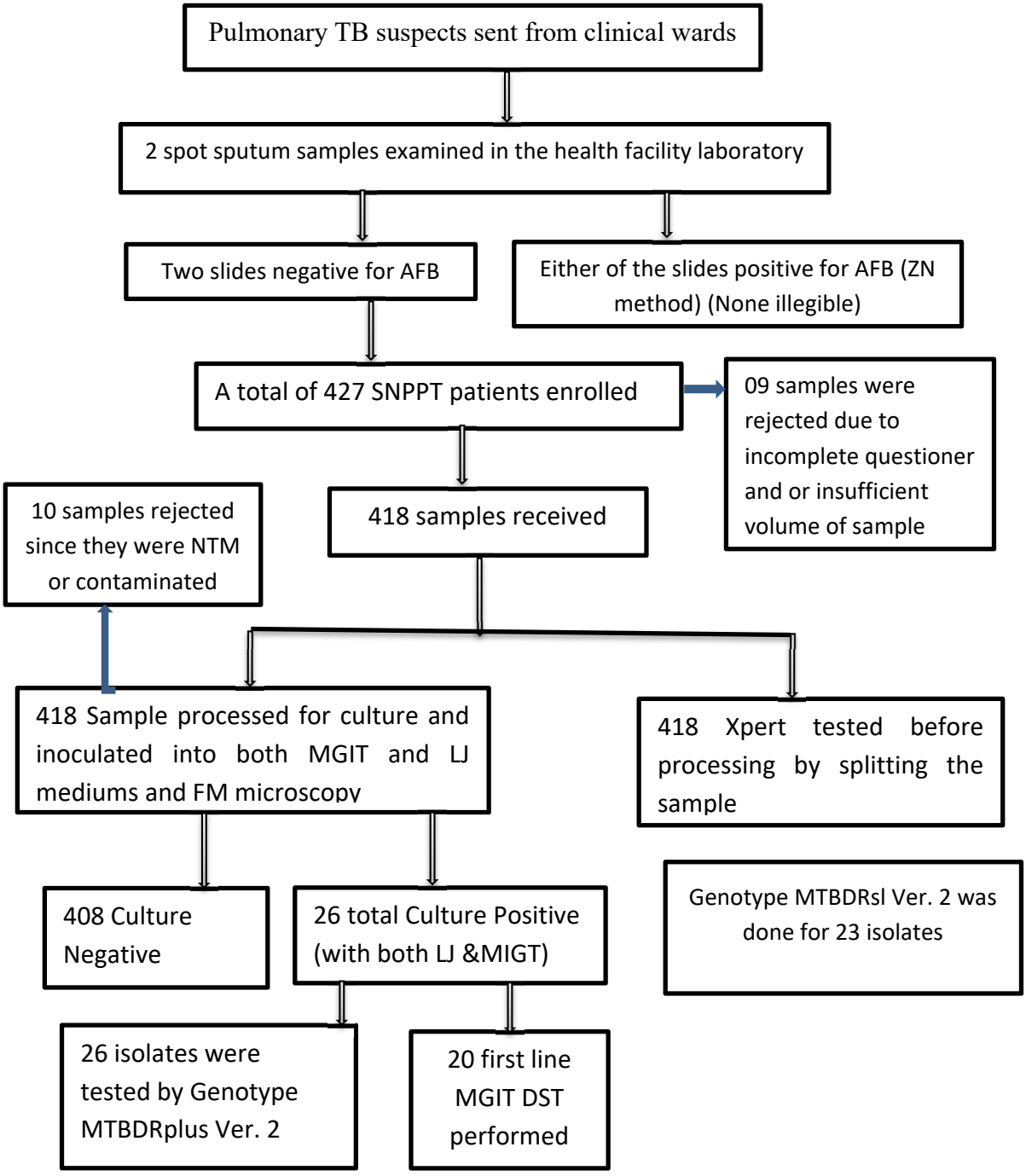
5.6 Data collection procedure:

Onsite training was given for all laboratory staffs, and clinicians about the project, for each respective study sites. Data was collected using structured questioner about socio-demographic information, medical status, clinical presentations and comorbidities, behavioral and other risk factors from SNPPT patients. Consent and or assent were asked from each participant before interview. Laboratory investigation data was also taken from study registration book, printouts, and culture work sheet.

5.6.1 Specimen collection

Two spot sputum specimens (in Spot-Spot sample collection strategy) were collected from, presumptive smear negative pulmonary tuberculosis patients and checked for AFB using ZN method in the health facilities. All patients who were smear negative was enrolled according to the inclusion criteria, and gave sputum sample with wide mouthed, clean, 50 ml capacity, sterile, disposable containers, polypropylene centrifuge tubes, translucent, screw capped container after detail patient instruction for sample collection at the health facility level. It was expected to collect sample with volume >= 3 ml; but those sample less than 3 ml was rejected, wrong sample containers, containers with leakage, breakage, and with incomplete questioner were rejected. Specimen was collected for the purpose of the study and from left over samples as well. Specimens collected was stored at 2-8^{o}c until transported. The specimen was transported using triple packaging, which was recommended for sputum sample transportation. Specimens arrived at the National Tuberculosis Reference Laboratory, was rechecked for fulfilling criteria and was stored again at 2-8^{o}c in the refrigerator if it was not processed immediately.

Figure 2: Flow chart of the study



NB: Any positive or Resistant strain found were reported to the physician for further patient management

5.6.2 Laboratory Analysis methods and procedures

All the laboratory investigations were done in the Ethiopian Public Health Institute, National Tuberculosis Reference Laboratory (NTRL). From the specimen collected, we performed AFB microscopy, Xpert MTB/RIF assay directly from the sample before processing for culture, Liquid culture (MGIT 960) and Solid culture (LJ media) for detection. For Drug Susceptibility Testing we used MTBDRplus ver. 2, MTBRDsl ver.2 and phenotypic MGIT DST and any result found positive was reported to the Physician requested the test for the benefit of the patient. All the laboratory analysis was done according to the National Tuberculosis Reference Laboratory SOPs (Annex 12-18). The NTRL is also star IV laboratory and ISO 15189 certified by Xpert MTB/RIF assay.

5.6.2.1 Acid-Fast Bacilli Microscopy (ZN Method) Preparation and Staining

The purpose of AFB microscopy is to detect acid-fast bacilli (AFB) by microscopic examination of clinical specimens and cultures. Both living and dead (viable and non-viable) bacilli can be stained and be counted. A semi-quantitative grading system was used to report the number of AFB observed in stained smears.

Principle: The property of acid-fastness is based on the presence of mycolic acids in the cell wall of mycobacterium. Primary stain (fuchsin) binds to cell-wall mycolic acids. Intense decolourization (strong acid or acid/alcohol) does not release the primary stain from the cell wall and the mycobacterium retain the red colour of Fuchsin – hence acid-fastness. Counterstaining (with methylene blue) provides a contrasting background (blue colour) (47); for detail procedure see Annex 12.

5.6.2.2 Light-Emitting Diode (LED) Fluorescence Microscopy

Light-emitting diodes (LEDs) provide a relatively inexpensive light source for fluorescence microscopy. LED microscopes or attachments require less power than conventional fluorescence microscopes and can run on batteries. Also, the bulbs have a long half-life and do not release potentially toxic products if they are broken. LED microscopy surpasses that of conventional Ziehl–Neelson microscopy by an average of 10% (17).

Principle: The property of acid-fastness of mycobacteria is based on the presence of mycolic acid in their cell wall. Primary stain (auramine) binds cell wall mycolic acids. Intense decolourization (strong acids, alcohol) does not release primary stain from the cell wall and AFB keep the fluorescent bright yellow color of auramine. Potassium permanganate is used to quench fluorescence in the background, but it provides little contrast for focusing, for this reason sometimes stains are preferred, of which Blue Ink may be the best (47); for detail procedure see Annex 13.

5.6.2.3 Xpert MTB/RIF Assay

The Xpert MTB/RIF assay is an automated, cartridge-based nucleic acid amplification test (NAAT) that uses the multi-disease GeneXpert platform. The Xpert MTB/RIF assay is performed directly on sputum, processed sputum sediment and selected extra pulmonary specimens from adults and children. The Xpert MTB/RIF assay simultaneously detects *M. tuberculosis* and rifampicin resistance in less than 2 hours, the sensitivity of the Xpert MTB/RIF assay for detecting TB is similar to that of liquid culture (sensitivity, 88% when compared with liquid culture as a reference standard); the specificity is also high (99%), for smear-negative culture-positive TB, the pooled sensitivity of Xpert MTB/RIF has been found to be 68% (48). The superior performance of Xpert MTB/RIF in detecting TB over that of microscopy makes it a particularly useful tool for case-finding among people living with HIV. As a tool for detecting rifampicin resistance, Xpert MTB/RIF has a sensitivity of 95% and specificity of 98% when compared with phenotypic reference standards, the biosafety precautions required for Xpert MTB/RIF are similar to those for smear microscopy, and the training is minimal, which allows the technology to be used at relatively low levels in a laboratory network (17).

Principle: The Xpert MTB/RIF assay allows for the rapid detection of *MTB* and Rifampicin (RIF) Resistance by combining automated extraction, amplification and detection on a single system. RIF is one of the first line anti-TB drugs and is also a surrogate marker for multi-drug resistant TB (MDR-TB). The assay amplifies a portion of the "rifampicin resistance determining region" of the *rpoB* gene, the most common site for RIF mutations, in real-time, using two sets of primers. Fluorescent probes are then used to differentiate between wild-type and mutant strains so that if one or more probes do not bind, this indicates the presence of a mutation and therefore RIF resistance. A sample processing control (SPC) consisting of spores from *Bacillus*

globigii, is included in the assay as an internal control to ensure adequate processing of the sample as well as to monitor the presence of PCR inhibitors. A probe check control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability (48, 49); for detail procedures see Annex 14.

5.6.2.4 Mycobacterial Culture:

Mycobacteria can be cultured in specific solid or liquid media. Bacterial growth can be identified visually or by automated detection of its metabolism. All positive mycobacterial cultures must be tested to confirm the identification of *M. tuberculosis* complex (MTBC). Conventional solid or liquid culture is required to monitor the treatment of patients with MDR-TB. Culture and identification of *M. tuberculosis* provide a definitive diagnosis of TB as well as significantly increasing in the number of cases identified when compared with microscopy, often an increase of 30–50%, provides the necessary isolates for conventional DST (17).

Sample Processing using 3 % NALC-NaOH-Na citrate Method: Processing sputum specimens has two objectives: decontamination of bacteria other than mycobacteria and liquefaction of mucous and organic debris in the specimen. Although there are several techniques available, none of them are ideal, meaning none of them will selectively destroy only contaminating flora and achieve complete liquefaction of the specimen. A reasonable compromise is to destroy as much of the contaminating bacteria as possible while harming as few mycobacteria as possible. All sputum specimens will be processed in this manner for preparation of AFB smears and liquid (MGIT) / solid (LJ) cultures.

Principle: N-acetyl-L-cysteine (NALC), a mucolytic agent, is used for rapid digestion, which enables the decontaminating agent, NaOH, to be used at lower final concentration (in sputum). NALC loses activity rapidly in solution, so it is made fresh daily. Sodium citrate exerts a stabilizing effect on the NALC by chelating heavy metal ions present in the specimen. The phosphate buffer neutralizes the NaOH and dilutes the homogenate to lessen the viscosity and specific gravity prior to centrifugation. Mycobacteria have a low specific gravity and may remain buoyant during centrifugation. A relative centrifugal force of 3,000 *x g* (not 3,000 rpm – the centrifuge must be calibrated) for 15 minutes is adequate to sediment mycobacteria. The rate at which mycobacteria sediment is critically dependent on time of centrifugation and

relative centrifugal force applied to the specimen. A longer centrifugation time can offset a lower relative centrifugal force, but increased centrifugation time increases the temperature of the specimen, which leads to additional killing of mycobacteria (hence, a refrigerated centrifuge will be used)(47); for detail procedure see Annex 15.

5.6.2.5 Drug Susceptibility Testing (DST):

In the study for those found to be positive for MTB, we have performed first line LPA (MTBDRplus Ver.2) and first line phenotypic (MGIT) DST then, for those found to be resistant for any of the first line anti-TB drugs we proceeded for 2nd line LPA.

5.6.2.5.1 First Line LPA (Genotype MTBDRplus Ver.2):

DNA Extraction: Extraction was performed by Genolyse DNA extraction method which is a chemical technique that provides a substantial decrease in time to prepare DNA from clinical samples or culture isolates. The time required to extract DNA is less than half that of the mechanical method. Extracted DNA was then amplified to facilitate detection of mycobacterial drug resistance. DNA extraction is a procedure whereby DNA is fetched from bacterial cells or fragments of bacterial cells to be used for molecular biology analysis. With the Genolyse chemical method test, this implies that: the bacterial cells in the decontaminated patient sample or culture samples are chemically broken to expose the DNA by using a lyses buffer); for detail procedure see Annex 16.

Reagent Preparation and Master Mix: The two ready-made reagents Amplification Mix A (AM-A), which contains PCR buffer, nucleotides, Taq polymerase and Amplification Mix B (AM-B), contains salts, specific primers, and dye were used. Reagent preparation and master mix rooms are different and unidirectional work flow was applied to prevent contamination. In the Addition room, in the PCR hood, after decontamination, cleaning and releasing UV for the appropriate time, 5µl DNA was added to the respective labeled PCR tubes containing 45 µl of prepared reagent. No DNA was added to the reagent control and assured that all PCR tubes were tightly closed); for detail procedure see Annex 16.

Amplification and Detection: Genotypic DST is used for rapid confirmation of drug resistance tuberculosis in suspected patients groups. MDR-TB diagnosis can be made in as short as 48

hours as compared to conventional DST which can take as much as 1-2 months. Genotypic DSTs were designed as alternative methods to improve the speed of diagnosis of drug resistant-TB, especially MDR/XDR-TB. Resistance to first line drugs develops through sequential accumulation of mutations in genes targeted by the respective drugs. Several genes were linked to resistance to TB drugs, the most known and used are *KatG* and promoter region of *InhA* for INH, *rpoB* for rifampicin resistance. Mutations in specific codons were identified and used for detecting resistance to specific drugs. Genotype MTBDRplus is based on the DNA-strip technology and consist of three steps: DNA extraction from cultures or clinical specimens, amplification of the target gene with biotinylated primers and a reverse hybridization.

All reagents needed for amplification are included in the master mix and are optimized for the PCR step of MTBDR*plus* test. The AM-A contains Taq polymerase, PCR buffer and nucleotides. The nucleotides acts as DNA precursors (the four deoxynucleoside triphosphates, dATP, dCTP, dGTP and dTTP) which will be used as building blocks during the elongation of the single stranded DNA. DNA polymerase (Hot Start *Taq*) is required to elongate the DNA molecule by facilitating the incorporation of the free nucleotides onto the end of the primer, according to the complementary base on the single stranded target DNA. The AM-B contains biotinylated primers for the amplification of specific regions of the mycobacterial chromosome. The Mg^{2+} in the salts forms soluble complexes with the free nucleotides allowing for the DNA polymerase to 24ecognize them as substrates during the amplification procedure. The membrane strips used in the hybridization or detection step are pre-coated with specific probes complementary to the amplified nucleic acids. After chemical denaturing, the single amplicons bind to the probes. Highly specific binding of complementary DNA strands is ensured by stringent conditions which result from the combination of buffer composition and a certain temperature. Thus, the probes reliably discriminates several sequence variations in the gene regions examined. The streptavidin-conjugated alkaline phosphatase binds to the amplicons' biotin via the streptavidin moiety. Finally, the alkaline phosphatase transforms an added substrate into a dye which becomes visible on the membrane strips as a coloured precipitate. For amplification and detection NTRL SOPs used; for detail procedure see annex 16.

5.6.2.5.2 First Line MIGT DST (Phenotypic DST):

Susceptibility testing in the MGIT 960 system is based on the same principles as isolation from sputum (detection of growth). DST is performed using an AST (antibiotic susceptibility testing) set, which consists of a Growth Control tube and one tube for each drug. A known concentration of drug is added to a MGIT tube, along with the specimen, and growth is compared with a drug-free control of the same specimen. If the drug is active against the mycobacterial isolate (isolate susceptible), growth will be inhibited and fluorescence will be suppressed in the drug-containing tube; meanwhile, the drug-free control will grow and show increasing fluorescence. If the isolate is resistant, growth and its corresponding increase in fluorescence will be evident in both the drug-containing and the drug-free tube. The MGIT 960 system monitors these growth patterns and can automatically interprets results as susceptible or resistant. An isolate is defined as resistant if 1% or more of the test population grows in the presence of the critical concentration of the drug; for detail procedure see Annex 18.

5.6.2.5.3 Second Line DST by Genotype MTBRDsl Ver.2

For second line LPA all the procedures starting from DNA extraction up to detection are the same with the first line LPA, what makes it different is the content of the reagents, probes, the target sequence which are going to be detected and the Interpretation of results. Target gene sequences are rpoB for rifampcin, gyrA and gyrB for floroquinones, rrs for injectables and eis for low level Kanamycin; for detail procedure see Annex 17.

5.7 Variables

5.7.1 Dependent variable

- ✓ Presence of M.TB and MDR TB Among TB suspected smear Negative patients

5.7.2 Independent variables

- ✓ Age
- ✓ Sex
- ✓ Marital status
- ✓ Occupation
- ✓ Family size
- ✓ Housing condition
- ✓ Educational level
- ✓ HIV status
- ✓ Known Chronic malignances
- ✓ Smoking
- ✓ Alcohol consumption
- ✓ TB Contact
- ✓ MDR Contact

5.8 Quality Assurance and Quality Control

Reagents, and culture medias prepared within the laboratory or purchased as readymade which have effect on the test result was checked for sterility and performance characteristics using well characterized control slide or strains such as; A positive and negative control smear slides was included in each batch of new lots of reagents prepared, 2% of a batch of 100 tubes of MGIT Culture media was tested with different dilutions of M. tuberculosis (H37Rv). Specimens was rejected if breakage or leakage specimen, incomplete patient information and mislabeling, delayed specimen more than five days after collection, inappropriate container, insufficient volume of sample (less than 3ml). Processing reagents (NaOH, Phosphate buffer) was sterilized before use. Each batch of samples that was processed in the laboratory used start and end control cultures. All laboratory investigations were done by experienced laboratory technologists in the National Tuberculosis Reference Laboratory in EPHI. Each of culture the procedure was also performed in a class II Bio-Safety Cabinet that have triple merit (keep the environment, keep the product and the personnel). Equipment preventive maintenance, temperature monitoring, monitoring of critical microbiological practices, and instrument function checkups was done. The laboratory performance is also continuously monitored by Proficiency Testing (PT) for all test methods. All laboratory analysis was done based on Ethiopian national TB, HIV and leprosy management guideline and WHO approved methods.

