

**ADDIS ABABA UNIVERSITY,  
COLLEGE OF HEALTH SCIENCE  
SCHOOL OF ALLIED HEALTH SCIENCE  
DEPARTMENT OF MEDICAL LABORATORY SCIENCE**



**COMPARATIVE PERFORMANCE BETWEEN XPERT MTB/RIF ASSAY, LED FLUORESCENCE MICROSCOPY, AND ZN LIGHT MICROSCOPY FOR DETECTION OF MYCOBACTERIUM TUBERCULOSIS IN SPUTUM SAMPLES IN ADDIS ABABA, ETHIOPIA**

**By: Daniel Dejene (BSc)**

**Advisors:**

BiniamTaye (BSc, MPH, PHD candidate, Assistant Professor), College of health Sciences, Department of Medical Laboratory Sciences, Addis Ababa University

KassuDesta (BSc, MSc, PHD candidate, Assistant Professor), college of health Sciences, Department of Medical Laboratory Sciences, Addis Ababa University

**Co-Advisor:** AbrehamTesfaye (BSc, MSc, PHD candidate) AARHRL

**A THESIS SUBMITTED TO COLLEGE OF HEALTH SCIENCES, DEPARTMENT OF MEDICAL LABORATORY SCIENCES. IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CLINICAL LABORATORY SCIENCES (CLINICAL LABORATORY MANAGEMENT AND QUALITY ASSURANCE)**

May 2014  
Addis Ababa

**ADDIS ABABA UNIVERSITY,  
COLLEGE OF HEALTH SCIENCE  
SCHOOL OF ALLIED HEALTH SCIENCE  
DEPARTMENT OF MEDICAL LABORATORY SCIENCE**

Comparative performance between Cepheid Xpert MTB/Rif assay, LED fluorescence microscopy, and ZN Light microscopy for detection of Mycobacterium Tuberculosis in sputum samples in Addis Ababa, Ethiopia

By: Daniel Dejene (BSc)

Department of Medical Laboratory sciences, College of Health Sciences,  
Addis Ababa University

Approved by: the Examining Board

---

Chairman, Department Graduate Committee

---

Advisor

---

Advisor

---

Examiner

---

Examiner

## **Acknowledgement**

First of all I would like to thank the Almighty God, who gave, gives and will give not what I am needed but what deserve to me.

I wish to acknowledge University of Addis Ababa College of Health Sciences Department of Medical Laboratory Technology for gives us a chance to take part in this interesting program and valuable support in academic and research process. My deepest gratitude and appreciation goes to my advisors Mr. Kassu Desta, and Mr. Biniam Taye for their unreserved guidance, constructive suggestions and comments from the stage of topic selection to come up with this final success.

My sincere appreciation also extends to my co-advisor Mr. Abreham Tesfaye, Head of AAHRL and all laboratory staffs of AAHRL for their priceless support by materials and advice. I am also grateful to Addis Ababa health bureau and the respective health facilities for permitting this project to be carried out in AAHRL and to the participants for their role in the project.

The last but not the least my great thanks extend to Mr. Kindu Amsalu from KOICA and Dereje Assefa from SMLT for their advice and constructive comments and technical support throughout the entire process of this study. Finally, I am always proud of my family specially my wife and my daughters for giving me their time and moral support.

# Table of content

| Contents                                                                  | Page |
|---------------------------------------------------------------------------|------|
| ACKNOWLEDGEMENT .....                                                     | I    |
| TABLE OF CONTENT .....                                                    | II   |
| ACRONYMS .....                                                            | V    |
| ABSTRACT .....                                                            | VII  |
| 1. INTRODUCTION .....                                                     | 1    |
| 1.1. Back Ground .....                                                    | 1    |
| 1.2. Statement of the problem .....                                       | 2    |
| 2. RATIONALE OF THE STUDY .....                                           | 3    |
| 3. LITERATURE REVIEW .....                                                | 4    |
| 4. OBJECTIVES OF THE STUDY .....                                          | 10   |
| 4.1. General objective: .....                                             | 10   |
| 4.2. Specific objectives; .....                                           | 10   |
| 5. METHODOLOGY .....                                                      | 11   |
| 5.1. Study Area and study period: .....                                   | 11   |
| 5.2. Study Design: .....                                                  | 11   |
| 5.3. Study Population: .....                                              | 11   |
| 5.4. Study Subjects: .....                                                | 12   |
| 5.5. Inclusion .....                                                      | 12   |
| 5.6. Exclusion criteria .....                                             | 12   |
| 5.7. Sample Size and Sampling Procedures: .....                           | 12   |
| 5.7.1. Sample Size: .....                                                 | 12   |
| 5.7.2. Sampling Procedures: .....                                         | 13   |
| 5.7.3. Data collection procedure .....                                    | 13   |
| 5.8. Laboratory methods .....                                             | 13   |
| 5.8.1. Sample collection .....                                            | 13   |
| 5.8.2. Direct smear preparation and staining .....                        | 14   |
| 5.8.3. Examination and grading of ZN and fluorescent stained slides ..... | 14   |
| 5.8.4. Xpert MTB/RIF assay laboratory procedure .....                     | 14   |
| 5.8.5. Laboratory procedure for sputum culture and identification .....   | 15   |
| 5.8.6. Safety Precautions .....                                           | 17   |
| 5.8.7. Quality control .....                                              | 17   |