5.9 Data entry and analysis methods

The data collected from each questionnaire and the laboratory investigation was doubly entered and cleaned by using Epi -data statistical software version 3.0 and exported to IBM SPSS software version 20.0 for analysis. By using central measurement, dispersion and frequency distribution descriptive statistics was done to characterize the study participants and the result of the study was also presented by using tables and figures. The prevalence of TB was calculated with its 95% CI. Binary logistic regression also done to determine the association between dependent variables and possible risk factors. Variables which show significance at P-value of 0.2 during univariate analysis were selected for multivariable analysis. The strength of association was measured by using odds ratio and P-value of less than 0.05 was considered as statistically significant during multivariable analysis. Sensitivity, Specificity, Positive Predictive value (PPV), and Negative Predictive value (NPV) and was determined for Xpert MTB/RIF assay, taking culture as a gold standard.

5.10 Ethical Consideration

This project was reviewed and approved by the department of Medical Laboratory Sciences research and ethics review committee (DRERC), school of allied health sciences, College of Health Sciences; Addis Ababa University and by Institutional Review Board (IRB) of Ethiopian Public Health Institute. Sample was collected after receiving informed consent from each participant or the guardians. Patient confidentiality was kept and there was no any enforcement for those not willing to participate in the study. Each selected facility was asked for permission with legal letter to conduct this research. Unique patient identifier was used rather than patients name for data collections. The results of the laboratory investigations of each specimen were kept locked and reported to each clinician requested the test in each facility for treatment and further management of the patients.

5.11 Result Dissemination

Findings of this study will be distributed to Ethiopian Public Health Institute (EPHI), Addis Ababa University, college of health sciences, school of allied health sciences, department of medical laboratory science, and Addis Ababa city administration Health Bureau. In addition, it will be published in the international Journal

6. Results

6.1 Socio demographic characteristics of participants

418 patients' epidemiological and bacteriological data were analyzed. Majority of the patients were from adult out-patient-department (OPD) (Figure 3).

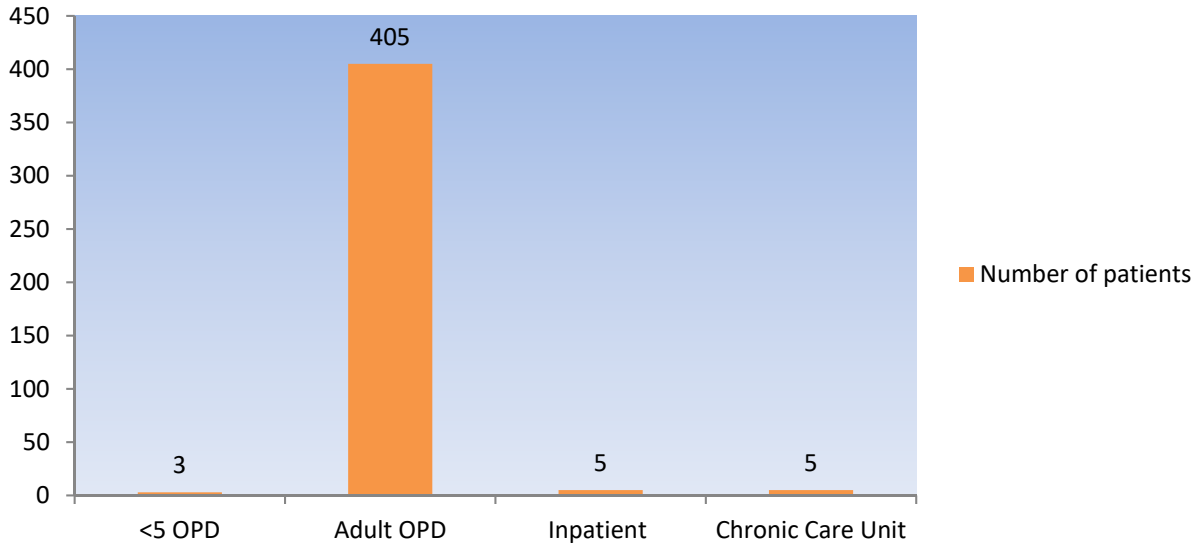


Figure 3: A bar graph showing study participant proportion by department

Majority (55.3%) patients were females. The mean age of participants was 35.68 (± 17.12) years. Most of the participants (29.8%) were in the age group 25-34 years, and children under 15 account for 4.8% of the participants. The predominant religion was Orthodox Christians (77.3%), followed by Muslims (13.6%). Nearly 55% of the participants' educational status was below grade 7. The participants had different occupation; the most frequent was house wife (21.1%) and the second frequent work was daily laborer (18.4%). Two hundred eighteen (52.3%) participants were married whereas 159 (38.1%) were single (Table 1)

Table 1: Socio demographic characteristics of study participants in Addis Ababa, August 01-December 30, 2017

	Variables	Frequency	Percent (%)
Sex	Male	187	44.7
	Female	231	55.3

	Total	**418**	**100**
Age Group	0-14	20	4.8
	15-24	94	22.4
	25-34	125	29.8
	35-44	61	14.6
	45-54	53	12.6
	55-64	27	6.4
	>65	38	9.3
	Total	418	100
Religion	Orthodox	323	77.3
	Muslim	57	13.6
	Protestant	34	8.1
	Catholic	2	0.5
	Other	2	0.5
	Total	**418**	**100**
Level of Education	None(No Formal Education)	112	26.9
	Standard grade 1-7	116	27.9
	Standard grade 8-10	88	21.2
	Standard grade 11-12	53	12.7
	Diploma(level 1-4 Certificate)	26	6.3
	First Degree	21	5.0
	Second Degree & above	0.0	0.0
	Total	**416**	**100**
Work status	Farmer	31	7.5
	Daily Laborer	76	18.4
	House wife	87	21.1
	Driver	10	2.4
	Teacher	7	1.7
	Student	46	11.2
	Merchant	14	3.4
	Health Care Worker	3	0.7
	Government employee	35	8.5
	Self-employed	60	14.6
	Other	43	10.4
	Total	**412**	**100**
Marital Status	Single	159	38.1
	Married	218	52.3
	Divorced	22	5.3
	Widowed	16	3.8
	Separated	2	0.5
	Total	**417**	**100**

6.2 Clinical Presentation, Comorbidities and Associated risk factors

Signs and symptoms, comorbidity and other risk factors were assessed in the study. Eighty eight percent (367/417) of the participants had cough as a cardinal chief compliant, and 112 (30.5%) of them with productive cough. HIV status was known only for 211 patients. HIV positivity rate was 27%. Fourteen participants were known to have diabetes mellitus (DM). Thirty five patients had either TB or MDR-TB contacts (8.4%) (Figure 4-5 & Table 2).

Figure 4: Clinical presentations among study participants

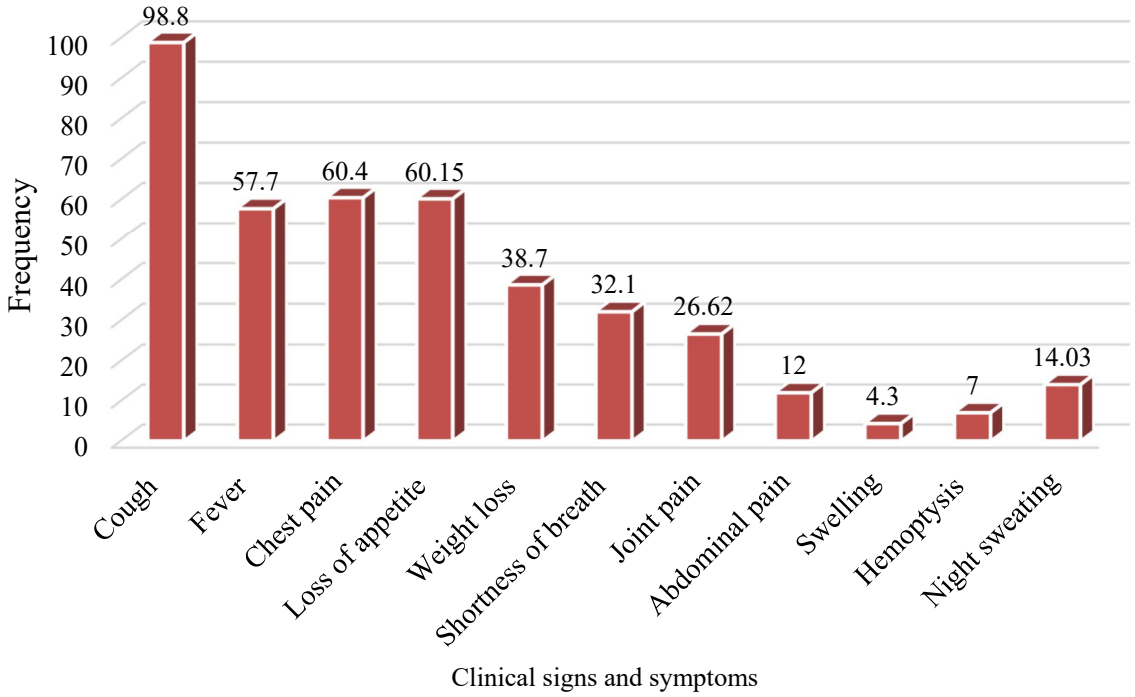
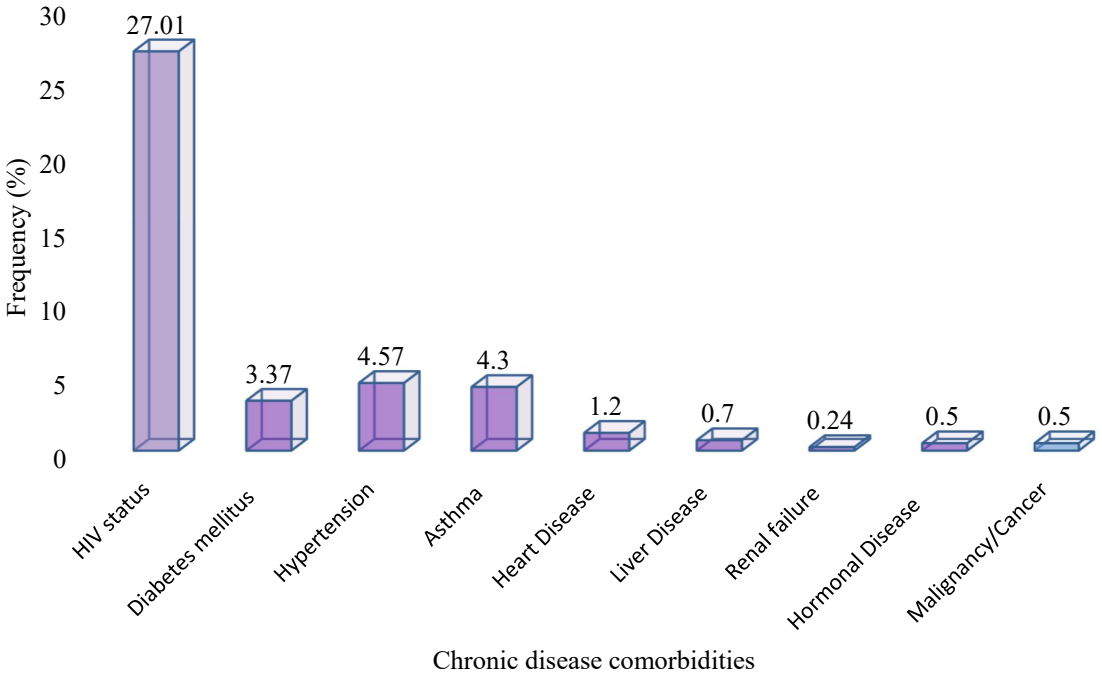


Table 2: Behavioral and other risk factors

Risk factors	Frequency	Percent (%)
Miner	11	2.7
Prisoner/Prisoner staff	2	0.5
Migrant	11	2.7
TB/MDR TB contact	35	8.45
Alcohol Consumption	122	29.3
Smokers	26	6.3
Chewing Khat	57	13.7

Figure 5: A bar graph demonstrating frequency of chronic comorbidities among study participants



6.3 Prevalence of Smear Negative Pulmonary Tuberculosis (SNPT)

SNPT prevalence among presumed smear negative PTB patients was 6.5% (95 % C.I, 4.103-8.816). Twenty six patients were smear negative and culture confirmed / by either of the culture techniques (LJ and MGIT), SNPT cases (Table 3).

Table 3: Detection of TB using different diagnostic tools in presumptive smear negative pulmonary TB patients from August 01 - December 30, 2017.

Diagnostic Method	Negative Result No. (%)	Positive Result No. (%)	Total No. (%)	Contaminated (%)	NTM (%)
Xpert MTB/RIF assay	391 (93.5)	27 (6.5)	418 (100)	NA	NA
MGIT culture result	354 (92.65)	24 (6.35)	378 (100)	34 (8.25)	06
LJ culture result	376 (94.47)	22 (5.53)	398 (100)	18 (4.3)	01
LJ and MGIT culture result	382 (93.6)	26 (6.4)	408 (100)	03 (0.73)	07

More TB cases were detected in male than female patients. The positivity rate among male and female was 10.2% and 3.5%, respectively. There was significant difference in TB prevalence among male and female (*p=0.005*). High rate of TB detection was observed in the age range 15-24 years (8.5%) and 25-34 years (8.9%) using Xpert MTB/RIF assay. About 20 children (<15 years age) were in the study, but none of them had laboratory confirmed TB. The study has included all age groups above two years old, but all positive SNPT cases were detected from the age groups between 15-64 years which is the economically productive age group. SNPT prevalence was 6.2% among Orthodox Christians, 8.8% and 5.9% among Muslims and Protestants respectively. Majority (80.77 %) of the total SNPT cases were found to be positive in those who were below grade 12, but it was not statistically significant (*P=0.496*). From the total of 411 responded participants, the most common work status (21.1%) was house wife, however SNPT prevalence was low (1.2%). The second frequent work (18.4 %) was daily laborer with 7.9% SNPT prevalence, and the least frequent (three) was health worker, with higher SNPT prevalence (33.3%) and there was association *(P<0.006)*. The odds of having SNPT was 68.7 times higher in health care workers as compared to others, (AOR 68.7; 95 % C.I, 2.447-1929.15, *p= 0.013*). From 417 participants interviewed, 218 (52.3%) were married, from which 11 (40.74%) was TB positive, 159 (38.1%) were single that most of MTB positive cases found, 14 (51.9%) (*p=0.460*) (Table 4).

Table 4: Socio demographic characteristics of study participants and level of Association (Chi square)

	Variables	Xpert Positive (N=27)	Xpert Negative (N=391)	P-value
Sex	Male	19	167	0.005
	Female	8	223	
	Total	**27**	**390**	
Age Group	0-14	0.0	20	0.237
	15-24	8	86	
	25-34	11	113	
	35-44	05	56	
	45-54	01	52	
	55-64	02	25	
	>65	0.0	38	
	Total	**27**	**390**	
	Orthodox	20	303	0.935

Religion	Muslim	5	52	
	Protestant	2	31	
	Catholic	0.0	02	
	Other	0.0	02	
	Total	**27**	**390**	
Level of Education	None(No Formal Education)	06	106	0.496
	Standard grade 1-7	04	112	
	Standard grade 8-10	06	82	
	Standard grade 11-12	05	48	
	Diploma(level 1-4 Certificate)	03	23	
	First Degree	02	18	
	Second Degree & above	0.0	0.0	
	Total	**26**	**389**	
Work status	Farmer	01	30	0.006
	Daily Laborer	06	70	
	House wife	01	86	
	Driver	0.0	10	
	Teacher	0.0	07	
	Student	0.0	46	
	Merchant	0.0	14	
	Health Care Worker	01	02	
	Government employee	02	32	
	Self-employed	07	53	
	Other	07	36	
	Total	**25**	**386**	
Marital Status	Single	14	145	0.460
	Married	11	206	
	Divorced	02	20	
	Widowed	0.0	16	
	Separated	0.0	02	
	Total	**27**	**389**	

Of 417 participants, 345 (82.73%) were new cases and 23 (6.67%) had confirmed TB. On the other hand, 72 (17.27%) participants were retreatment cases and 4 (5.56%) were positive for TB. There was significant difference in TB detection among new and retreatment cases ($p<0.001$).

Among clinical signs and symptoms this study has investigated; weight loss ($p<0.001$), shortness of breath ($p=0.034$) and loss of appetite ($p=0.023$), having association with the case in univariate analysis. In the multivariate analysis these symptoms are not statistical significant; loss of appetite (AOR 0.778; 95 % C.I, 0.248-2.438, P=0.666), weight loss (AOR 0.340; 95 % C.I, 0.108-1.069, p=0.065) and shortness of breath (AOR 0.493; 95 % C.I, (0.181-0.1.343), p=0.167).

Known chronic malignancies and comorbidities were not significantly associated with SNPT depending on these study findings. HIV-TB co-infection was 5.3%. From behavioral and other risk factors that showed association in the univariate model, only being migrant were statistically significant factor for the case (AOR 0.121; 95 % C.I, (0.20-0.730) p=0.021) (Table 6).

Table 5: Clinical presentation, comorbidities and associated risk factors for SNPT & SNMDR TB

Variables	Yes/No	Xpert Negative frequency (%)	Xpert Positive frequency (%)	P-value
Signs and symptoms				
Cough	Yes	385 (93.4)	27 (6.6)	0.554
	No	05 (6.4)	0 (0.0)	
Fever	Yes	236 (92.5)	19 (7.5)	0.317
	No	153 (95 %)	8 (5.0)	
Chest pain	Yes	232 (92.1)	20 (7.9)	0.134
	No	158 (95.8%)	07 (4.2)	
Loss of appetite	Yes	222 (91.4)	21 (8.6)	0.034
	No	168 (96.6%)	06 (3.4)	
Weight loss	Yes	141 (87.6)	20 (12.4)	< 0.001
	No	248 (97.3 %)	07 (2.7)	
Shortness of breath	Yes	120 (89.6)	14 (10.4)	0.023
	No	270 (95.4%)	13 (4.6)	
Joint pain	Yes	102 (91.9)	09 (8.1)	0.414
	No	288 (94.1)	18 (5.9)	
Abdominal pain	Yes	48 (96.0)	02 (4)	0.448
	No	342 (93.2)	25 (6.8)	
Swelling	Yes	17 (94.4)	01 (5.6)	0.871
	No	373 (93.5)	26 (6.5)	
Hemoptysis	Yes	25 (86.2)	04 (13.8)	0.098
	No	364 (94.1)	23 (5.9)	
Night sweating	Yes	59 (95.2)	03 (4.8)	0.847
	No	356 (85.2)	24 (5.74)	
Known comorbidities				
HIV status	Positive	54 (94.7)	03 (5.3)	
	Negative	154 (91.7)	14 (8.3)	
	Unknown	197 (95.2)	10 (4.8)	
Diabetes mellitus	Yes	13 (92.9)	01 (7.1)	0.92
	No	376 (93.5)	26 (6.5)	
Hypertension	Yes	19 (100)	0(0.00)	0.24
	No	370 (93.2)	27 (6.8)	
Asthma	Yes	18 (100)	0(0.00)	0.253
	No	371 (93.2)	27 (6.8)	
Heart Disease	Yes	05 (100)	0(0.00)	0.553

	No	384 (93.4)	27 (6.6)	
Liver Disease	Yes	03 (100)	0(0.00)	0.647
	No	386 (93.5)	27 (6.5)	
Renal failure	Yes	01(100)	0(0.00)	0.792
	No	388 (93.5)	27 (6.5)	
Hormonal Disease	Yes	02 (100)	0(0.00)	0.709
	No	387 (93.5)	27 (6.5)	
Malignancy/Cancer	Yes	02 (100)	0(0.00)	0.709
	No	387 (93.5)	27 (6.5)	
Other risk factors				
Miner	Yes	04 (100)	0(0.00)	0.596
	No	383 (93.4)	27 (6.6)	
Ex-miner	Yes	07 (100)	0(0.00)	0.481
	No	380 (93.4)	27(6.6)	
Current smoker	Yes	10 (83.3)	02 (16.7	0.149
	No	377 (93.8)	25 (6.2)	
Ex-smoker	Yes	10 (90.9)	01 (9.1)	0.727
	No	377 (93.5)	26 (6.5)	
Prisoner/Prison staff	Yes	02 (100)	0(0.00)	0.708
	No	385(93.4)	27 (6.6)	
Migrant	Yes	8 (72.7)	03 (27.3)	0.005
	No	379 (94.0)	24 (6.0)	
Contact with TB patient	Yes	25 (92.6)	02 (7.4)	0.847
	No	362 (93.5)	25 (6.5)	
MDR-TB contact	Yes	07 (87.5)	01 (12.5)	0.489
	No	380 (93.6)	26 (6.4)	
Alcohol consumption	Yes, currently	59 (86.8)	09 (13.3)	0.042
	Yes, previously	52 (96.3)	02 (3.7)	
	Not at all	279 (94.6)	16 (5.4)	
Smokers	Yes, currently	11 (84.6)	02 (15.4)	0.174
	Yes, previously	11 (84.6)	02 (15.4)	
	Not at all	361 (94.0)	23 (6.0)	
Chewing Khat	Yes, currently	22 (88.0)	3 (12.0)	0.038
	Yes, previously	27 (84.4)	05 (15.6)	
	Not at all	341 (94.7)	19 (5.3)	

Table 6: Showing adjusted odds ratio of those socio demographic factors, clinical presentation and risk factors having association in the univariate analysis.

Variables		Xpert Results		Adjusted Odds Ratio	*P-value*
		Positive N (%)	Negative N (%)	AOR (95% C.I)	
Occupation	Farmer	01 (3.2)	30 (96.8)	1	
	Daily Laborer	06 (7.9)	70 (92.1)	2.407 (0.255-22.723)	0.443
	House wife	01 (1.1)	86 (98.9)	0.635 (0.033-12.243)	0.763
	Driver	0 (0.00)	10 (100)	0 (0.00)	
	Teacher	0 (0.00)	07 (100)	0 (0.00)	
	Student	0 (0.00)	46 (100)	0 (0.00)	
	Merchant	0 (0.00)	14 (100)	0 (0.00)	
	Health Care Worker	01 (33.3)	2 (66.7)	68.7 (2.447-1929.15)	0.013
	Government employee	02 (5.9)	32 (94.1)	2.38 (0.188-30.159)	0.503
	Self-employed	07 (11.7)	53 (88.3)	3.031 (0.318-28.886)	0.335
	Other	07 (16.3)	36 (83.7)	6.311 (0.662-60.122)	0.109
Sex	Male	19 (10.2)	167 (89.8)	1	
	Female	8 (3.5)	223 (96.5)	0.321 (0.097-1.062)	0.063
Loss of Appetite	Yes	21 (8.6)	222 (91.4)	1	
	No	6 (3.4)	168 (96.6)	0.778 (0.248-2.438)	0.666
Weight Loss	Yes	20 (12.4)	141 (87.6)	1	
	No	07 (2.7)	248 (97.3)	0.340 (0.108-1.069)	0.065
Shortness of Breath	Yes	14 (10.4)	120 (89.6)	1	
	No	13 (4.6)	270 (95.4)	0.493 (0.181-0.1.343)	0.167
Migrant	Yes	03 (27.3)	08 (72.7)	1	
	No	24 (06)	379 (94)	0.121 (0.20-0.730)	0.021
Alcohol Consumption	Yes, currently	09 (13.2)	59 (86.8)	1	
	Yes, previously	02 (13.7)	52 (96.3)	0.254 (0.046-1.399)	0.116
	Not at all	16 (5.4)	279 (94.6)	0.956 (0.311-2.939)	0.937
Chewing Khat	Yes, currently	03 (12)	22 (88)	1	
	Yes, previously	05 (15.6)	27 (84.4)	1.324 (0.221-7.922)	0.759
	Not at all	19 (5.3)	341 (94.7)	0.570 (0.127-2.565)	0.464

6.4 Prevalence of Smear Negative Multidrug Resistant Tuberculosis

A total of 26 TB cases were confirmed by culture. All clinical isolates were tested by using Genotype MTBDRplus Ver. 2, first- line MGIT DST and Genotype MTBDRsl Ver.2. Among these isolates, 3 were MDR-TB cases (resistant to both isoniazid and rifampicin) and 7 were resistant to isoniazid. All rifampicin resistant (RR) cases by GeneXpert were found to be MDR-TB cases by Genotyping and MGIT DST methods. Twenty three cases were isolated from new TB patients and two cases were rifampicin resistant. The remaining three cases were from previously treated patients and one was rifampicin resistant case. From 23 new SNPT cases, 06 (26.08%) were resistant for isoniazid. From 03 retreatment cases, 01 (33.33%) was resistant for isoniazid (p=0.79). All of SNMDR TB 03/26 were men (p=0.264) and in the age group of 15-34 years old (p=0.814). Drug susceptibility testing was performed for 23 (88.46%) of the isolates for second line anti-TB drugs using line probe assay (Genotype MTBDRsl Ver.2), to check for any resistance but all isolates were susceptible (Table 7).

Table 7: Genotypic and phenotypic drug susceptibility testing pattern of SNPT isolates

Drug susceptibility testing	Drugs		Status (Resistant/Sensitive)	
Genotype MTBDRplus Ver. 2			**Resistant No. (%)**	**Sensitive No. (%)**
	Rif		03 (11.54)	23 (88.46)
	INH		07 (26.9)	19 (73.1)
	MDR TB		03 (11.54)	23 (88.46)
	Drugs	**Final drug concentration tested**	**Drug status (Sensitive/ Resistant)**	
			Resistant No. (%)	**Sensitive No. (%)**
First line MGIT DST	STR	1.0 µg/ml	03 (15%)	17 (85%)
	INH	0.1 µg/ml	03 (15 %)	17 (85)
	RIF	1.0 µg/ml	02 (10%)	18 (90 %)
	ETM	5.0 µg/ml	01 (5%)	19 (95%)
	MDR TB		02 (10%)	18 (90)

First Line MGIT DST; the reason for variation with LPA is that 06 isolates from which 1 MDR Isolate, 03 were INH resistant by LPA was not finalized due to time shortage, still under process.

6.5 The sputum quality and Xpert MTB/RIF assay result (Sputum from SNPPTPs)

Saliva was the leading sample quality (35.3%) with the positivity rate 6.1%. Mucoid was the second most type of sputum (34.1%) with the positivity rate 4.2%. However, the positivity rate was high (33.3%) among the blood stained type of sputum samples. There was no TB case detected in muco purulent and bloody sputum samples. The univariate analysis revealed sample quality has significant association with GeneXpert positivity (*p=0.028*). In 95 % C.I, blood stained samples showed 7.67 ((OR 7.667; 1.642- 35.8), *p=0.002941*) times more positivity against saliva (Table 8).

Table 8: Positivity of Xpert MTB/ RIF assay in different sputum sample qualities

Variables		**Xpert MTB/RIF Result**		***P-value***	**Odds Ratio in 95 % CI.**	
		Negative no. (%)	**Positive no. (%)**		**OR**	***P - value***
Sputum Quality	Purulent	106 (92.2)	09 (7.8)	0.028	1.302 (0.4995, 3.393[1])	0.5885
	Mucoid	137 (95.8)	06 (4.2)		0.6715 (0.2327, 1.93)	0.4589
	Muco-purulent	02 (100)	0(0.00)		------	
	Saliva	**138 (93.9)**	**09 (6.1)**		**REF**	
	Blood stained	06 (66.7)	03 (33.3)		7.667 (1.642, 35.8[1])	0.002941
	Bloody	01 (100)	0(0.00)		-----	
	Total	417 (100)			------	

6.6 Performance characteristics of Xpert MTB/RIF assay, for the diagnosis of SNPT in comparison with culture and RR TB compared to Genotype MTBDRplus Ver. 2

Depending our data collected, 01 sample was LJ positive while it was MGIT negative, on the other hand 04 samples were positive by MGIT but 02 was negative and 02 contaminated by LJ. In addition to this, 02 samples which were negative by Xpert MTB/ RIF assay; 01 negative and 01 positive by MGIT, but both positive by LJ method. Moreover, 03 samples which were Xpert MTB/RIF positive, samples were culture negative. This might be due to cross contamination during GeneXpert processing or harsh decontamination during culture processing. Based on the above findings performance characteristic of Xpert MTB/ RIF was determined by taking culture

(LJ +MGIT) as a gold standard. Therefore, the diagnostic Sensitivity (True positive rate), specificity (true negative rate), positive and negative predictive values of GeneXpert was 92.86% (95 % C.I: 83.32-100%), 99.74%, (95 % C.I: 99.24-100%), 96.29% (95 % C.I: 89.17-100%) and 99.49% (95 % C.I: 98.78-100%) relative to culture. The overall agreement was 99.28% (95 % C.I: 98.48-100%) (Table 9&10). All RR cases by Genotype MTBDR plus Ver. 2 were detected with Xpert MTB/RIF assay.

Table 9: Two by two table Xpert Versus TB culture

Diagnostic methods		**Culture result (LJ +MGIT)**		**Total**
		Positive	Negative	
Xpert MTB/RIF	Positive	26	01	27
	Negative	02	390	392
	Total	28	391	419

Table 10: Xpert MTB/ RIF assay performance characteristics

Variables	Formula	Xpert Performance Characteristics	95 % C.I
Sensitivity	100 x [TP/ (TP+FN)	26/(26+02)*100= 92.86%	83.32-100%
Specificity	100 x TN/(FP+TN)	390/(01+390)*100= 99.74%	99.24-100%
PPV	100 x TP/ (TP+FP)	26/(26+01)*100= 96.29%	89.17-100%
NPV	100 x TN/ (TN+FN)	390/(390+02)*100= 99.49%	98.78-100%
Overall agreement	100 x (TP +TN)/ (TN + TP + FN + FP)	(26+390)/(26+02+01+390)* 100= 99.28	98.48-100%

7. Discussion

According to WHO, more than 9 million people who become ill with TB each year; more than 3 million are not diagnosed, treated, or officially registered by national TB programmes and also emergence of MDR TB makes the situation worse. Collectively, these "missed" millions are a global public health failure (16). Similarly, diagnosis of MTB in developing countries largely depends on smear microscopy which has poor sensitivity (37), resulting in increased smear negative pulmonary tuberculosis (SNPT) that remains undiagnosed, or delay in diagnosis and treatment. SNPT represents 30–60% of all pulmonary TB cases. The mortality of these patients can reach 25% in populations with high prevalence of HIV infection, which may be largely related to delay in diagnosis. Patients with SNPT were found to be less infectious and to have a lower mortality but a significant proportion (i.e. 50-71%) progressed to active disease justifying treatment. Left undiagnosed and untreated a person can infect as many as 15-20 individuals per year (20-25). SNPT and SNMDR TB prevalence in Addis Ababa is not well known. Therefore, determining the magnitude and distribution of SNPT and smear negative MDR TB in different settings can contribute in the national efforts for fighting TB.

Our study findings showed that the prevalence of smear negative pulmonary tuberculosis among presumptive smear negative pulmonary tuberculosis patients was 27/418 (6.5%) (95 % C.I, 4.103-8.816), which was Xpert MTB/ RIF confirmed and 26/408 (6.4 %) %) (95 % C.I, 4.003-8.743), by a collaborative diagnostic performance of BACTEC MGIT 960 and LJ culture. The difference between GeneXpert and culture results might be due to cross contamination during Xpert MTB/RIF sample processing or harsh decontamination during culture processing. From the total, majority (55 %) of the study participants were female, but more than two times SNPT (10.2 %) were detected from men (p=0.005) which is in line with the fact that TB is more common in men than women (1). The present study revealed higher smear negative PTB prevalence than a report from Vietnam which was 5.6% (smear negative culture positive PTB) (50). Our study reveals lower prevalence, as compared to a finding by a study conducted in 8 prisons in Amhara region, Ethiopia in the year 2013 that the overall prevalence of culture and/or Xpert test positive smear-negative pulmonary TB cases in the study was 8% (51). Another studies in Karamara hospital, Jigjiga, Ethiopia, conducted from December 2013 to May 2014, shown 7.9% SNPT prevalence; this result was the combined result of Xpert MTB/ RIF and

culture-positive (52). A study conducted in Adama, Ethiopia in 2013, also showed a higher SNPT prevalence that was 12.1% (34) than our study results and this study is similar with our study finding in that men were more affected by SNPT than females. Other study conducted in St. Peter TB specialized hospital Addis Ababa, Ethiopia in the year between November 2004 and October 2005, confirmed a higher prevalence that is 12.46% pulmonary TB isolated from smear negative pulmonary patients (36) than ours. These differences may be due to different reasons like geographical variation, change in sample collection strategy, difference in time that the study conducted, change in TB diagnostic and treatment algorithm and diagnostic method differences. Another justification for these differences might be, our study tried to cover a wide range of facilities including private health institutions. Although the study included all age groups, above two years, all (100%) of the SNPT were detected in the age group 15-64 years' participants which are the economically productive age group of the population. This finding is in line with WHO estimation, that states TB affects mostly adults in the economically productive age groups; around two-thirds of cases are estimated to occur among people aged 15–59 years (1)

The second most important finding of this study is the prevalence of smear negative multidrug resistant TB. According to the findings from the 26 isolates from smear negative presumptive pulmonary TB patients 03 (11.54 %) were MDR TB. From these SNMDR TB isolates 02/23 (8.7 %) were among new cases and 01/03 (33.3%) was from retreatment presumptive SNPT cases. Our study showed higher SNMDR TB prevalence in comparison with a study in Iran among tuberculosis patients in between the year 2011-2013, which was 4.3% (2.38% in the new TB treatment group and 23.1% in the retreatment groups) (53). The present study demonstrated low MDR TB prevalence as compared to a finding by a study in Belarus which was 47% SN MDR-TB: 33% among new and 73% previously treated patients (33). But, our study showed high prevalence of SN MDR TB in comparison with a study in India that was conducted with newly diagnosed sputum-positive pulmonary tuberculosis between February 2008 and December 2009 demonstrated very low prevalence that 1.13% MDR-TB (54). No Smear negative MDR TB was detected in a study conducted in St. Peter hospital, Addis Ababa, Ethiopia (36). In our study among 27 Xpert MTB/ RIF confirmed MTBC cases 03 (11.11%) were Rifampicin resistant (RR) cases which is a bit higher when compared to a study conducted in Karamara hospital, Jigjiga,

Ethiopia which was 3/38 (7.9%) RR strains were identified from smear-negative culture-positive samples (52). These variations of prevalence of SN MDR TB, and RR TB from our study result may be due to geographical location, population difference, time, study settings and method variation. Using Genotype MTBDRplus ver.2, Isoniazid (INH) mono resistance was 07/26 (26.9%). All RR SNPT isolates that Xpert MTB/ RIF assay diagnosed as RR were found to be MDR TB from which 33.3% had MDR TB patient contact history. Drug susceptibility testing was performed for 23 (88.46%) of the isolates for second line anti-TB drugs using Genotype MTBDRsl ver.2, to check for any resistance, but all isolates were susceptible. This study is different from other study conducted in Kenya, which showed resistance to first line and second line anti-TB drugs, but no MDR or XDR TB was detected (55).