|                                                                                                                                              |    |
|----------------------------------------------------------------------------------------------------------------------------------------------|----|
| 5.8.8. Data Management, handling and analysis .....                                                                                          | 17 |
| 5.8.9. Ethical considerations .....                                                                                                          | 18 |
| 6. RESULTS.....                                                                                                                              | 19 |
| 6.1. Socio-demographic characteristics of the study population .....                                                                         | 19 |
| 6.2. Comparison of results of direct ZN microscopy, direct LED-FM microscopy and Xpert<br>MTB/RIF assay for M.tuberculosis Detection .....   | 20 |
| 6.3. Comparison of results of LED ZN microscopy, direct LED-FM microscopy and Xpert<br>MTB/RIF assay with culture method to detect MTB. .... | 22 |
| 6.4. AFB quantification reported by direct smear technique and XpertMTB/RIF assay and sputum<br>consistency .....                            | 22 |
| 6.5. Test characteristics of the direct ZN microscopy, LED fluorescent Microscopy and GenXpert<br>test according to sputum consistency.....  | 24 |
| 7. DISCUSSION .....                                                                                                                          | 26 |
| 7.1. Strengths and limitations of the study .....                                                                                            | 29 |
| 7.1.1. Strength.....                                                                                                                         | 29 |
| 7.1.2. Limitation .....                                                                                                                      | 29 |
| 8. CONCLUSION AND RECOMMENDATION .....                                                                                                       | 30 |
| 8.1. Conclusion.....                                                                                                                         | 30 |
| 8.2. Recommendations .....                                                                                                                   | 30 |
| 9. REFERENCES:.....                                                                                                                          | 32 |
| 10. ANNEXES .....                                                                                                                            | 37 |
| Annex-I: Request paper .....                                                                                                                 | 37 |
| Annex-II: Laboratory data .....                                                                                                              | 37 |
| Annex-III: IUATLD/WHO recommended grading of sputum microscopy and Xpert MTB/RIF<br>assay MTB/RIF results. ....                              | 39 |
| Annex- IV: Ziehl-Neelsen and Fluorochrome Staining Reagent and culture sample<br>decontaminating reagent preparation.....                    | 40 |
| Annex V: Information sheet for study subjects (English version).....                                                                         | 43 |
| Annex VI: Informed consent form (Amaric version) .....                                                                                       | 44 |
| Annex VII: Declaration .....                                                                                                                 | 46 |

## List of tables

|                                                                                                                                                                                                   |    |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 1. Distribution of study subjects attending at Addis Ababa regional health and research laboratory according to age and sex (n = 358) in Addis Ababa, 2014.....                             | 19 |
| Table 2. Frequency distribution for HIV status of the participants with positive MTB detection pattern in Addis Ababa, 2014. ....                                                                 | 19 |
| Table 3. Percent Comparison between direct ZN and LED-FM microscopy, ZN microscopy and Xpert MTB/RIF assay, and LED-FM microscopy and Xpert MTB/RIF assay to detect MTB in Addis Ababa, 2014..... | 20 |
| Table 4. Test characteristics of the direct ZN microscopy, Xpert MTB/RIF assay and LED fluorescent Microscopy against LJ culture results (n=167) in Addis Ababa, 2014.....                        | 21 |
| Table 5. Performance parameters of direct LED fluorescent microscopy, direct ZN smear microscopy and MTB/RIF assay against culture results. (n=167) in Addis Ababa, 2014.....                     | 22 |
| Table 6. Distribution of quantified reported results of LED-FM, ZN microscopy technique and Xpert MTB/RIF assay in Addis Ababa, 2014.....                                                         | 23 |
| Table 7. Distribution of reported results of LED-FM, ZN microscopy technique and Xpert MTB/RIF assay against consistency of sputum.....                                                           | 24 |
| Table 8. MTB complex detection by Xpert MTB/RIF assay, ZN smear microscopy and LED-FM smear microscopy among culture confirmed cases against their treatment history pattern (n=167) .....        | 25 |