Clinical features are significant in supporting the diagnosis of smear-negative TB even though it has poor sensitivity and specificity (37). Our study demonstrates that smear-negative TB inmates showed different clinical presentations, such as cough, fever, and chest pain, loss of appetite, weight loss, and shortness of breath, joint pain, abdominal pain, hemoptysis, and night sweating. Furthermore, cough, fever, weight loss, chest pain and night sweating are also symptoms in smear-positive cases, as it was reported in previous studies (51). Shortness of breath, (AOR 0.493; 95 % C.I, 0.181-0.1.343, p=0.167), weight loss (AOR 0.340; 95 % C.I, 0.108-1.069, p=0.065) and loss of appetite (AOR 0.778; 95 % C.I, 0.248-2.438, p=0.666) are significant clinical features of SNPT cases in our study in the univariate analysis, but doesn't not have association in the multivariate model. However, another study reported that smear-negative patients were less probable to suffer from fever and weight loss than their smear-positive counterparts (56).

HIV comorbidity is a known confounder factor for the diagnosis of TB (17). Our finding demonstrated 27% HIV positivity rate and 5.3 % SNPT- HIV co-infection; but it doesn't show a significant association and the comorbidity is much lower than the finding reported in Brazil which was 41% comorbidity (19). Our finding also showed that 7.1% SNPT among known diabetes mellitus patients and this finding is a bit higher compared to a study finding from St. Peter Hospital Addis Ababa, Ethiopia which was 6.7 % (57). This difference may be due to difference in study participants (they have done it on active TB patients), geographical coverage and time.

From all demographic, behavioral and other risk factors that the study has assessed, sex (p=005), work status or occupation (p=0.006), being a migrant (p=0.005), alcohol consumption (p=0.042), chewing Khat (p=0.038), has association with the case in a univariable analysis; a study conducted in Ethiopian prisons has showed alcohol consumption as a risk factor but chewing Khat was not associated with active TB disease (58). From the multivariable analysis performed; the odds of having SNPT, is 68.7 times higher in health care workers as compared to others, (AOR 68.7; 95% C.I, 2.447-1929.15, p=0.013). None migrants have 0.121 times less likely to have smear negative pulmonary TB in comparison with migrants, (AOR 0.121; 95 % C.I, 0.20-0.730, p= 0.021). Further and detail researches should be conducted concerning underlying factors for smear negative, Xpert/ MTB RIF and / culture positive pulmonary cases.

Our findings demonstrated the need to exercise caution in collecting sputum for Xpert MTB/RIF and in interpreting results because sputum quality may affect test yield and sensitivity. A study in Uganda has showed similar findings. Saliva sample was the most common sample submitted to our study and also to Uganda's. But in our study blood stained sample showed the highest sensitivity and purulent sample the second, but saliva revealed the most sensitive and yield sample for Uganda's study. In addition to this, they demonstrated blood stained sample as the lowest sensitivity (59).

Performance characteristics of Xpert MTB/ RIF assay was determined by taking culture as a gold standard. Therefore, the diagnostic Sensitivity (True positive rate), specificity (true negative rate), positive and negative predictive values of GeneXpert was 92.86% (95 % C.I: 83.32-100%), 99.74%, (95 % C.I: 99.24-100%), 96.29% (95 % C.I: 89.17-100%) and 99.49% (95 % C.I: 98.78-100%) relative to culture. The overall agreement was 99.28% (95 % C.I: 98.48-100%). Xpert MTB/RIF has detected all rifampicin resistant TB as compared to Genotype MTBDRplus Ver. 2. A study in Peru, has shown a better diagnostic sensitivity which was 97.8% (95% CI 88.4–99.6) (44/45), less specificity (97.7% (95% CI 91.9–99.4)), positive predictive value (95.7% (95% CI 85.5–98.8)) and the negative predictive value (98.8% (95% CI 93.6–99.8)), but it was performed among TB suspected HIV positive cases (40). The present study has shown better sensitivity, specificity, PPV and NPV of Xpert MTB/RIF assay as compared with a study in Thailand which was sensitivity of 83.9% (95% CI: 66.3-94.5) and a specificity of 92.1% (95% CI: 83.6-97), PPV of 81.3% (95% CI: 63.6-92.8) and NPV of 93.3% (95% CI: 85.1-97.8) (60).

Our study demonstrated almost two times higher sensitivity in comparison with a study in Jigjiga, Ethiopia which was 48.6 % (95 % CI: 32.5–64.8 %) for detection of smear-negative culture positive TB (52).

8. Strength of the study

In this study almost all methods available in the country were used to diagnose SNPT. Contamination rate was within the expected range and laboratory quality controls were performed and all found acceptable. Multiple DOTs sites in Addis Ababa including both from the private and governmental health facilities were considered in the study. I addition to all the above, this study is the first to determine smear negative TB and MDR TB prevalence and associated risk factors in the same day TB diagnosis strategy among presumptive smear negative PTB patients in Ababa, Ethiopia as far as our knowledge concerned.

9. Limitations of the study

- It was difficult to get sputum sample from children especially in under 5 years old
- Phenotypic DST not done for PZA and for 6 isolates for SIRE drugs due to shortage of PZA set and time

10. Conclusion

Based on the findings overall prevalence of smear negative pulmonary tuberculosis in Addis Ababa was 6.5 % by Xpert MTB/ RIF assay and 6.4 % by culture, with the same day diagnosis strategy. In this study Xpert MTB/RIF assay has shown comparable sensitivity and specificity with culture for the diagnosis of SNPT and excellent agreement for the detection of RR cases as compared to Genotype MTBDRplus Ver.2. Therefore, with the expansion of Xpert MTB/ RIF assay it is possible to diagnose more SNPT cases, and facilitate early detection and treatment initiation. Men were diagnosed with SNPT more than two times higher, compared to women. All SNPT were diagnosed in the economically productive age groups (15-64 years old), which indicates working with more attention to these populations is important. On the other hand, high prevalence (11.54 %) of smear negative multidrug resistant tuberculosis (SNMDR TB) was detected. Mono resistant is also high; 8.7 % for RIF and 26.08 % for Isoniazid resistance from new cases, 33.33 % for both drugs in retreatment cases were resistant implying that continuing using smear microscopy as the first diagnostic test for TB may lead to programmatic failure. Sputum sample quality is an important factor observed which matters for the diagnosis of smear negative pulmonary TB by GeneXpert, that proper patient instruction for sample collection is quit essential.

11. Recommendations

It is found that smear negative PTB is still high, smear negative MDR TB is emerging and mono resistance for first line anti- TB drugs is increasing among SNPPT patients in Addis Ababa. This demands a different strategy from the previous to ban smear negative TB and MDR TB disease. This study demonstrated that Xpert MTB/ Rif showed better diagnostic performance, that the TB control program in Addis Ababa, should consider it for the diagnosis and early treatment of smear negative pulmonary TB and SN MDR TB. In addition to preventive measures, availing TB culture and drug susceptibility testing facilities to peripheral health facilities is crucial. Proper patient instruction for sputum sample collection should be strictly followed and all type sputum sample qualities including saliva should be analyzed for GeneXpert. Other further studies on comorbidities and behavioral risk factors should be conducted in large scale. In this study it was difficult to get enough and appropriate sputum sample from children, so we recommend other researchers further study SNPT prevalence in children considering other samples too.

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8C27%3Fp_p_id%3D101_INSTANCE_ng3U%26p_p_lifecycle%3D0%26p_p_state%3Dnormal%26p_p_mode%3Dview%26p_p_col_id%3Dcolumn1%26p_p_col_count%3D4

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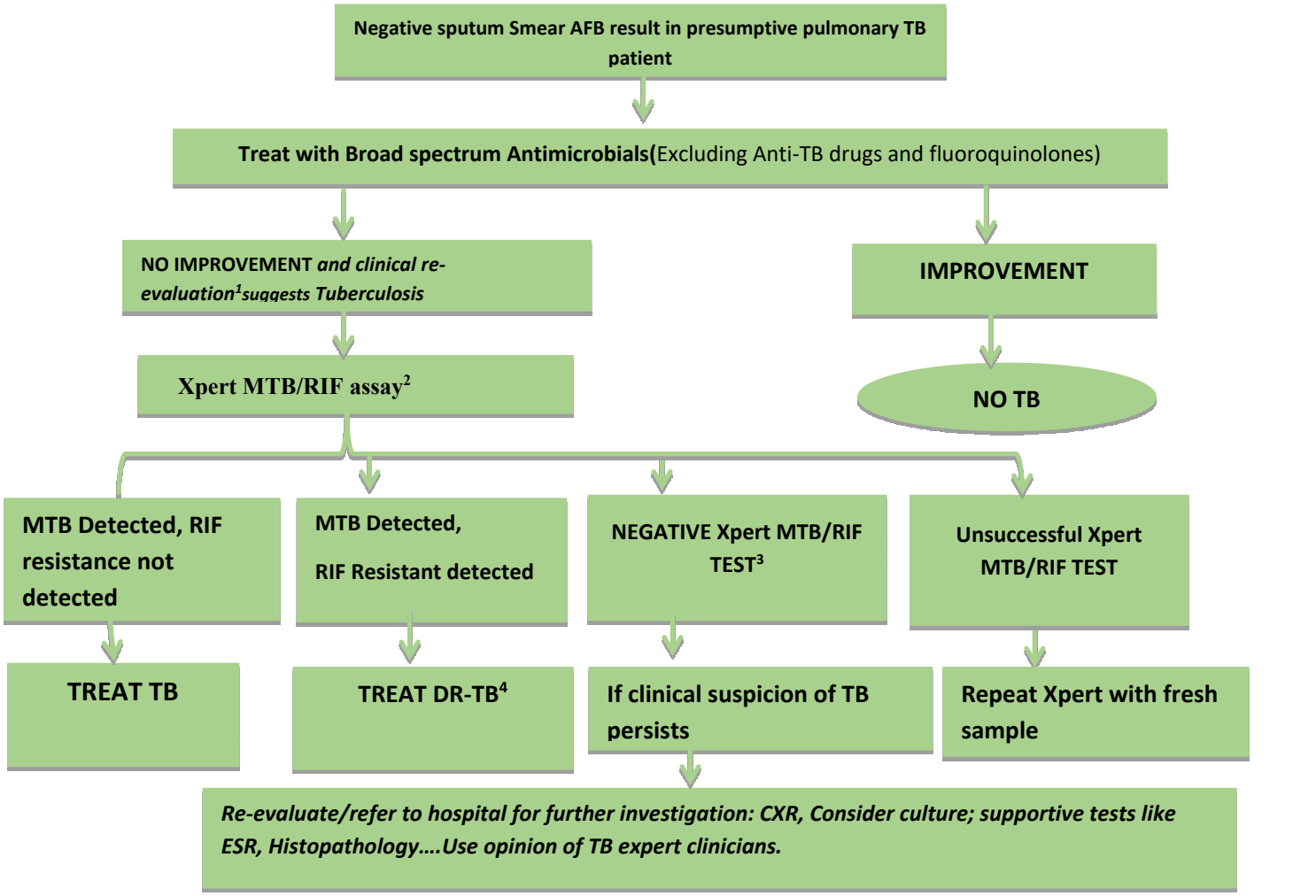
13. Annexes

Annex 1: Study sites in Addis Ababa Region and amount of samples taken

S. No	Health facilities	Quarter Report (August 01-October 30, 2016)	Approximate expected % of sample to be taken from the site	Total samples collected
1	Mercy Care Ethiopia HC (Gullele)	36	35*413/1131= 12.78	35
2	Kofie Woreda 01 HC	31	11.32	31
3	Felege Meles HC (Addis Ketema)	11	4	11
4	Akaki Kality Hc	61	22.64	62
5	Janmeda HC (Arada)	21	7.67	21
6	Goro HC (Bole)	70	25.93	71
7	Hidase HC (Gullele)	12	4.38	12
8	Guto Meda HC (Gullele)	30	10.95	30
9	Philipos HC (Kolfie)	19	6.94	19
10	Kolfie Woreda 6 HC	12	4.38	12
11	Woreda 5 HC (Nefas Silk Lafto)	77	28.48	78
12	Woreda 10 HC (Nefas Silk Lafto)	27	9.86	27
13	Merry Joy (Yeka Woreda 01 Hc)	1	0.37	1
14	Kidus Gabriel G/hospital (Bole)	2	0.73	2
15	Kadisko G/Hospital (Bole)	1	0.37	1
16	Tezena Hospital (Kolfie)	5	1.83	5
Total		416	100 %	418

Annex 2: Diagnostic algorithm for Presumptive pulmonary TB patients with Negative sputum results for AFB microscopy (adopted from Ethiopian national TB, HIV and leprosy management 2016 guideline)

Fig 4. Diagnostic algorithm for Presumptive pulmonary TB patients with Negative sputum results for AFB microscopy



[1] *clinical re-evaluation includes focused history, contact history, persistence/ worsening of symptom & sign consistent with TB.*

[2] *if Xpert MTB/RIF assay is not available; repeat AFB microscopy, take Chest radiograph and request other indicated supportive tests to assist the clinician to diagnose TB.*

[3] *Negative Xpert result cannot rule out presence of Active pulmonary TB.*

[4] *RR-TB result in patients with low risk for DR-TB needs to be re-confirmed with Xpert test on fresh specimen, and*

- if result shows RR TB again treat with Second line drug;

- if result shows MTB but No RR TB, treat with first line drugs anddo Culture and conventional DST.

Annex 3: Data collection tool, English Version

Prevalence of *Mycobacterium Tuberculosis* and *Multidrug Resistance Tuberculosis* and Associated Risk Factors Among Smear Negative Presumptive Pulmonary *Tuberculosis* Patients by GeneXpert Assay in Addis Ababa, Ethiopia

Instruction

General instruction for the interviewer

1. Request the willingness of the respondent for interview after reading the consent form and confirm by signature of the respondent.
2. Read each question to the respondent. Do not read the answers in response category unless otherwise directed to do so.
3. Circle only one response for each question, unless otherwise directed to do so.
4. Write responses for other "other (specify)" options clearly, using the space provided.
5. Follow all skip patterns
6. Record all information in pen. Do not use pencil.
7. In all cases use Gorgonian Calendar for date variables
8. If the respondent refused to answer a question or to participate write a word "**Refused**" in available space in the response column and go on to the next question
9. If the respondent wishes to stop the interview, write "**Stop**" in any available space in the response category and conclude the interview.

Don't forget to fill the Identification Information correctly before the interview

A. Identification Information

Health Facility Code ☐☐ **Patient Code** ☐☐☐

Unique Survey Identification Number: ☐☐☐☐☐

(Health Facility Code + Patient Sequential No. for Study)

Date of identification (European calendar**)**:_____/_____/_____
DD/MM /YYYY

Department

1. Under five OPD **2**. Adult OPD **3**. Inpatients **4**. Chronic Care Unit (ART Clinic) **5.** Antenatal Care Unit **6**. Post-natal Care Delivery Unit

Medical registration number (MNR): _____

Name of interviewer: _____

Signature of Interviewer: _____

Section A Patient Demographic Data				
A.1	Patient's age in complete year:		☐☐ years if Known use **99**	
A.2	Patient's Sex:		Male……1 Female……...…….2	
A.3	Patient Address	Region	_____	
A.4		Zone/Sub City	_____	
A.5		Woreda	_____	
A.6		Kebele	_____	
A.7	Religion of the Respondent		Orthodox …….……1 Muslim…….....2 Protestant…….…….3 Catholic …….....4 Other(Specify)_____ 5	
A.8	What is your highest level of education:		None(No Formal Education)……..1 Standard grade 1-7……..2 Standard grade 7-10……3 Standard grade 10-12……..4 Diploma(level 1-4 Certificate)…….5 First Degree(Bsc, BA)…….6 Second Degree(Msc,MA)……...….7 PhD……...….....8 Specific grade for grade 1-12_____	
A.9	What is your work status (professional):		Farmer.……1 Daily Laborer……...2 House wife...…… ……..3 Driver…….……4 Teacher…….……...5 Student……6 Police/Military……7 Merchant……..8 Health Care Worker ……...9 Government employee……...….10 Self-employed…….....11 Other specify_____12	

A.10	*If the respondent age is less than 18 years don't ask this question* What is your marital status	Single…….……1 Married…….……...2 Divorced…...…… ……..…...3 Widowed…….……4 Separated…….……..…...5	
A.11	How do characterized your residence area	Overcrowded……...1 Not crowded……...……2	
A.11.1	Family size/house	A. 1-3 B. 4-5 C. 6 and Above	
A.11.2	Area of the house you are living in (in meter square)?	_____	
A.11.3	Number of windows of your house?	A. 0 B. 1 C. 2 D. 3 E. 4 F. 5 and Above	
A.11.4	Number doors of your house?	A. 1 B. 2 C. 3 D. 4 E. 5 and above	
A.12	How you characterized your working area?	Overcrowded…….....……...1 Not crowded…….2	
A.12.1	Number of staffs working in the same room with you?	A. 1-3 B. 4- 5 C. 6 and above	
A.12.2	Number of windows of your office?	A. 1 B. 2 C. 3 D. 4 E. 5 and above	
A.12.3	Number doors of your office?	A. 1 B. 2 C. 3 D. 4 E. 5 and above	
A.12.4	Area of the office you are working in?	_____	
A.13	What is the means transportation that you used frequently for your daily activity	Private Car…….…….....1 Taxi……..2 City bus……3 Bajiaj……4 Bik/Motor cycle……...……5 Animal drawn-cart……....6 On foot……7 Other_____8	
Section BClinical Presentation			
B.1	What is the chief complaint of the patient	1._____ 2._____	
B.2	Duration of the symptoms	_____ Days	

B.3	Have you previously treated for these symptoms	Yes……..……1 No……...……2	
B.4	What was the diagnosis	Respiratory Tract Infection……..……1 Other Bacterial infection……..…2 Pain(Muscle, Joint)…….….3 Other specify_____ 4 Unknown……..5	
B.5	Where did you been treated	Government Health facility……..1 Private health facility……....2 NGO Facilities.……3	
B.6	Type of the facility	Clinic……1 Health center……2 Hospital……3 Drug bender/Pharmacy……4	
B.7	Duration of treatment	_____ Days	
B.8	Treatment outcome	Cured but relapsed……...1 Aggravated……...2 Reduced but till there is the symptoms……3	
B.9	what additional symptoms do you have	**Yes** **No** 1. Cough …… ☐ ☐ 2. Fever ……..... ☐ ☐ 3. Chest pain …… ☐ ☐ 4. Loss of appetite ……. ☐ ☐ 5. Weight loss……. ☐ ☐ 6. Shortness of birth……... ☐ ☐ 7. Joint pain…… ☐ ☐ 8. Abdominal pain……. ☐ ☐ 9. Swelling……. ☐ ☐ 10. Hemoptysis…… ☐ ☐ 11. Other_____ 12. Other_____ 13. Other_____	
B.10	Physical examination finding	S.N **Yes** **No**	