## Acronyms

|        |                                                           |
|--------|-----------------------------------------------------------|
| AARHRL | Addis Ababa Region Health and research Laboratory         |
| AAU    | Addis Ababa University                                    |
| AFB    | Acid-Fast Bacilli                                         |
| AHRI   | Armour Hansen Research Institute                          |
| AIDS   | Acquired Immune Deficiency Syndrome                       |
| BCG    | BacilleCalmetteGuérin                                     |
| BSC    | Biosafety Cabinet                                         |
| BSL    | Biosafety Level Laboratory                                |
| CDC    | Center for Disease Control and Prevention                 |
| CFU    | Colony Forming Unit                                       |
| CI     | Confidence Interval                                       |
| CSA    | Central Statistical Authority                             |
| CSF    | Cerebro-spinal fluid                                      |
| Ct     | Cycle threshold                                           |
| CXR    | Chest X-ray                                               |
| DNA    | Deoxyribonucleic Acid                                     |
| DOTS   | Directly Observed Therapy Short course                    |
| DST    | Drug Sensitivity Testing                                  |
| FM     | Fluorescent microscopy                                    |
| FMOH   | Federal Ministry of Health                                |
| HBCs   | High-Burden Countries                                     |
| HIV    | Human Immunodeficiency Virus                              |
| IRB    | Institutional Review Board                                |
| IUATLD | International Union against Tuberculosis and Lung Disease |
| LED    | Light Emitting Diode                                      |
| LJ     | Lowenstein-Jensen                                         |

|        |                                       |
|--------|---------------------------------------|
| MDG    | Millennium Development Goals          |
| MDR    | Multi Drug Resistant                  |
| MGIT   | Mycobacterium Growth Indicator Tube   |
| MTBC   | Mycobacterium Tuberculosis Complex    |
| NAATs  | Nucleic Acid Amplification Tests      |
| NPV    | Negative predictive value             |
| NTM    | Non-tuberculosis mycobacterium        |
| NTP    | National Tuberculosis program         |
| PCR    | Polymerase Chain Reaction             |
| PPV    | Positive predictive value             |
| PTB    | Pulmonary Tuberculosis                |
| QC     | Quality Control                       |
| ROC    | Receiver Operating Characteristic     |
| rpoB   | RNA polymerase $\beta$                |
| SOP    | Standard operating procedures         |
| SPC    | Sample processing control             |
| TB     | Tuberculosis                          |
| UV     | Ultra Violet                          |
| WHO    | World Health Organization             |
| XDR TB | Extremely Drug Resistant tuberculosis |
| ZN     | Ziehl-Neelsen                         |



## Abstract

**Background:** In Ethiopia, the main tool used to diagnosis pulmonary tuberculosis is Ziehl-Neelsen microscopy. The emergence of drug-resistant forms of tuberculosis which needs more resources to detect, to successfully treat and effectively reduce the burden is among the top challenges in the country. Tuberculosis case detection among vulnerable population groups including children, HIV positives and other high risk groups remains low due to insensitive diagnostic tools. Due to its low sensitivity, Ziehl-Neelsen microscopy does not detect all positive TB cases among TB suspects; as a result tuberculosis transmission by false negative cases increases. Therefore, there is a need to assess the incremental value of new diagnostic tool like Xpert MTB/RIF assay and Light Emitted Diode Fluorescent microscopy for tuberculosis detection in our setting.

**Objective of the study:** To determine the performance of Xpert MTB/RIF assay in comparison with Light Emitted Diode Fluorescent microscopy and Ziehl-Neelsen smear microscopy for detection of Mycobacterium Tuberculosis in sputum samples in Addis Ababa.

**Methods and Materials:** A prospective cross-sectional study from December, 2013 to April, 2014 was conducted in AAHRL to compare the performance of light emitting diode fluorescent microscopy (LED-FM), Xpert MTB/RIF assay and Ziehl-Neelsen(ZN) smear microscopy in the diagnosis of pulmonary tuberculosis (PTB) Adult patients. LJ culture media was used as the gold standard for sub-samples. All data was entered into an excel spreadsheet, then transfer and analyzed using SPSS version 20. Descriptive statistics was performed and data presented in tables. McNamara's and Kappa statistics was used for proportion and the percent agreement analysis.

**Results:** A total of 358 pulmonary tuberculosis and MDR-TB (Multi drug resistance tuberculosis) suspected patients were enrolled from December, 2013 to April, 2014 in Addis Ababa Health research and Laboratory with mainly young adults of median age 30.5. After excluding 8 samples that were invalid by Xpert MTB/RIF assay 350 samples were analyzed statistically and The percent agreement between Light Emitted Diode Fluorescent microscopy and Ziehl-Neelsen smear microscopy, Xpert MTB/RIF assay and Light Emitted Diode Fluorescent microscopy, Xpert MTB/RIF assay and Ziehl-Neelsen smear microscopy for MTB detection showed that  $k=75\%(95\%CI, 67\% \text{ to } 81\%)$ ,  $p<.0005$ ,  $k=81.8\%(95\%CI, 81\% \text{ to } 82.6\%)$ ,

$p < .0005$  and  $k = 72.6\%$  (95% CI, 61.6% to 82.6%),  $p < .0005$ , respectively. The percent agreement of the three tests showed that there were an overall percent agreement of 87.4%, 84.6%, and 90.9% between Light Emitted Diode Fluorescent microscopy and Ziehl-Neelsen microscopy, Xpert MTB/RIF assay and Ziehl-Neelsen microscopy, and Light Emitted Diode Fluorescent microscopy and Xpert MTB/RIF assay respectively. In the sub group study the sensitivity, specificity, PPV and NPV achieved with direct Ziehl-Neelsen microscopy, Light Emitted Diode Fluorescent microscopy and Xpert MTB/RIF using culture as a gold standard in 167 (47%) samples were (76.9%, 96%, 97.8% and 64%), (81.2%, 90%, 95%, 67.2%) and (89.7%, 92%, 96.3%, 86.2%) respectively.

Conclusion: Xpert MTB/RIF assay has better yield than Light Emitted Diode Fluorescent smear microscopy and Ziehl-Neelsen smear microscopy with incremental yield of 12.8% and Light Emitted Diode Fluorescent smear microscopy has better yield than Ziehl-Neelsen smear microscopy with incremental yield of 4.8% therefore Xpert MTB/RIF assay is a highly accurate diagnostic tool for MTB detection, which achieves a substantial reduction in diagnostic delay With overall performance characteristics that are superior to Light Emitted Diode Fluorescent smear microscopy and Ziehl-Neelsen smear microscopy.

Keywords: Light Emitting Diode Fluorescent Microscopy (LED-FM), Ziehl-Neelsen (ZN), Xpert MTB/RIF assay.

# 1. Introduction

## 1.1. Back Ground

TB (Tuberculosis), a bacterial disease caused by *Mycobacterium tuberculosis*, and occasionally by other species of *Mycobacterium tuberculosis* complex which include *M. africanum* (West African 1 and West African 2), *M. canettii*, *M. bovis*, *M. caprae*, *M. microti*, *M. pinnipedii* and the attenuated for vaccine purpose *M. bovis* BCG. All are considered as AFB (acid fast bacilli), non-spore forming, and non-motile slow growing bacterium. However some morphological and biochemical differences are present which can be useful for identifying specific members of the MTC (*mycobacterium tuberculosis* complex) [1, 2].

Tuberculosis remains a major public health problem worldwide. In 2012, an estimated 8.6 million people developed TB of which only 66% were notified and 1.3 million died, of these 320 000 deaths were among HIV-positive people. Geographically, the burden of TB is highest in Asia and Africa. India and China together account for almost 40% of the world's TB cases. About 60% of cases are in the South-East Asia and Western Pacific regions and Africa has 27% of the world's cases. Worldwide, 3.6% of new cases and 20% of previously treated cases were estimated to have MDR-TB. India, China, the Russian Federation and South Africa have almost 60% of the world's cases of MDR-TB. The highest proportions of TB patients with MDR-TB are in Eastern Europe and central Asia. In the same year, there were an estimated 309 600 of new and 1.72 million previously treated MDR-TB cases. Among notified MDR TB patients only 84 000 cases were confirmed MDR TB [3].