		1 Shifting of trachea ☐ ☐ 2 Abnormal birthing Sound ☐ ☐ 3 Dullness During percussion ☐ ☐ 4 Tenderness ☐ ☐ 5 Swelling of lymph-nodes ☐ ☐ 6 Discharge from the swelling area ☐ ☐ 7 Other _____ 8 Other_____ 9 Other_____	
B.11	Diagnostic test done	S.N | Tests | Done Y/N | Abnormal finding(Y/N)/Result 1 AFB 2 ESR 3 WBC 4 Differential count 5 Hgb 6 Gram stain 7. X-ray	
C. Past Illness			
C.1	Have you any known chronic illness	S.N | Yes | No 1 Diabetes mellitus ☐ ☐ 2 Hypertension ☐ ☐ 3 Asthma ☐ ☐ 4 Heart Disease ☐ ☐ 5 Liver Disease ☐ ☐ 6 Renal failure ☐ ☐ 7 Hormonal Disease ☐ ☐ 8 Malignancy/Cancer ☐ ☐ 9 Other _____	

C.2	Patient HIV status	Known RVI Patient……..…1 Not Known……..……..…..2	***If " 1" go to D1***
C.3	If the patient is not Known RVI patient, HIV test is offered?	Yes…….……..…..1 No…….....2	
C.4	If HIV test is offered, offered test was	Accepted…….…….……1 Refused…….…...2	***If "No" go to D1***
C.5	Patient HIV Test Result	Reactive ……..1 Non-Reactive……..2 Invalid…….3	
C.6	Date of HIV test	_____/_____/_____ DD/MM/YYYY	
D. TB Treatment History			
D.1	Are you a member of one of the following groups (***Circle all that apply***)	**Yes = 1 No= 2** 1. Health Care Worker……..1 2 2. Miner…….....1 2 3. Ex-miner……....1 2 4. Current smoker……1 2 5. Ex-smoker…….....1 2 6. Prisoner/Prison staff……....1 2 7. Migrant …….....1 2 8. Contact with TB patient……1 2 9. MDR-TB contact…….....1 2	
D.2	Have you ever taken INH prophylaxis	Yes…….1 No…….2	***If "No", go to D.4***
D.3	For how long has IPT been taken (in weeks)?	☐☐ Weeks	
D.4	Have you previously treated for TB	Ye…….……..…..1 No…….....2	***If " 2" go to D8***
D.5	What was the outcome	Cure/Treatment Complete……..…….1 Failure…….……..2 Defaulter/Lost to follow-up…….3	***If " 1 and 2" go to D8***
D.6	If D.5 is 3 for how long did you discontinue your past TB treatment?	☐☐ Weeks	

D.7	What were the main reasons for not adhering to TB treatment?	**Yes No** Pill burden……...1 2 Alternative treatment……..1 2 Side effect……...1 2 Distance of health facility……1 2 Stigma……..1 2 Other(specify)_____	
D.8	What is the current classification	New……..1 Failure……...2 Relapse……..…...3 Return after default…….…..4 Uncertain…….….5	
E. Behavioral Risk Factors			
E.1	Did you ever chew Khat	Yes Currently ……..…..1 Yes Previously……...2 No at all…….…….....3	
E.2	If "**Yes"**for how long did you chew khat	☐☐ Month ☐☐ Year	
E.3	If "**Yes Currently**" how often did you chew Khat	Daily…….1 Every other day…….2 Weekly……...3 Every other week……...4 Occasionally …….…….5 Other…….6	
E.4	Did you ever take alcohol	Yes Currently ……..…..1 Yes Previously……...2 No at all…….…….....3	
E.5	If **"Yes"** for how long did you drink alcohol	☐☐ Month ☐☐ Year	
E.6	What type of alcohol drink did you frequently used	Local drinks (Tela, Teji&Areke) ……1 Beer…….2 Wine drinks……..3 Liquors……4	
E.7	How many bottle/cups did you drink on one episode	_____ Cup……..1 Bottle……...2	

E.8	If "**Yes Currently**" how often did you take alcohol	Daily……1 Every other day……2 Weekly……...3 Every other week……...4 Occasionally …….…….5 Other……6	
E.9	Did you ever smoke cigarette	Yes Currently ……..…..1 Yes Previously……...2 No at all…….…….....3	
E.10	If **"Yes"** for how long did you smoke cigarette	☐☐ Month _____ ☐☐ Year _____	
E.11	If "**Yes Currently**" how often did you smoke	Daily……1 Every other day……2 Weekly……...3 Every other week……...4 Occasionally …….…….5 Other……6	
E.12	How many cigarettes did you smoke during one episode	_____	
E. 13	What other things you smoke ?	_____	

Thank you very much for your participation!

Annex 4. Questioner Amharic Version

ስሚርማክሮሰኮፕምርመራተሰርቶላቸውየተቢተዋህሲያንባልተገኘባቸውየሳንባቲቢተጠርጣሪህሙማንላይየቲቢእና መድሀኒትየተላመደቲቢስርጭት,

ተጽእኖእናአጋላጭሁኔታዎችንጅንኤክስፐርትበመጠቀምበአዲስአበባ፣ኢትዮጵያማጥናት

መመሪያ: ቃለመጠይቅከማድረጋችንበፊትየምንከተለውጠቅላላመመሪያዎች

1. ስለጥናቱአላማበማስረዳትየተሳታፊውን/ዋንፈቃደኝነትመጠየቅፈቃደኝነታቸውንምበፊርማቸውማረጋገጥ።
2. እያንዳንዱንጥያቄለተሳታፊው/ዋማንበብ።የመልስምርጫዎችንካልተጠየቅህበስተቀርአታንብብላቸው።
3. ለእያንዳንዱጥያቄአንድመልስብቻአክብብ።
4. ሌላካለሚለውንየምላሽምርጫ፣በተሰጠውቦታላይበትክክልማስቀመጥ።
5. Follow all skip patterns
6. ሁሉንምበስክርቢቶሙላእርሳስአትጠቀም።
7. የቀንአቆጣጥሮችንበሙሉበግሪጎሪያንአቆጣጠርሙላ።
8. ምላሽሰጭውየሆነጥያቄለመመለስ/ለመሳተፍፈቃደኛካለሆነበተፈቀደውቦታላይ“**Refused**”ብለህ/ሽበመጻፍወደቀጣዩጥያቄ/ ረድፍእለፍ።
9. ተሳታፊውቃለምልልሱንለማቋረጥፍላጎትካለውከደረስህበትቦታላይ“**Stop**” ብለህ/ሽበመጻፍቃለምልልሱንአጠናቅ።
10. ከቃለምልልሱበፊትየተሳታፊውንመለያቁጥር / ኮድበትክክልመሙላትህን/ሽንአትርሳ/ሽ።

ሀ. መለያኮድ

የጤናተቋዋሙመለያኮድ **የታካሚውመለያኮድ**

ልዩሰርቬ/የጥናቱመለያቁጥር: -

(የጤናተቋዋሙመለያኮድ + የጥናቱተሳታፊተከታታይቁጥር)

ታካሚውየተለየበትቀን (እ. ኤ. አ):_____/_____/_____

ቀን/ ወር / ዓመት

ዲፓርትሜንት :

1. ከ5 አመትበታችምርመራክፍል 2. የአዋቂዎችምርመራክፍል 3. ኤ.አር. ክፍል 4. ቅድመወሊደ 5. ድህረወሊድ 6. ማዋለጃ 7. ተኝቶህክምናክፍል

የታካሚውየህክምናካርድቁጥር: _____

ቃለምልልስአድራጊውባለሙያስም: _____

ክፍልሀ. የታከሚውዲሞግራፊመረጃ				
ሀ.1	የታካሚውእድሜ (በሙሉአመት):		☐☐ አመት	
ሀ.2	የታካሚውጸታ:		ወንድ……1 ሴት……..……2	
ሀ.3	የታካሚ ውአድራሻ	ክልል	_____	
ሀ.4		ዞን/ክፍለከተማ	_____	
ሀ.5		ወረዳ	_____	
ሀ.6		ቀበሌ	_____	
ሀ.7	የታካሚውሃይማኖት		ኦርቶዶክስ…….……1 ሙስሊም…….....2 ፕሮቴስታንት……..….3 ካቶሊክ …….....4 ሌላ (ይገለጽ)_____ 5	
ሀ.8	የደረስህበት/ሽበትከፍተኛየትምህርት ደረጃ:		ምንምአልተማርሁም(ምንምመደበኛትምህርትአልተማርሁም) ….....…..1 መደበኛ 1 -7 ክፍል ……..2 መደበኛ7-10 ክፍል ……3 መደበኛ10-12 ክፍል……..4 ዲፕሎማ(ደረጃ 1-4 ሰርቲፊኬት)…….5 የመጀመሪያዲግሪ (BSc, BA)…….…6 ሁለተኛዲግሪ (Msc, MA)……...….7	

		ዶክተሬትዲግሪ (PhD)……...….....8 1-12ኛክፍልትክከለኛክፍላቸውይገለጽ_____	
U.9	ስራሽ/ህ (ሙያ) ምንዲንነው? ካንድበላይሊመልሱይችላሉ	አርሶአደር……1 የጉልበትሰራተኛ……...2 የቤትእመቤት....…… ……..3 ሾፌር…….……4 መምህር…….……...5 ተማሪ……6 ፖሊስ/ ወታደር…….7 ነዳዴ……...8 የጤናባለሙያ .....……...9 የመንግስትሰራተኛ…...……...….10 የግልስራ……...…….....11 ሌላካለይገለጽ_____12	
U.10	የጋብቻሁኔታ (እድሜያቸውከ18 በታችከሆኑማለፍይችላሉ)	ያላገባ/ች……1 ያገባ/ች…….……...2 የተፋታ/ች…...…… ……..…...3 ባልየሞተባት/ ሚስትየሞተችበት……4 ሌላካለይገለጽ…….……..…...5	
U.12	የመኖሪያአካባቢሽን/ህንእንዴትትገልጹታላችሁ?	የተጨናነቀ……...1 ያልተጨናነቀ……...……2	----------

	አብረውየሚኖሩቤተሰብብዛት/ 1 ቤት	ሀ. 1-3 ለ. ሐ. 4-5 መ. 6 እናበላይ	
	የቤትዎስፋትበሜተርስኩዌር (M*M)?	----------	
	የቤትዎመስኮትብዛት?	ሀ. 0 ለ. 1 ሐ. 2 መ. 3 ሠ. 4 እናበላይ	
	የቤተዎበርብዛት?	ሀ. 0 ለ. 1 ሐ. 2 መ. 3 ሠ. 4 እናበላይ	
ሀ. 13	የስራአካባቢሽን /ህንእንዴትትገልጹታላችሁ?	የተጨናነቀ ……...1 ያልተጨናነቀ……...……2	
	የስራባልደረቦችብዛት/ 1 ክፍል	ሀ. 1-3 ለ. 4-5 መ. 6 እናበላይ	
	የቢሮስፋትበሜትርስኩዌር	----------	
	የሚሰሩብትቤትመስኮትብዛት	ሀ. 0 ለ. 1 ሐ. 2 መ. 3 ሠ. 4 ረ. 5 እናበላይ	
	የሚሰሩብትቤትበርብዛት	ሀ. 1 ለ. 2 ሐ. 3 መ. 4 ሠ. 5 እናበላይ	
ሀ.14	ለቀንተቀንስራሽአዘውትረሽ/ህየምት ጠቀመው/ሚውየተራንስፖርትዘዴ ምንድንነው?	የግልመኪና…….…….....1 ታክሲ……..2 የከተማአውቶበስ……3 ባጃጂ……4 ብስክሌት/ሞተርብስክሌት……...……5 ጋሪ……....6 በእግር……7 ሌላከለገለጽ_____8	
ክፍልለ፡የበሽታምልክቶችንበተመለከተ			

ለ.1	ከህመሙምልክቶችውስጥታከሚው/ዋዋናችግርህመምምልክትምንድንነው?	1._____ 2._____	
ለ.2	የህመምምልክቱለምንያህልጊዜቆየብዎት?	_____ ቀናት	
ለ.3	ከዚህበፊትለዚህህመምምልክትታክመውያውቃሉ?	አዎ……..……1 አይ……...……2	
ለ.4	በሽታውመንድንነውተባሉ?	የመተንፈሻአካልኢንፌክሽን/ በሽታ……..……1 ሌላየባክቴሪያኢንፌክሽን……..…2 ህመም (የጡንቻ, የመገጣጠሚአካል)…….….3 ሌላከሆነይገለጽ_____ 4 በግልጽአልተዎቀም……..5	
ለ.5	የትነውየተከሙት?	የመንግስትጤናተቋም……..1 ግልጤናተቋዋም……....2 መንግስታዊያልሆነጤናድርጂት(NGOfacility)… …..3	
ለ.6	ምንአይነትጤናተቛምነው?	ከሊኒክ……1 ጤናጣቢያ……2 ሆስፒታል……3 ፋርማሲ……4 ሌላካለይገለጽ----------5	
ለ.7	ለምንያህልቀንታከሙ?	_____ ቀናት	
ለ.8	የህክምናውውጤትምንሆነ?	ድኝነበር፣ተመልሶአገረሸብኝ…-……...1 አገረሸብኝ……...2	

		ትንሽቀንሰሷል፣ህመሙግንአሁንምአለ……3	
ለ.9	ምንሌላተጨማሪየህመምስሜቶችይሰማዎታል ?	**አዎ** **አይ** 14. ሳል…… ☐ ☐ 15. ትኩሳት…… ..... ☐ ☐ 16. የደረትህመም …… … ☐ ☐ 17. የምግብፍላጎትማጣት……. ☐ ☐ 18. የክብደትመቀነስ……. ☐ ☐ 19. ቶሎቶሎመተንፈስ……... ☐ ☐ 20. የመገጣጠሚያህመም…… ☐ ☐ 21. የሆድህመም……. ☐ ☐ 22. እብጠት……. ☐ ☐ 23. ደምመትፋት…… ☐ ☐ 24. ሌላ_____ 25. ሌላ_____ 26. ሌላ__ _____	
ለ.10	የአካላዊምርመራውጤቶች	**ተ ቁ** **አዎ** **አይ** 1 የጉሮሮመሸንቆር ☐ ☐ 2 በሚተነፍሱበትጊዜያልተለመደድምጽ ☐ ☐	

		3	Dullness During percussion	☐	☐	
		4	የመንደቅደቅስሜት	☐	☐	
		5	የሊንፍኖዶችእብጠት	☐	☐	
		6	ከእባጭአካባቢየሚወጣፈሳሽመ ኖር	☐	☐	
		7	ሌላ _____			
		8	ሌላ_____ _____ ____			
		9	ሌላ_____ _____			
ለ.11	የላቦራቶሪምርመራተስረቶላችኋል?	ተ.ቁ	የላብ ምርመራ	ተሰርቷልአዎ/አይ	ያልተለመደውጤት አዎ/አይ (ውጤቱይገለጽ)	
		1	ኤ.ኤፍ.ቢ			
		2	ኢ.ኤስ.አር			
		3	ጠቅላላነጭደምህዋሳትቆጠራ			
		4	ነጭደምህዋሳትአይነትልየታቆጠራ			
		5	ሄሞግሎቢን			
		6	ግራምስቴን			

		7. X-Ray	
ሐ. ከዚበፊትየነበረህመም			
ሐ.1	ከዚበፊትየሚታወቅየቆየህመምአለብዎት	ተ.ቁ 1 የስኪርህመም ☐ ☐ 2 ደምግፊት ☐ ☐ 3 አስም ☐ ☐ 4 የልብበሽታ ☐ ☐ 5 የጉበትበሽታ ☐ ☐ 6 Renal failure ☐ ☐ 7 የሆርሞንበሽታ ☐ ☐ 8 ካንሰር ☐ ☐ 9 ሌላ/ ይገለጽ_____	
ሐ.2	የኤችአይቪሁኔታ	የታወቀየኤችአይቪታካሚ……..…1 ኤችአይቪሁኔታአይታዎቅም……..…..2	***መላሱ“ 1” ከሆነወደመ 1ይሂዱ***
ሐ.3	የኤችአይቪሁኔታማይታዎቅከሆነለበሽታውምርመራተደርጎለዎታል?	አዎ…….……..…..1 አይ…….....2	
ሐ.4	የኤችአይቪምርመራከተደረገልዎትምርመራውን	ተቀብየዋለሁ…….……..……1 አልተቀበልሁትም…….…...2	አልተቀበልሁት ምከሆነወደመ1

			ሂድ
ሐ.5	የታካሚውኤችአይቪምርመራውጤት	ፖዚቲቭ……..1 ኔጋቲቭ……..2 ኢንቫሊድ…….3	
ሐ.6	የኤችአይቪምርመራየተደረገበትቀን	_____/_____/_____ ቀን/ ወር/ አመት	
መ. የታካሚውየቲቢህክምናታሪክ			
መ.1	ከሚከተሉትየትኛውግሩፕአባልነህ/አንተንይመለከታል (***የሚመለከትዎትንሁሉያክብቡ***)	**አዎ= 1 አይ=2** 10. የጤናባለሙያ……..…..1 2 11. ማዕድንአውጭ…….....1 2 12. ድሮማዕድንአውጭየነበረ…....1 2 13. አሁንየሚያጨስ……..….1 2 14. በፊትየሚያጨስ…… ... ….....1 2 15. ታራሚ/ ታራሚከነበረጋርየሚኖር.....1 2 16. ስደተኛ …….....1 2 17. ከቲቢታካሚጋርየሚኖር/የሚሰራ…1 2 18. ኤም.ዲ. አርቲቢታካሚጋርየሚኖር/የሚሰራ...1 2	
መ.2	INH prophylaxis ወስደህታውቃለህ	አዎ……1 አይ……2	አይከሆነመ.4 ጋርይለፉ