Tuberculosis is a major cause of morbidity and mortality as well in Ethiopia and a case detection rate is limited to 35.8 %. WHO 2012 estimate shows TB prevalence of 237/100,000 and 1700 and 550 MDR-TB cases among new and retreatment cases respectively and also the national TB prevalence survey 2011 shows a prevalence of 277/100,000 of Bacteriological confirmed TB and is 108/100000 smear positive TB, TB Incidence (all forms): 258/100,000, TB mortality: 18/100,000 and new infection rate 19.7%. MDR-TB burden in Ethiopia is not well known but based on drug resistant survey in 2003-2005 it is estimated to be 1.6% and 11.8% MDR-TB among new and previously treated TB cases respectively[ 4, 5, 6].

## 1.2. Statement of the problem

A major reason for poor TB case detection and enrolment to treatment is the inadequacy of diagnostic tools. For decades, the mainstay of TB diagnosis has been sputum-smear microscopy for patients with suspected pulmonary TB, followed by chest radiography in those with negative sputum smears. Furthermore, smear microscopy cannot diagnose MDR-TB. For this reason some laboratories in Ethiopia have been upgraded in recent years. These have now the capacity to do Drug Susceptibility testing and to assist clinicians with advanced culture techniques to come to a fast and adequate diagnosis [5, 6]. WHO recommended that Xpert MTB/RIF should be used as the initial diagnostic test in persons suspected of having HIV-associated TB and in those at risk of MDR-TB. It may also be used as a follow-on test to microscopy, especially for patients with smear-negative specimens [7]. So Xpert MTB/RIF assay has been added to the tools of the TB laboratory, and this opens new perspectives for research and for clinical decision making.

According to WHO (world health organization) 2011 report, globally 3.2% of incident cases of TB (290,000) are estimated to have MDR-TB. There are 27 identified high burden countries that carry 86% of the world MDRTB burden, Ethiopia is one of them[8]. Tuberculosis (TB) remains a leading infectious cause of death worldwide, contributing to over 1.4 million deaths annually; yet 35% of all cases go undetected, and an additional 7% are diagnosed too late to prevent death . The burden of TB is most profound in sub-Saharan Africa, where incidence rates are over twice the global average, and the Millennium Development Goals' 2015 target of a 50% reduction in TB prevalence and mortality from 1990 rates is unlikely to be reached [9, 10, 11]. A key contributor to this burden of morbidity and mortality is poor diagnosis; only 60% of new TB cases in Africa are ever detected. Inadequate case detection reflects, in part, the limitations of sputum smear microscopy (low sensitivity) [12].

Early treatment of tuberculosis (TB) is hindered by the lack of rapid, accurate diagnostic modalities that can be applied in resource-limited settings (RLS). Sputum smear microscopy which is the cheapest and the most available method of TB diagnosis identifies TB in less than half of patients with HIV/ TB co-infection .Access to mycobacterium culture is limited and where available results are often delayed by several weeks [13]. The 2010/11 Ethiopian national TB Prevalence survey showed that smear positive cases accounted for only 43% of culture positive cases. This indicates the need for more sensitive and specific diagnostics for improving the diagnosis of smear negative TB cases. [14]

## **2. Rationale of the study**

TB case detection in Ethiopia is about half of the World Health Organization (WHO) global target, standing at 36% according to FMOH administrative report for the year 2009/10. The detection rate remains low as compared with WHO target of 70 % case detection. The limited diagnostic capacity for TB in the country remains a challenge to improving case detection rates [4]. Ethiopia has the seventh highest burden of TB globally and ranks third in Africa. TB is the eighth leading cause of hospital admissions and the third leading cause of hospital deaths in Ethiopia. DOTS strategy, which is the backbone of global TB control aimed at the diagnosis of 70% of new smear positive TB cases and achieving 85% cure can be effective through accurate laboratory diagnosis methods [14].