መ.3	ለምንያህልጊዜነው IPT የወሰድከው (በሳምንትይገለጽ)?	☐☐ ሳምንት	
መ.4	የቲቢህክምናወስደህታውቃለህ?	አዎ……1 አይ……2	*“ 2” ከሆነወደመ. 8*
መ.5	የህክምናውውጤትምንድንነበር?	መዳን/ መድሃኒትወስዶመጨረስ……..……1 ፌይለር……..……..2 ዲፎልተር/ህክምናክትትልማቋረጥ…….3	***If “ 1 and 2” go to D8***
መ.6	የመ.5 መልስ 3 ከሆነለምንያህልነውየቲቢህክምናውን ያቋረጥከው?	☐☐ ሳምንት	
መ.7	የቲቢህክምናውንበትክክልእንዳትወስድያደረገህዋናምክንያትህምንድንነው?	አዎ = **1** አይ=2 የመድሃኒቱተጽዕኖ……...1 2 ሌላአማራጭህክምናስላገኘሁ……..1 2 የመድሃኒቱየጎንዮሽጉዳት……...1 2 የጤናተቋሙርቀት……1 2 እስቲግማ/stigma……..1 2 ሌላ(ይገለጽ)_____	
መ.8	አሁንየታካሚውኬዝሁኔታ	አዲስ……..1 ፌለር/Failure……...2 እንደገናማገርሸት……...…...3 ካቋረጡበኋላየተመለሱ……..…..4 አይታወቅም…….….5	

ሠ. ባህሪያዊአጋላጭሁኔታዎች			
ሠ.1	ጫትቅመህታውቃለህ?	አዎአሁንእቅማለሁ……..…..1 አዎድሮ……...2 ቅሜአላውቅም…….....3	
ሠ.2	**ሠ**.1መልሱአዎከሆነለምንያህልጊዜ?	ወር☐☐ ☐☐	
ሠ.3	አዎአሁንእቅማለሁ”ከሆነበምንያህል ድግግሞሽነውየሚቅሙት?	በየቀኑ……1 አንዳንድቀንእያለፍሁ…….2 በየሳምንቱ……...3 አንዳንድሳምንትእያለፍሁ……...4 አልፎአልፎ …….……5 ሌላካለይገለጽ…….6	
ሠ.4	አልኮሆልጠጥተውያውቃሉ	አዎአሁንእየጠጣሁነው……..…..1 አዎድሮ……...2 ጠጥቸአላውቅም…….....3	
ሠ.5	**ሠ**.4መልሱአዎከሆነለምንያህልጊዜ?	☐☐ ☐☐ ወርዓመት	
ሠ.6	አዘውትረህየምትጠቀመውአልኮሆል ምንድንነው?	በአካባቢውየሚገኙመጠጦች (ጠላ፣ጠጅእናአረቂ-----1 ቢራ/ ድራፍት (ሁለቱንም)…….2 ወይን…….-……..3 ውስኪ……4 ሌላካለይጠቀስ---------- 5	

ሠ.7	ምንያክልጠርሙስ/ መለኪያበአንድጊዜትጠጣለህ?	መለኪያ ……..1 ጠርሙስ……...2	
ሠ.8	አዎአሁንእጠጣለሁ”ከሆነበምንያህል ድግግሞሽነውየሚጠጡት?	በየቀኑ……1 አንዳንድቀንእያለፍሁ…….2 በየሳምንቱ……...3 አንዳንድሳምንትእያለፍሁ……...4 አልፎአልፎ ……..……5 ሌላካለይገለጽ……6	
ሠ.9	ሲጋራአጭሰውያውቃሉ	አዎአሁንአጨሳለሁ……..…..1 አዎድሮ……...2 አጭሼአላውቅም…….....3	
ሠ.10	ሠ.9 መልሱአዎከሆነለምንያህልጊዜ?	ወር☐☐ ☐☐	
ሠ.11	አዎአሁንአጨሳለሁ” ከሆነበምንያህልድግግሞሽነውየሚያ ጨሱት?	በየቀኑ……1 አንዳንድቀንእያለፍሁ…….2 በየሳምንቱ……...3 አንዳንድሳምንትእያለፍሁ……...4 አልፎአልፎ ……..……5 ሌላካለይገለጽ……6	
ሠ.12	በአንድጊዜምንያህልሲጋራያጨሳሉ?	_____	
ሠ.13	ሌሎችየሚያጨሷቸውዕጾችካሉ	----------	

Annex 5. English Version Patient Information and Consent Form

My name is Waganeh Sinshaw and I am MSc student in Clinical Laboratory Science (Diagnostic and Public Health Microbiology Specialty track) at Addis Ababa University College of Health Science, School of Allied Health Sciences, and department of Medical Laboratory Science. I am doing a research on "Prevalence of *Mycobacterium Tuberculosis* and *Multidrug Resistance Tuberculosis* and associated risk factors among Smear Negative presumptive pulmonary Tuberculosis patients by Xpert MTB/RIF Assay in Addis Ababa, Ethiopia."

Purpose: the main aim of this study is to determine the prevalence of Mycobacterium tuberculosis (MTB) and Multidrug resistance tuberculosis (MDR TB) among smear negative pulmonary patients by using different diagnostic technologies like Xpert MTB/RIF assay and to determine different associated risk factors. This study will also evaluate Xpert MTB/RIF assay diagnostic performance, for the diagnosis of smear negative pulmonary tuberculosis cases. The results of this study will substantially contribute in the planning, interventions and control of TB and Drug Resistance TB to reduce TB morbidity and mortality in Addis Ababa, and it is important to see other diagnostic technologies than smear microscopy for early diagnosis and management of smear negative TB and DR TB. The study can be used as an initial reference for other studies. Based on the findings we will recommend for concerned bodies.

Participation: I am asking you to participate voluntarily in this study; if you agree to participate you will be asked to sign a consent form and response to short questionnaire interview and give sputum specimen.

Risks associated: With this study there are no risks associated during sample collection procedures and with your participation in the study.

Benefits: If there is any positive finding in laboratory examination the result will be reported to your physician for appropriate treatment and management.

Confidentiality: All information you give and data obtained from laboratory analysis will be kept confidential and will be communicated only to responsible figure. Formats containing data will be kept locked.

Sharing the result: Report will be written about the finding of the study, either through publication or any other means. The result will not bear any information relevant to your personality in anyway.

Address:

If you have any questions and or Comments please use this address.

Address of the Principal Investigator:

Waganeh Sinshaw,

Addis Ababa University, College of Allied Health Sciences, Department of Medical Laboratory Science or Ethiopian Public Health Institute,National Tuberculosis Reference laboratory Addis Ababa, Ethiopia, Arbegnoch Street, P.O.Box 1242

Mobile Phone: +251913737808/912920670

Tel (Office): +251 112780845

Consent Declaration Form:

I have read this Patient Information and Consent Form, or this form has been read to me. I have had the opportunity to ask, and I have received answers to any questions I had regarding the study. I understand that if I have any additional questions, I may contact my treating clinician. I understand there is no serious invasive procedure at the beginning as well as at the end of the study. I agreed on the terms and the conditions indicated above. And I have received a copy of this Patient Information and Consent Form.

Participant's	Clinician's Name:	For those who cannot Read and write
		Witness
Signature:	Signature	Signature:
Date	Date	Date:

Annex 6. የምርምር ጥናት ማብራሪያ /አማርኛ ቅጂ

ዋጋነህ ስንሻው እባላለሁ። የአዲስ አበባ ዩኒቨርስቲ የጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ዲፓርትመንት የክሊኒካል ላቦራቶሪ ሳይንስ (የምርመራ እና የማህበረሰብ ጤና ሕክምና ማይክሮባዮሎጂ) የማስተርስ ዲግሪ የ3ኛ ኣመት ተማሪ ነኝ። ጥናቱ ስሚር ማክሮሰኮፕ ምርመራ ተሰርቶላቸው የተቢ ተዋህሲያን ባልተገኝባቸው ህሙማን ላይ የቲቢ እና መድሀኒት የተላመደ የሳምባ ቲቢ ስርጭት, ተጽእኖ እና አጋላጭ ሁኔታዎችን በአዲስ አበባ ለማወቅ የሚካሄድ ጥናት ነዉ።

<u>የጥናቱ ተሳታፊዎች የመረጃ ቅጽ</u>

ሀ. **<u>የጥናቱ ዓላማ</u>**፡- የስሚር ማክሮሰኮፕ ምርመራ ተሰርቶላቸው የተቢ ተዋህሲያን ባልተገኝባቸው ህሙማን ላይ የቲቢ እና መድሀኒት የተላመደ የሳምባ ቲቢ ስርጭት ተጽእኖ እና አጋላጭ ሁኔታዎችን ለማወቅ የተሻለ የላቦራቶሪ መመርመሪያ ቴክኖሎጂ በመጠቀም በአዲስ አበባ ከተማ የሚካሄድ ጥናት ነዉ። ይህ ጥናት ጂን ኤክስፐርት የተባለውን ቲቢ እና መድሀኒት የተላመደ ቲቢ መመርመሪያ ቴክኖሎጂ ስሚር ማክሮሰኮፕ ኔጋቲቭ ኬዞችን ለተቢ ተዋህሲያን እና መድሀኒት የተላመደ ቲቢ ዝርያ የመመርመር አቅም መገምገምንም ይጨምራል።

ለ. **<u>ፈቃደኝነት</u>**፡-እርስዎን በጥናቱ በሙሉ ፍቃደኝነት እንዲሳተፉ እየጠየቅን በጥናቱ ላይ ለመሳተፍ ፍቃደኛ ከሆኑ ለሚቀርብልዎትን መጠይቅ ምላሽ ከሰጡ በኋላ የአክታ ናሙና ሁለት ጊዜ እንዲሰጡ ይጠየቃሉ::

ሐ. **<u>የሚያገኙት ጥቅም</u>**፡- በሽታ አምጪ ተህዋሳያን በተሻለ የላቦራቶሪ መመርመሪያ ቴክኖሎጂ መኖራቸው ተረጋግጦ ተገቢውን ህክምና እንዲያገኙ የላቦራቶሪ ውጤት ወደ ሀኪም ተልኮ ተገቢዉን ህክምናና ክትትል ያገኛሉ።

መ.**<u>የሚያሰከትለዉ ጉዳት</u>**፡- በዚህ ጥናት በመሳተፍዎ በእርስዎ ላይ የሚያስከለዉ ምንም አይነት ችግር የለም በጣም ይጠቀማሉ።

ሠ. **<u>ሚስጥራዊነት</u>**፡-የእርስዎ የግል መረጃ በሙሉ ሚስጥራዊነቱ የተጠበቀ ይሆናል።

ረ. **<u>ውጤቱን ስለመጠቀም</u>፡**-ከዚህ ጥናት በኋላ የበሽታውን ስርጭት, ተጽእኖ እና አጋላጭ ሁኔታዎችን በተመለከተ ሪፖርት ይፃፋል፡፡ሆኖም የእርስዎን ማንነት የሚገልፅ መረጃ የማይካተት ሲሆን ችግሩን ለማሳወቅ ብቻ የሚውል ነው፡፡

አድራሻ፡-

ማንኛውም ጥያቄ ወይም ጥርጣሬ ካለዎት ይህንን አድርሻ ይጠቀሙ፡

የዋናው ተመራማሪ አድራሻ፡ - ዋጋነህ ስንሻው

አዲስ አበባ ዩኒቨርስቲ የጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ዲፓርትመንት

ሞባይል፤ +251912920670/ +251913737808 ኢሜል፡- wagamels@gmail.com

<u>የስምምነት ማረጋገጫ ቅጽ</u>

ይህ የተሳታፊዎች መረጃ እና ስምምነት ቅጽ አንብቢለሁ ወይም ተነቦልኛል፡፡ ጥናቱን በተመለከተ ጥያቄዎችን የመጠየቅ እድሉ ነበረኝ፡፡ የጠየቅሁዋቸው ጥያቄዎችም በአግባቡ ተመልስውልኛል፡፡ ተጨማሪ ጥያቄዎች ካሉኝም ሃኪሜን ጠይቄ መረዳት እንደምችል ተረድቻለሁ፡፡ ስለዚህ ከላይ በተጠቀሱት ሁሉ ተሰማምቻለሁ፡፡

የተሳታፊ ፊርማ፡- ---------- ቀን፡ ----------

ማንበብ እና መጻፍ ለማይችሉ እማኝ ስም፡ ---------- ፊርማ፡ ---------- ቀን፡----------

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ቃለ መጠየቁን ያደረገው ሐኪም/ የላብ ባለሙያ ስም፡ ---------- ፊርማ፡ -------- ቀን፡----------

Annex 7: Parental Consent Form for the Participation of Children aged <18

Your signature below indicates that you have read or listened to and understand the information provided to you about the study. Before you sign, please confirm that you understand the following:

- Purpose of the study
- Study procedures, including
  - Interview
  - Sputum sample collection
- Process for feedback of results
- Risks and benefits of participating in the study
- Right to refuse or stop participation at any time
- Confidentiality and privacy concerns
- Who to contact if you have questions

By signing, you are making a decision to allow your child (son/daughter/ child/infant/adolescent youth) to participate in this study. If you decide that you wish your child to withdraw or discontinue participation in the study, you may do so at any time.

I have read and/or listened to the description of the study and I understand what the procedures are and what will happen to my child in the study. I agree to allow him/her to participate in it. I know that my child can quit the study at any time.

_____

Name of (son/daughter/child/infant/adolescent youth)

_____ _____

Signature of Parent(s) or Legal Guardian Date

_____ _____ _____

Name Signature of interviewer Date

Annex 8: ከ 18 አመት በታች ላሉ ልጆች የወላጆች የስምምነት ቅፅ በአማርኛ

ሰለ ጥናቱ የተሰጡት ኢንፎርሜሽን እንዳነበቡ ወይም ግንዛቤ እንዳገኙ የሚከተለዉ ፊርማዎ ያመለክታል። ከመፈረምዎ በፊት የሚከተሉትን መገንዘበዎን ያረጋግጡ ፡ -

- የጥናቱ ዓላማ
- የጥናቱ የሚከተሉትን ቅደም ተከተሎች
  - ቃለ መጠየቅ
  - የአክታ ምረመራ
- ስለዉጤት ግብረመልስ
- በጥናቱ በመሳተፍዎ ያሉትን ጥቅሞች እና ጉዳቶች
- በፈለጉት ጊዜ ጥናቱን ማቆም እንደሚችሉ
- ምስጢራዊነቱን
- ለጥያቄ ማንን ማግኘት እንደሚችሉ

በጥናቱ ለመሳተፍ መወሰንዎን በመፈረም ያረጋግጣሉ። ላለመሳተፍ ወይም ለማቋረጥ ከወሰኑ በማንኛዉም ጊዜ ይችላሉ

በተሰጠኝ ገለፃ መሰረት በጥናቱ ሂደት በእኔ ላይ ሊደረጉ የሚችሉ ኹነቶችን ተረድቻለሁ' በጥናቱ ለመሳተፍ ወስኛለሁ' በፈለኩት ጊዜ ማቋረጥ እንደምችል ተገንዝቢያለሁ'

_____ _____ _____ የልጅ ሙሉ ስም

የወላጅ ወይም የህጋዊ አሳዳጊ ፊርማ ቀን

ቃለ መጠይቅ ያደረገው ሃኪም ስም፡ --------- ፊርማ---------- ቀን----------

Annex 09: Assent form for child between <18 years of age

I have read and/or listened to the description of the study and I understand what the procedures are and what will happen to me in the study. I have received permission from my parent(s)/guardian(s) to participate in the study, and I agree to participate in it. I know that I can quit the study at any time.

_____ _____

Signature of Child Date

_____ _____

Signature Date

Annex 10: ከ18 ዓመት በታች ላሉ ልጆች የስምምነት ቅፅ

በተሰጠኝ ገለፃ መሰረት በጥናቱ ሂደት በእኔ ላይ ሊደረጉ የሚችሉ ኹነቶችን ተረድቻለሁ' በጥናቱ ለመሳተፍ ከወላጆቸ/አሳዳጊዎቸ ፈቃድ አግኝቻለሁ ስለዚህ ለመሳተፍ ፈቃደኛ ነኝ' በጥናቱ ለመሳተፍ ወስኛለሁ በፈለኩት ጊዜ ማቋረጥ እንደምችልም ተገንዝቢያለሁ።

_____ _____

ፊርማ/የልጅ ቀን

_____ _____

የቃለ መጠይቅ ደረገው ሃኪም ስምና ፊርማ ቀን

Annex 11. Assurance of the Principal Investigator

Declaration

I the undersigned candidate, declare that this thesis is my original work and has not been presented for a degree in this or any other university and all resources used for this thesis have been acknowledged.

Name of the student: **Waganeh Sinshaw Ayenew**

Signature: _____

Date: _____

Approval of Advisor

Name of the primary advisor: **Adane Bitew**

Signature: _____

Date: _____

Approval of the Advisor

Name of the primary advisor: **Abebaw Kebede**

Signature: _____

Date: _____

Annex 12: AFB (ZN method) microscopy procedure

Step	Action
1.	**place the slides with smear upwards on the staining rack over a sink about finger-width apart**
2.	**Add 1% carbol-Fuchsin staining solutions over the smears**
3.	**Prepare the torch by dipping its cotton wool end in burning spirit and light it**
4.	**Heat all slides keeping the torch a little below them until steam arises**
5.	**Repeat it twice at intervals of 3-5 minutes. do not let staining solution dry on the slides**
6.	**Leave the heated stain on the slide a minimum of 05 minutes.**
7.	**Tilt each slide using forceps to drain off the staining solution**
8.	**Rinse the slides well with clean water from a beaker.**
9.	**Pour decolourising solution over the smears covering them completely**
10.	**Allow to act for 3 minutes**
11.	**Tilt each slide with forceps to drain off the acid**
12.	**Gently rinse each slide again with clean water. do not splash adjacent slides.**
13.	**If needed, repeat until all macroscopically visible stain has been washed away.**
14.	**Flood smear with methylene blue solution for 1 minute.**
15.	**Gently rinse each slide with water. Do not splash adjacent slides**
16.	**Tilt each slide with forceps to drain off excess water**
17.	**Clean back of slide with moist paper**
18.	**Using forceps place it on draining rack. Always keep smears out of direct sunlight.**
19.	**Examine slides after it has dried**

Recording

	Observation	Reporting
Result Interpretation	No AFB found in at least 100 fields	**Negative**
	1-9 AFB in100 fields	**Record exact number of bacilli**
	10 - 99 AFB per 100 fields	**1+**
	1 to 10 AFB per field (check 50 fields)	**2+**
	More than 10 AFB per field (check 20 fields)	**3+**

Annex 13: FM microscopy procedure

Step	Action
1.	**Place the slides on the staining rack over a sink and keep distance between every slide at least 1cm.**
2.	**cover the smears completely with 0.1% auramine solution**
3.	**do not heat**
4.	**leave for 20 minutes**
5.	**wash the slides well with distilled or running water**
6.	**pour the 0.5% acid solution over them**
7.	**allow to act for 3 minutes**
8.	**gently rinse each slide again with distilled or tap water**
9.	**Flood smear with 0.3% methylene blue solution for 1 minute. (Potassium per manganate can be used as a quenching reagent)**
10.	**wash off with distilled or running water**
11.	**stand the slide on edge to drain, and air dry on the slide rack out of strong light**
12.	**keep stained smears in the dark (box or folder) till reading, and read as soon as possible since fluorescence fades quickly**

Examination of Fluorescent Stained slides

Examine fluorescent-stained slides within 24 hours of staining. Save all sputum smears in slide boxes.

1. With a fluorescent microscope, scan the entire smear with the 20X objective (with 10X eyepiece for a total of 200X magnification).
2. Using 20X magnification, one 2 cm length is equivalent to 30 fields, which is sufficient to report a negative result.
3. Occasionally use the 40X objective to see more detailed bacterial morphology. Confirming morphology at higher magnification avoids a false-positive report due to fluorescing debris.
4. Mycobacteria appear as rod-shaped, coccoid, or filamentous bacilli. They may be vary in shape and staining intensity, and may occur singly, in small groups containing a few bacilli, or as large clumps.

5. Count bacilli in the number of fields appropriate for the degree of positivity (e.g., the higher the smear positivity, the fewer fields counted). Average the count for the number of fields and record on the lab worksheet the appropriate result according to the table.

Interpretation and reporting with Grading Scale for ZN & Fluorescent Stained slides

IUATLD/WHO SCALE (1000x field =HPF) Result	MICROSCOPY SYSTEM USED		
	BRIGHTFIELD (1000x magnification; one length = 2cm = 100 HPF)	**FLUORESCENCE (200-250x magnification; one length = 30 fields = 300 HPF)**	**FLUORESCENCE (400x magnification; one length = 40 fields = 200 HPF)**
Negative	Zero AFB / 1 length	Zero AFB / 1 length	Zero AFB / 1 length
Scanty	1-9 AFB / 1 length or 100 HPF	1-29 AFB / 1 length	1-19 AFB / 1 length
1+	10-99 AFB / 1 length or 100 HPF	30-299 AFB / 1 length	20-199 AFB / 1 length
2+	1-10 AFB / 1 HPF on average	10-100 AFB / 1 field on average	5-50 AFB / 1 field on average
3+	>10 AFB / 1 HPF on average	>100 AFB / 1 field on average	>50 AFB / 1 field on average

*The number of AFB indicates how infectious the patient is. It is important to record exactly what you see. ** Confirmation required by another technician or prepare another smear, stain and read.

Annex 14: Xpert MTB/ RIF Assay procedure

Step	Action
1	**Check the sample integrity. If food particles are present – Proceed but be careful not to add food particles to the Xpert cartridge**
2	**Visually inspect and estimate the volume of specimen in the collection container**
3	**Open the lid of the collection container and pour Sample Reagent buffer (supplied in kit) to the specimen in a 2:1 (SR buffer: specimen) volume ratio**
4	**Close the lid of the container tightly and vortex for 15 seconds.**
5	**Allow the mixed specimen to stand for 15 minutes at room temperature**
6	**Shake the container once during the 15 minute incubation i.e. after 10 minutes**
7	**At the end of 15 minutes if the sample is still viscous, shake the sample again and leave it for another 5 min until it is properly liquefied**
8	**This mixture can be kept for up to 8 hours at 2-8ºC, in case repeat testing is required**

Loading the processed specimen into an Xpert cartridge

Step	Action
1	**Check the sample integrity. If food particles are present – Proceed but be careful not to add food particles to the Xpert cartridge**
2	**Visually inspect and estimate the volume of specimen in the collection container**
3	**Open the lid of the collection container and pour Sample Reagent buffer (supplied in kit) to the specimen in a 2:1 (SR buffer: specimen) volume ratio**
4	**Close the lid of the container tightly and vortex for 15 seconds.**
5	**Allow the mixed specimen to stand for 15 minutes at room temperature**
6	**Shake the container once during the 15 minute incubation i.e. after 10 minutes**
7	**At the end of 15 minutes if the sample is still viscous, shake the sample again and leave it for another 5 min until it is properly liquefied**
8	**This mixture can be kept for up to 8 hours at 2-8ºC, in case repeat testing is required**

Step	Action
1	**Remove an MTB/RIF Xpert cartridge from its wrapper, taking care NOT TO TOUCH the back of the cartridge. Once the foil wrapper is removed, the specimen must be added to the cartridge within 30 minutes**
2	**Label the side of the Xpert cartridge with the sample ID. Do not write or place sticker over cartridge barcode**
3	**Open the lid of the cartridge**
4	**Open the lid of the collection container containing the processed specimen**
5	**Open a sealed sterile pipette (supplied in kit) without touching the tip.**
6	**Use the pipette to aspirate >2ml of the specimen (just above 2ml mark on pipette) and slowly dispense it into the open port of the Xpert MTB/RIF cartridge. Do not add less than 2ml of mixed specimen to the cartridge**
7	**Close the cartridge lid firmly**
8	**Dispose the specimen collection container, pipette and leftover SR buffer into a suitable waste bin**
9	**Take the Xpert cartridge to the bench with the GeneXpert instrument**. NOTE: **the test must be started within 30min of adding the specimen to the cartridge.**
10	**Load the cartridge with the specimen to the GeneXpert machine**

Result Interpretation:

- MTB Not Detected – MTB target DNA is not detected hence the test is negative for TB
- MTB Detected RIF Resistance not Detected- MTB present is not resistant to Rifampicin
- MTB Detected RIF Resistance Detected - MTB present is resistant to Rifampicin
- Invalid – Repeat test
- Error – Repeat test
- No Result – Repeat test

Annex 15: Culture and Identification testing procedure

Pre-Specimen processing preparation:

- Label the sample and request form with culture number
- Check all the material required for sample processing is in place
- Arrange sample, and required material in the biological safety cabinet

Step	Action
1	• Use the TB processing checklist to collect supplies, reagents, and specimens • Label the specimen, slide and LJ media (with lab code, date inoculated) • Disinfect BSC and perform daily maintenance • Clean slides with 70% alcohol • Set up BSC for specimen processing with absorbent liner, disinfectant, and waste containers
2	• Sort specimens into batches using sterile water blanks as the first and last negative processing controls • Batch size was based on the centrifuge load including negative processing controls

Preparation for process using NALC-NaOH

Sputum sample

Step	Action
1.	Check the volume of the sputum (at least 2 ml, not more than 5 ml) (if the volume of the sample was above 5ml, transferred 5ml of the purulent part to another falcon tube and proceed this allocate for the subsequent steps)
2.	Add equal volumes of NALC-NaOH solution. Use aliquots of NALC-NAOH(1 vial of NALC-NAOH per one specimen)
3.	Tighten cap of container and vortex slowly
4.	Shake intermittently to aid homogenization and decontamination

5.	Invert each bottle to ensure that NALC-NaOH solution contacts all the sides and inner portion of caps.
6.	Keep at 20°C – 25°C for 15 min for decontamination
7.	Fill the tube with phosphate buffer up to 45 ml mark on the tube. . Use aliquots of phosphate buffer(1 vial of of PBS per one specimen)
8.	Mix-well or vortex
9.	Centrifuge at 3,000 ×g for 15 minutes
10.	Carefully pour off the supernatant into a discard container containing 5% sodium hypochlorite or other germicide. Make sure the final concentration of bleach is 1% after pouring off the supernatant.
11.	Prepare smear on slide labelled with culture number for microscope
12.	Re suspend the deposit with 2ml PBS.

Inoculation

MGIT Media

Step	Action
1.	Mark each MGIT tube with laboratory number.
2.	Using repeater pipette Add 0.8 ml of the PANTA solution to each MGIT tube just prior to inoculation.
3.	with disposable Pasteur pipette, Add 0.5 ml of a well-mixed processed and/or direct specimen to the appropriately labelled MGIT tube
4.	Tightly recap the tube and mix by inverting the tube several times.
5.	Wipe tubes and caps with a Mycobactericidal disinfectant.
6.	Leave inoculated MGIT tubes at room temperature for 30 min.
7.	Scan the inoculated MGIT tube and load into the slot identified by the MGIT 960
8.	Check MGIT 960 daily for indicator lights flagging positive and negative cultures
9.	Incubate MGIT tubes until the instrument flags them as positive (red flag) or negative (green flag)

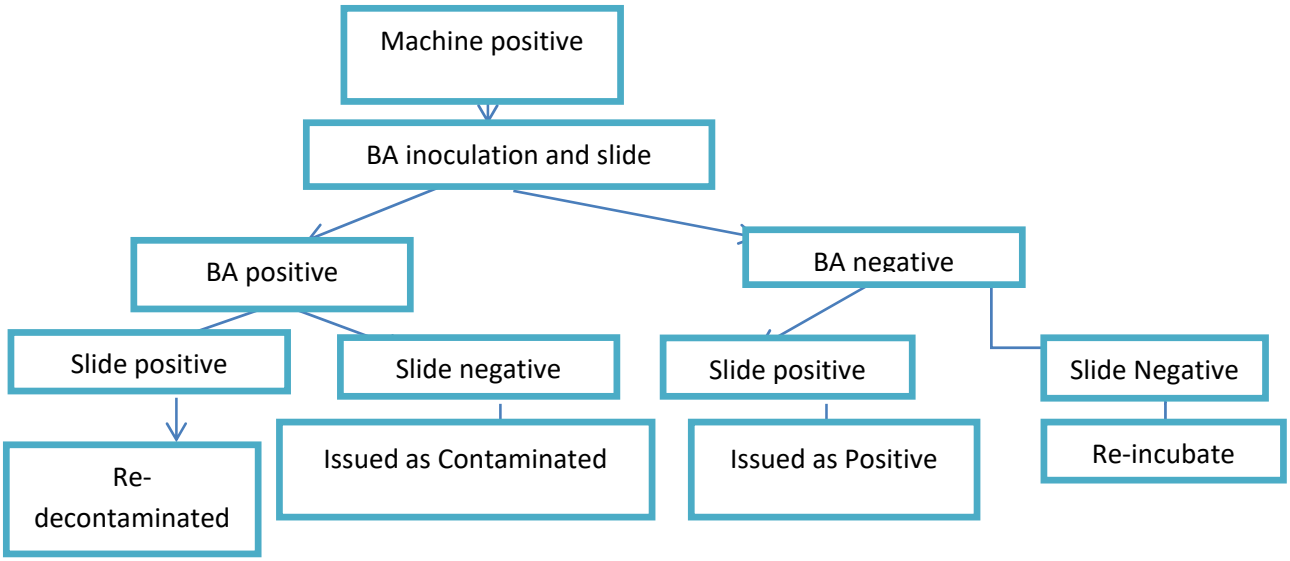
10	Positive and negative tubes will be issued by pull the respective drawer and press “positive or negative button as needed”, the machine displayed by the indicator light changing to green at the exact location of the tube in the instrument drawer
11	Remove the tube and scan
12	Continue MGIT culture work up.

LJ Media

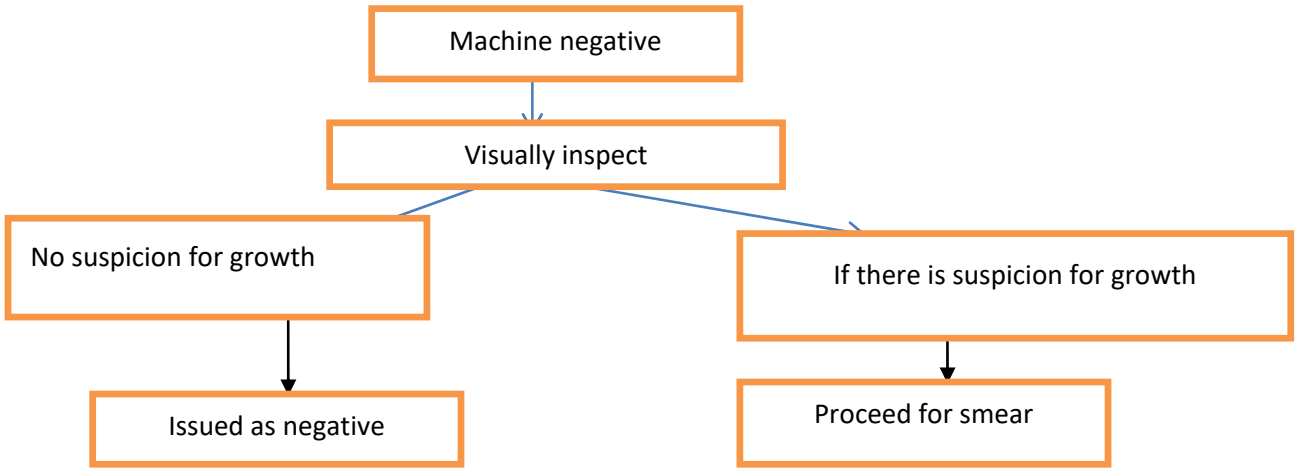
Step	Action
1.	Mark each LJ tube with laboratory number
2.	Decant excess water from the media
3.	Inoculate 2 to 3 drops of sediment and/or direct preparation in to two LJ media
4.	Wipe tubes and caps with a Mycobactericidal disinfectant.
5.	Loose the cap slightly and put the LJ tube in slant position facing upward in the incubator
6.	Make sure the fluid cover the surface
7.	Keep in a slant position for one week in the incubator (35 – 37 ^{o}C temperature)
8.	Check LJ two times a week for any contamination and fast grower Mycobacterium /NTM
9.	Read LJ tubes weekly until a positive growth obtained
10	If the LJ tube have growth and enough (>50 matured colony) for subsequent work , quantify the colony
11	If the LJ tube have growth and not enough for subsequent work write “P” on the work sheet and wait 1 to 3 week till enough growth obtained
12	Positive tube will be kept in the incubator till (maximum of 1 week) the subsequent work is done
13	If the LJ tube have no growth in 8th week issued as negative
14	Negative tube will be safely discarded from the laboratory after autoclaving

MGIT Work Ups:

Machine positive MGIT tube



When machine negative MGIT tube:



(38).

Annex 16: First line LPA procedure

Procedures for DNA extraction:

From decontaminated smear positive sediments;

Steps	Actions
1	Log specimen numbers onto a DNA extraction worksheet and record date and initials
2	Work on no more than 10 sediments and 2 controls at a time
3	Assemble sediments for the batch in the BSC
4	Vortex thoroughly for 15-20 seconds each of the 50ml conical tubes containing the sediments
5	Using a sterile disposable Pasteur pipette, transfer 500µl of each decontaminated sample to labelled 1.5ml screw cap tube
6	Load the 1.5ml screw cap tubes in a micro-centrifuge with aerosol-tight rotor.
7	Centrifuge for 15 minutes at 10,000 RCF or 10263RPM
8	Unload tubes from the microcentrifuge and carefully carry the tubes to the BSC
9	Discard supernatant from each tube by use of a 1000µl adjustable pipette
10	Resuspend each pellet in 100µl Lysis Buffer (A-LYS)
11	Mix the contents of each tube by use of a sterile tip followedby thorough vortexing for at least 15 to 20 seconds
12	Incubate the tubes for 5 minutes at 95 °C in a thermoblock
13	Add 100 µl Neutralisation Buffer (A-NB) and vortex the samplefor 5 seconds
14	Load the tubes into a microcentrifuge and spin for 5 minutesat 13,000 x g
15	Carefully carry the tubes to the BSC. Uncap tubes one at a time, and transfer 100µl of DNA-containing supernatant to a sterile 1.5ml screw cap tube
16	Store DNA at 2-8 °C for not more than 7 days. For longer storage, keep at -20 °C

DNA extraction from liquid culture isolates

Step	Action
1	Using a sterile disposable Pasteur pipette, transfer 1000µl of each thoroughly mixed liquid culture sample to labelled 1.5ml screw cap tube
2	Proceed similarly to the procedure for **decontaminated smear positive sediments**, from step 6 to 16

DNA extraction from solid culture isolates

Step	Action
1	Pipette 100µl lysis buffer (A-LYS) into sufficient number of 1.5ml screw cap tubes (1 tube per culture)
2	Use 1µl sterile disposable inoculation loop to collect bacteria from solid media with sufficient growth
3	Inoculate the bacteria into the lysis buffer. Break the clumps by aid of the inoculation loop
4	For 15-20 seconds thoroughly vortex to adequately mix
5	Proceed similarly to the procedure for **decontaminated smear positive sediments**, from step 12 to 16

Quality Control: H37Rv ATCC 25177 strains for positive control and molecular grade water for negative control was used during extraction.