Therefore, there is a need of highly sensitive, specific and feasible technique for combating TB in Ethiopia and to achieve the target set for the Millennium Development Goals (MDG) by 2015 [15]. Even though Xpert MTB/RIF assay is a promising innovation in routine TB diagnosis owing to its high sensitivity, specificity and rapid turnaround time of only two hours, it needs to be evaluated in Ethiopian context, So this study is aimed to compare Xpert MTB/RIF assay with LED-FM and Light microscopy for detection of Mycobacterium Tuberculosis in sputum samples in the case of Addis Ababa, as a result this study will add value as base line data for further study and gives information for the program owners and policy makers.

### 3. Literature Review

In middle- and high-income countries, development continued with innovations in microscopy like light emitting diode (LED) microscopes, microbiological culture (for example, rapid automated liquid culture systems, like the Becton Dickinson MGIT 960 (Becton Dickinson, Sparks, Maryland, USA), nucleic acid amplification systems for example, (Hain Lifesciences, Nehren, Germany) line probe assays and automated systems, such as the Cepheid Xpert MTB/RIF system (Cepheid, Inc., Sunnyvale, CA, USA) [9] .

Xpert MTB/RIF assay is a semi-quantitative nested real-time PCR in-vitro diagnostic test for the detection of Mycobacterium tuberculosis complex DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputa that are either acid-fast bacilli (AFB) smear positive or negative; The assay detects MTB and rifampicin resistance; conferring mutations of the *rpoB* gene using three specific primers and five unique molecular probes. It provides results in less than two hours and has minimal bio-safety requirements and training [16]. Real-time PCR is a method based on the detection and quantitation of PCR products as they accumulate which occurs by monitoring of a fluorescent signal in real time as PCR products accumulate. The signal which is emitted increases at a rate which is directly proportional to the amount of PCR product present in the reaction. A detectable increase in fluorescence above a baseline value indicates PCR product is being accumulated. The exact point at which this occurs is known as the Cycle threshold (CT) value [17].

Smear microscopy is a low-cost method and the frontline tool for TB diagnosis across the developing world, is effective in detecting the most infectious cases and can be performed in basic laboratories. The introduction of fluorescence microscopy has increased the sensitivity of the test, and procedural improvements have increased throughput. Despite these gains, detection is poor in specimens without a relatively high bacterial load; many cases remain undiagnosed. Extra-pulmonary TB diagnosis and DST cannot be performed with microscopy. So it remains the cornerstone of TB diagnosis in the majority of high burden settings. It is a quick, inexpensive, and specific method for TB diagnosis with a sensitivity of up to 80% in immune competent patients and 20% in HIV co-infected patients [18, 19].

A systematic meta-analysis review of the available data in smear microscopy comparison by WHO revealed that LED FM was more sensitive by 6% (95% CI, 0.1–13%), with no appreciable loss in specificity than Ziehl-Neelsen (standard) microscopy; and LED microscopy was 5% (95% CI, 0–11%) more sensitive and 1% (95% CI, -0.7% - 3%) more specific than conventional fluorescence microscopy [20]. Another systematic review by Steingart *et al.* [12] revealed that the sensitivity of FM was on an average 10% (95% CI = 5–15%) more than conventional microscopy with similar specificities. Similar results have been demonstrated in other settings, including in developing economies with high TB burden [21].

In 2011, the World Health Organization (WHO) recommended that light-emitting diode fluorescence microscopy (LED-FM) be phased in as an alternative to conventional light microscopy using Ziehl-Neelsen (ZN) stain for the detection of *Mycobacterium tuberculosis* acid-fast bacilli in sputum smears for the diagnosis of pulmonary tuberculosis (PTB) [13]. LED-FM using auramine phenol stain has been shown to be more sensitive (~10%) than conventional microscopy in controlled laboratory settings [22].

Conventional FM using a mercury vapour lamp is expensive with high maintenance cost and limited lifespan (typically 200–300 h). So Light-emitting diode (LED) FM gives a better advantage. LED technology provides a cheap and reliable light source with a usable lifespan of > 50,000 h; repeated on-and-off switching does not reduce its usable lifespan, and it does not pose a potential toxicity risk. The fact that LED FM does not require a darkened environment greatly enhances the practical operability of using it to provide decentralized diagnostic services [23, 24]. Compared to mercury vapour FMs, LED microscopes are less expensive and have lower maintenance requirement [25].

In a comparative study of cost and performance of LED FM microscopy with ZN microscopy among HIV-TB co-infected patients, LED FM showed similar overall sensitivity, but median reading time was much quicker with LED FM [26]. In another study in Indonesia, a country with high TB burden, lower specificity was reported in LED FM over ZN microscopy [27] (although both greater than 90%). Some studies reported similar specificities in certain settings [12]. Marais *et al.* also found high inter-reader kappa value for LED FM over conventional FM and light microscopy [28].

The Xpert MTB/RIF assay has analytic sensitivity of five genome copies of purified DNA, and 131 CFU/mL of *M. tuberculosis* spiked into sputum. No cross-reactivity with non-tuberculosis mycobacterium (NTM) was detected. TB and resistance to rifampicin were correctly detected when NTM DNA or mixed susceptible and resistant strains were present. The sample reagent, added in a 2:1 ratio to sputum, killed  $.6 \log_{10}$  CFU/mL of *M. tuberculosis* with 15 min of exposure and rendered .97% of sputum smear-positive samples negative by Lowenstein–Jensen culture. No infectious aerosols were detected following the Xpert MTB/RIF inoculation procedure and sample testing [29, 30]

A study conducted in South Korea to assess accuracy and turnaround time of XPERT/MTB RIF assay in sputum samples showed that among 681 patients in whom Xpert MTB/RIF assay was requested the sensitivity, specificity, PPV and NPV of Xpert MTB/RIF assay for diagnosis of PTB were 79.5% (124/156), 100.0% (505/505), 100.0% (124/124) and 94.0% (505/537), respectively. Those for the detection of rifampicin resistance were 57.1% (8/14), 100.0% (113/113), 100.0% (8/8) and 94.9% (113/119), respectively. The median TAT of Xpert MTB/RIF assay to the report of results and results confirmed by physicians in outpatient settings were 0 (0–1) and 6 (3–7) days, respectively [31].

A multicenter study done by the Foundation for Innovative and New Diagnostics (FIND) in Lima, Peru; Baku, Azerbaijan; Cape Town and Durban, South Africa; and Mumbai, India on direct MTB/RIF test performance showed that the sensitivity was 551 of 561(98.2%) patients with smear-positive tuberculosis and 124 of 171(72.5%) with smear-negative tuberculosis (72.5%) and a specificity of 604 of 609(99.2%) patients without tuberculosis. As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified 200 of 205(97.6%) patients with rifampin-resistant bacteria and 504 of 514 (98.1%) with rifampin-sensitive bacteria[32].

Another study done in Malaysia shows that Of 15 culture-positive TB cases, single Xpert assay accurately detected only eight previously undiagnosed TB cases, resulting in a sensitivity, specificity, positive predictive value and negative predictive value of 53.3% (95% CI 30.12-75.2%), 100% (95% CI 96.6-100%), 100% (95% CI 67.56-100%) and 94.0% (95% CI 88.2-97.1%), respectively. Only 1 of 15 (6.7%) active TB cases was smear-positive. [33].



A study done in India with different samples, the majority of specimens came from sputum, CSF, and pleural fluid of the patients shows that 9(2.16%) out of 416 specimens with valid rifampicin test were found to be rifampicin resistant, six from sputum, one from pus, one from CSF, and one from pleural fluid. All 26 specimens that were reported as “scanty AFB” in the sputum smear yielded a positive result in the Xpert MTB/RIF assay [34].

A study done in Indonesia among 404 tuberculosis suspects from the outpatient clinic and 256 from the HIV clinic shows that, mycobacterium culture was positive in 12.6% and 27%, respectively. The optimal sensitivity of LED-FM was achieved by using a threshold of 2 AFB/length. LED-FM had a higher sensitivity (75.5% vs. 54.9%,  $P = 0.01$ ) but lower specificity (90.0% vs 96.6%,  $P = 0.01$ ) compared to ZN microscopy. HIV was associated with a lower sensitivity but similar specificity. The average reading time using LED-FM was significantly shorter (2.2360.78 vs 5.8261.60 minutes,  $P = 0.01$ ), while costs per slide were similar. [35].