Reagent Preparation and Master Mix procedures:

Step	Action
1	Thoroughly apply freshly prepared 0.5% bleach solution to all surfaces of PCR hood and worktop. Allow 15 minutes contact time release UV for 15-20 minutes
2	Wipe with damp cotton wool
3	Apply 70% ethanol to all the surfaces PCR hood and worktop
4	Thaw the two amplification mixes, A and B (AM-A and AM-B) at room temperature.
5	Gently invert to mix the contents of each tube, AM-A and AM-B

6	Carefully remove sufficient PCR tubes from stock pack, place in a PCR tube rack in the PCR hood. Ensure that the lids are closed.
7	Determine the number of samples to be amplified, and positive and negative control samples plus one contingency
8	Label PCR tubes accordingly.
9	Prepare a master mix containing AM-A and AM-B, 10:35 ratio respectively in a sterile screw cap tube (1.5ml) according to the number of samples and controls
10	Mix the master mix gently.
11	Pipette 45 µl of the master mix to each labelled PCR tube
12	Ensure all PCR tubes are tightly closed. Close the PCR tube rack
13	Place the stock reagents AM-A and AM-B back in the freezer at -20 °C
14	Wipe pipettes, PCR hood surfaces and worktop with 0.5% bleach followed by 70% ethanol

DNA addition

Step	Action
1	Thoroughly apply freshly prepared 0.5% bleach solution to all surfaces of PCR hood or worktop. Allow 15 minutes contact time
2	Wipe with damp cotton wool
3	Apply 70% ethanol to all the surfaces PCR hood
4	Obtain DNA samples from the refrigerator or freezer and allow to reach room temperature
5	Assemble PCR tubes containing 45µl master-mix reagent
6	Add 5µl of DNA to corresponding mastermixPCR tubes in the PCR hood. NB- No DNA should be added to the reagent control
7	Check that all PCR tubes are tightly closed
8	Wipe pipette, PCR hood surfaces with 0.5% bleach followed by 70% ethanol

Amplification

Step	Action
1	Thoroughly apply freshly prepared 0.5% bleach solution to work top surfaces.
2	Wipe with damp cotton wool
3	Apply 70% ethanol to all work top surfaces
6	Transfer PCR tubes to middle section of thermal cycler. Avoid placing tubes at the edges as this can result in evaporation of the contents during heating
7	Check program parameters and follow menu options to start appropriate program
8	Run the specific program • **Ver.2-cul** for samples from solid or liquid culture, • **Ver.2-dirsample** for decontaminated smear positive sediments
9	After the cycles are complete, proceed to the detection stage. If detection cannot be performed on same day, store PCR tubes with amplicons at 4°C for a maximum of 7days

Amplification

Step	Action
1	Thoroughly apply freshly prepared 0.5% bleach solution to work top surfaces.
2	Wipe with damp cotton wool
3	Apply 70% ethanol to all work top surfaces
4	For first use, set up the thermal cycler to the correct amplification profiles according to the instruction manual (see SOP on Use and Maintenance of Thermal Cycler).
5	If PCR tubes have bubbles at base, remove by swinging arm with tubes in hand in arc.
6	Transfer PCR tubes to middle section of thermal cycler. Avoid placing tubes at the edges as this can result in evaporation of the contents during heating
7	Check program parameters and follow menu options to start appropriate program
8	Run the specific program ,**Ver.2-cul** for samples from solid or liquid culture, **Ver.2-dirsample** for decontaminated smear positive sediments
9	After the cycles are complete, proceed to the detection stage. If detection cannot be performed on same day, store PCR tubes with amplicons at 4°C for a maximum of 7days

Detection

Step	Action
1	Pre-warm HYB and STR solutions (green and red) to 45°C in water bath (15 minutes total). Pre-warm RIN (rinse solution) and sterile distilled water to room temperature
2	Pre-warm TwinCubator to 45°C
3	Remove DNA strips from tube (shake strips down to end of tube then remove carefully holding the end of the strip with forceps) and mark them with provided pen according to detection worksheet.
4	Pipette 20µl DEN (denaturing solution) to one end of each well of a clean tray to be used
5	Add 20µl of corresponding amplified DNA sample to the denaturing solution in each well, and mix well by pipetting up and down 5 times
6	Incubate for 5 minutes at room temperature
7	Carefully add 1 ml HYB (hybridization solution) to each well and in the opposite end to the DEN/DNA mixture. Use single tip for each well. Do not splash mixture or contaminate neighbouring well
8	Gently tilt to shake and homogenize solution. Do not splash mixtures
9	Add each labelled strip to each well with coloured marker facing up. If strips turn over, re-position them with a fresh pipette tip. Strips must be completely covered by hybridization solution
10	Place tray on Twincubator and press "START" to incubate for 30 minutes at 45°C. From this point, press right arrow on Twincubator once to advance steps in protocol.
11	When alarm goes off, press right arrow key to stop
12	Remove HYB carefully by pipetting with individual sterile pipette tip or sterile disposable Pasteur pipette into a small plastic discard container containing undiluted bleach solution. Change tips or Pasteur pipettes between wells
13	Wipe off condensation that forms on Twincubator lid before every incubation step.
14	Add 1 ml STR (stringent buffer) per well and incubate for 15 minutes in Twincubator at 45°C. Press right arrow key to start.
15	When alarm goes off, press right arrow key. Completely remove STR as previously described for HYB removal.
16	Add 1 ml rinse solution (RIN) to each well. Press right arrow key to rinse the strips for 1 minute. When alarm goes off, press right arrow key. Completely remove RIN.
17	Add 1 ml of diluted Conjugate per well. Press right arrow to incubate at 37°C for 30 minutes on Twincubator

18	When alarm goes off, press right arrow to stop
19	Completely aspirate CON-D solution using Pasteur pipette
20	Add 1ml RIN per well. Press right arrow and incubate for 1 minute on Twincubator
21	When alarm goes off, press right arrow key to stop. Remove RIN completely and repeat Step 20
22	Remove RIN completelyand wash with 1 ml sterile distilled water per well on TwinCubator. Press right arrow key and incubate for 1 minute on TwinCubator
23	When alarm goes off, press right arrow key to stop. Remove water and add 1 ml of diluted substrate per well.
24	Place on Twincubator under aluminum foil for a maximum of 10 minutes. Look for colour reaction to indicate reaction completion after 4-5 minutes. If colour reaction is too weak, replace the foil and re-incubate for several more minutes, up to a maximum of 10 minutes
25	Wash twice for 1 minute with distilled water. Remove distilled water after each wash
26	Use forceps to transfer membrane strips to an absorbent paper and allow to air dry
27	Soak trays in 0.5% bleach for 15 minutes and rinse with distilled water
28	Clean pipettes, instruments and work area with freshly diluted 0.5% bleach, followed by 70% alcohol
29	Switch off the Twincubator after use

Interpretation of LPA strip results

Step	Action
1	Use forceps to transfer strips to the GenoType MTBDR*plus* Results Sheet provided with the kit
2	Align the bands Conjugate Control (CC) and Amplification Control (AC) on each strip with the respective lines on the sheet
3	Attach the strips to the results sheet using clear adhesive tape
4	Determine the band positivity and positions on each strip using the reference reading chart of the kit and mark the results on the worksheet
5	In order for a batch of results to be valid, the negative control strip must have a CC and AC band present, but no other bands must be visible.
6	If a positive result is obtained with the negative control, the whole batch must be repeated and measures taken to remove amplicon contamination from all rooms and equipment.
7	In order for patient results to be valid, CC (conjugate control) and AC (amplification control) bands must

	appear for every sample. The presence of TUB band indicates that *M.tuberculosis* complex is present in the sample
8	If CC is negative the conjugation or substrate reaction was unsuccessful either due to error in the procedure or due to problems with the reagent
9	If AC is positive, errors during extraction and amplification set-up and presence of amplification inhibitors in the specimen can be excluded
10	Signal of AC can be weak or even absent while results for other bands (TUB, rpoB, katG and inhA locus controls) may be positive. This might be due to competitive reactions between AC and TUB, ropB, katG, inhA during amplification. In this case, the strip can be evaluated.
11	A weak or missing AC band with negative test result for TUB, rpoB, katG and inhA locus controls may indicate potential mistakes during extraction and amplification set-up, or presence of amplification inhibitors. In this case, the test results are invalid
12	*rpoB* predicts RIF resistance, *katG* predicts high level INH resistance, *inhA* predicts low level INH resistance
13	The *rpoB, katG* and *inhA* each have a control band which must be present in order to interpret the results. Locus Control zones (*rpoB, katG, inhA*) detect a gene region specific for their respective genes. If the locus control zones are negative, then their respective mutation- specific positive bands cannot be considered for evaluation
14	A mutation in the relevant gene (and resistance to the relevant drug) is signified by either an absent wild type band and/or the presence of a mutant band for each gene cluster.
15	For results to be valid the bands (except CC) must be of intensity approximately equal to or greater than the intensity of the AC band.
16	If the TUB zone is negative, the tested bacteria does not belong to *M. tuberculosis* complex; therefore, presence or absence of any other bands (except CC and AC) cannot be considered for evaluation
17	When all wild type probes of a gene stain are positive and there is no detectable mutation within the examined regions, the tested strain may be considered sensitive for the respective antibiotic
18	In case of mutation, the respective amplicon cannot bind to the corresponding wild type capture probe on the strip due to the mismatch
19	The absence of a signal for at least one of the wild type probes may predict resistance to the respective antibiotic *indirectly*
20	Positive hybridization signal with a mutation-specific capture probe (for common mutations only!) may predict resistance to the respective antibiotic *directly*

	21	Presence of rare mutations that do not have mutation-specific capture probes may only be indicated by the lack of hybridization with one or more wild type probes

Annex 17: Second line LPA interpretation

Genotype MTBRD*sl* Ver 2.0 Interpretation

1. **Mutation Type for FQ**

- When gyrA WT1 is missing and gyrAMUT1 and gyrAMUT2 band absent
- When gyrA WT2 is missing and MUT1 band present
- When gyrA WT2 is missing and MUT2 band present
- When gyrB WT is missing and gyrB MUT1 or gyrB MUT2 band present
- When gyrA WT3 missing and gyrAMUT3A or gyrAMUT3B or gyrAMUT3C or gyrAMUT3D present

❖ **Interpretation:** Phenotypic resistant to FLQ

2. **Mutation Type for SLID**

- When rrs WT1 missing and rrs MUT1 present

❖ **Interpretation:** Phenotypic resistance to Capriomycin, Amikacin and Kanamycin

- When rrs WT1 missing and no rrs MUT1 is present

❖ **Interpretation:** Phenotypic resistant to Capriomycin, Kanamycin and Viomycin

- When rrs WT2 missing and rrs MUT2 is present

❖ **Interpretation:** Phenotypic resistant to Capriomycin, Kanamycin ,Viomycin and Amikacin

Mutation Type for Low level Kanamycin

- When eisWT1 missing and no eis MUT1 is present
- When eisWT2 is missing and eis MUT1 is present
- When eisWT2 is missing and no eis MUT1 is present
- When eisWT3 is missing and no eis MUT1 is present

Interpretation: Phenotypic resistant to low-level Kanamycin

NB: The **presence of wild type** and **absence of mutation** is interpreted as sensitive for those specific drugs.

Annex 18: First line MGIT DST procedure

Procedure

Dehydration of Lyophilized SIRE and PZA Drugs

Step	Action
1.	Using a sterile pipette or transfer pipette, reconstitute each of the SIRE (Streptomycin, Isoniazid, Rifampicin and Ethambutol) lyophilized drug vials with 4 ml of sterile distilled water.
2.	Reconstitute PZA drug vial with 2.5 ml of sterile distilled water.Use separate pipette for each drug.
3.	Aliquot in sterile cryovial tube by considering the average test done at each run
4.	Store aliquoted drugs at -20 ^{0}C up to 6 months or up to the date of original expiry, whichever comes first.
5.	Once thawed, discard any leftover drug and do not store or refreeze.

Inoculum preparation for MGIT DST

Preparation of inoculum from LJ Media

Step	Action
1.	Colonies from solid media may be used if they are no more than 15 days from the first appearance of positive growth.
2.	Using a sterile loop scrape as many colonies as possible trying not to remove any of the solid medium.
3.	Transfer the growth into a sterile tube containing 2-3drops of saline or distilled water and 8-10 sterile glass beads.
4.	Tighten the cap and vortex the tube for 2-3 minutes to break up any large clumps.
5.	The turbidity of the suspension should be greater than the McFarland number 1 standard.
6.	Let the suspension stand for 30 minutes undisturbed.
7.	Using a sterile pipette, carefully transfer the supernatant suspension into sterile tube containing 2-3drops of saline or distilled water

8.	The turbidity of this suspension should be greater than McFarland 0.5 standard.
9.	Adjust the turbidity of this suspension to McFarland 0.5 standard by adding sterile saline and adjusting visual comparison.
10.	Dilute 1.0 ml of this suspension in 4.0 ml of sterile saline and mix well. This 1:5 dilution will be used as the inoculum for DST

Preparation of 1:100 and 1:10 dilution (for SIRE and PZA growth control)

Step	Action
11.	Prepare the 1:100 by adding 100 µl of the seed (1-2 days old) or 1:5 dilution of 3-5 days old tube to 9.9 ml of saline
12.	Prepare the 1:10 dilution by adding 500 µL of the seed or 1:5 to 4.5 ml saline
13.	Repeat for all MGIT seed tubes or 1:5 dilutions.

Inoculation into SIRE set

Step	Action
1.	Label five MGIT tubes for each test isolate with GC (Growth Control), STR (streptomycin), INH (isoniazid), RIF (rifampicin), and EMB (ethambutol) and laboratory number.
2.	Place the tubes in the following sequence in the 5 tube AST set carrier, from left to right: GC, STR, INH, RIF, and EMB. N.B Epicenter label can be used for DST entry if AST carrier is not used
3.	Add 0.8 ml MGIT SIRE Supplement to each SIRE tube including growth control tube.
4.	Add 0.1 ml (100 µl) of reconstituted drug solutions to the corresponding labelled MGIT tubes.
5.	Do not add drugs to the MGIT GC tube.
6.	Using a sterile transfer pipette, dispense 0.5 ml from the top 1/3 of the 1:100 dilution tube in to the SIRE GC tube .
7.	Inoculate each labelled drug-containing tube with 0.5 ml of the inoculum prepared just inside the mouth of the tube so the inoculum runs down the inside of the tube.

8.	Immediately recap the tube tightly and mix by inverting the tube twice.
9.	Wipe all tubes and caps with a mycobactericidal disinfectant.

Inoculation into PZA set

Step	Action
1.	Label two PZA tubes for each test isolate with GC (Growth Control), or PZA (pyrazinamide).
2.	Place the tubes in the following sequence in the 2 tube AST set carrier, from left to right: GC, PZA. **N.B Epicenter label can be used for DST entry if AST carrier is not used**
3.	Add 0.8mL of PZA supplement to each PZA tube
4.	Add 0.1 ml (100 µl) of reconstituted PZA drug solutions to the corresponding labelled MGIT tubes.
5.	Do not add drugs to the PZA GC tube.
6.	Using a sterile transfer pipette dispense 0.5 ml from the top 1/3 of the **1:10** dilution tube in to the PZA GC tube
7.	Inoculate PZA drug-containing tube with 0.5 ml of the Inoculum prepared just inside the mouth of the tube so the inoculum runs down the inside of the tube
8.	Immediately recap the tube tightly and mix by inverting the tube twice.
9.	Wipe all tubes and caps with a mycobactericidal disinfectant.

Incubation

Step	Action
1.	Enter the inoculated set of DST specimens into the BACTEC 960 instrument using the AST set entry feature.
2.	Be sure that the tubes are loaded according to the order specified above
3.	Be sure that the caps are tightly closed
4.	Open the desired MGIT 960 drawer and press the “tube enter” key.
5.	The barcode scanner will light up.
6.	Scan the inoculated MGIT tube and load into the slot identified by the MGIT 960.

7.	Incubate MGIT tubes until the instrument flags them as positive
8.	Check MGIT 960 daily for indicator lights flagging positive
9.	Positive tubes will be displayed by the indicator light changing to green at the exact location of the tube in the instrument drawer.

Procedural Notes

- If the GC tubes become positive in less than 4 days or remain negative up to 21 days or some other conditions occur which may affect the test results, the instrument report will be as an Error ("**X**"). In such situations, the test needs to be repeated.
- If a laboratory routinely experiences x200 errors when performing DST in the local patient population, the 3-5 day culture can be used undiluted as a first step. Monitor this technique very closely to ensure that an excess of x400 errors is not subsequently produced.
- The turbidity should not be less than McFarland 0.5.
- The tube cap should be tightly closed.

Age of MGIT	Activities
1-2 days old	1. Positive MGIT cultures must have pure growth of *M. tuberculosis* (ZN positive, BAP negative, MPT/MTB 64 antigen test positive; in order to be tested for drug susceptibility.
	2. DST must not be set up on the same day a MGIT tube signals positive. The day a MGIT tube is positive by the instrument is considered **Day 0.**
	3. If MGIT tube**1-2 days old** after signaling positive, Vortex each seed tube for 1 minute to break up clumps
	4. Leave the tube undisturbed for about 20 minutes to allow large clumps to settle.
3-5 days old	1. If growth is on Day 3, 4, or 5, Vortex each seed tube for 1 minute to break up clumps
	2. Let the large clumps settle for 20 minutes.

		3. Dilute 1.0 ml of the positive broth in 4.0 ml of sterile saline (1:5 dilutions).
		4. Use this well–mixed, diluted culture for inoculation of the drug set.
	Longer than five days	1. Vortex MGIT broth for 1 min. Leave 20 minutes to allow any large clumps to settle.
		2. Supplement a new MGIT tube with 0.8 ml Growth Supplement *without* PANTA.
		3. Remove inoculum from the supernatant broth and make a 1:100 dilution of the positive MGIT tube into sterile saline
		4. Mix tube well by inverting gently several times.
		5. Inoculate new MGIT tube with 0.5 ml of the 1:100 diluted specimen.
		6. Cap tube tightly and mix well by inverting gently several times.
		7. Enter tube into MGIT 960 instrument and monitor until it becomes positive.
		8. Use new tube for DST within one to five days of positivity as described above.
Result Interpretation		• MGIT 960 system automatically interprets results as "**S**" (susceptible) or "**R**" (resistant). • An isolate is defined as RESISTANT: if 1% or more of the test population grows in the presence of the critical concentration of the drug. • An isolate is defined as SUSCEPTIBLE: if there is no growth or less than 1% of the test population grows in the presence of the critical concentration of the drug.