A study done in South Africa and Tanzania showed that Compared with the combined binary smear and culture results as a reference standard, the Xpert MTB/RIF assay had high sensitivity (97.0%, 95% CI 95.8—97.9), but poor specificity (48.6%, 45.0—52.2) The reduction in positivity rates with Xpert MTB/RIF were slower than those with the standard methods. The reduction in detection of quantitative M tuberculosis DNA with Xpert MTB/RIF correlated with smear grades ( $\rho = -0.74$ ;  $p < 0.0001$ ), solid culture grades ( $\rho = -0.73$ ;  $p < 0.0001$ ), and time to liquid culture positivity ( $\rho = 0.73$ ;  $p < 0.0001$ ).Boehme et al. documented with one sputum sample, the Gene Xpert had a sensitivity of 92% for all culture-positive specimens. This increased to 96% for two samples and 98% for three. Specificity on non-TB cases was 99% with one sputum sample, declining marginally to 98% with three samples. However, for culture-positive, smear-negative specimens, sensitivity using one sputum sample was 73% rising to 90% with three samples [36]. Similar study done in west Tanzania showed Xpert MTB/RIF Assay achieved 88.4% (95%CI=78.4% to 94.9%) sensitivity among patients with a positive culture and 99% (95%CI=94.7% to 100.0%) specificity in patients who had no TB [37].

Another study done in Uganda in 235 children shows that The Xpert MTB/RIF test had a sensitivity of 79.4% (95% CI 63.2 - 89.7) and a specificity of 96.5% (95% CI 93 - 98.3). The Xpert MTB/RIF test identified 13 of the 14 (92.9%) smear positive-culture positive and 14 of the 20 (70%) smear negative -culture positive cases. The Xpert MTB/RIF identified twice as many cases as the smear microscopy (79.4% Vs 41.2%) [38].

Xpert MTB/RIF assay evaluation study done in Saudi Arabia showed that Out of the 239 specimens investigated Compared with the culture, the Xpert MTB/RIF assay achieved 95.4% (95% CI: 89-100%) sensitivity, and 100% (95% CI: 93.6-100%) specificity for respiratory samples, while the sensitivity for non-respiratory specimens was 94.4% (95% CI: 90.2-98.5), and the specificity for non-respiratory specimens was 100% (95% CI: 95.8-100%). Overall, a 95.2% (95% CI: 87.6-100) sensitivity, and 100% (95% CI: 92.4-100%) specificity, was observed for the Xpert MTB/RIF assay compared with conventional methods for MTBC detection [39].

Another Nucleic Acid amplification assay comparison study in German showed that Among culture-positive samples (n = 68), overall sensitivity for detection of MTB complex was 74.6%, 73.8%, and 79.1% for Xpert MTB/RIF, CTM-MTB, and DTB, respectively. Within the subgroup of smear-negative TB samples (n = 51) sensitivity was 68% for Xpert MTB/RIF and CTM-MTB and 72% for DTB. Among smear-positive TB samples (n = 17), all (100%) were detected by DTB and 94.1% and 93.3% by Xpert MTB/RIF and CTM-MTB, respectively. Specificity was best for CTM-MTB (100%) and lowest for Xpert MTB/RIF (96.2%) due to misidentification of two NTM samples as MTB complex. CTM-MTB yielded the highest rate of invalid results (4.1%) (0.8% by Xpert MTB/RIF and DTB, respectively)[40].

Xpert MTB/RIF performance assessment study in Egypt shows that among (40) sputum specimens collected from adult patients having pulmonary tuberculosis from Chest and Ten (10) external control patients the conventional method, including Ziehl-Neelsen staining showed the presence of MTB in 77.5% and bacterial culture in 85% of the cases Whereas, the Xpert MTB/RIF test provided detection of 82.5%, in addition it correctly identified five out of six cases of RIF resistant MTB with sensitivity and specificity (83 and 100%) respectively, the resistant cases were all previously treated with RIF. Sensitivity, specificity, positive and negative predictive value of Xpert MTB/RIF in comparison to conventional culture was 97.14, 100, 100 and 85.7%, respectively. The control group showed no positive results with the Xpert MTB/RIF. The sensitivity of Xpert for smear positive, culture positive TB was 100% and in smear negative, culture positive TB was 66.6% while its specificity in both was 100%[41]

A total of 217 specimens submitted for routine smear and culture from three different sites within the western United States were used to evaluate the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA). Overall agreement compared to culture was 89% (98% for smear positives and 72% for smear negatives) for detection of *Mycobacterium tuberculosis* [42].

A national validation study done in Ethiopia by Ethiopian public health institute( EPHI) shows that the overall sensitivity, specificity, PPV and NPV of Xpert MTB/RIF assay in detecting MDR cases were 96.8%, 92.9%, 90.9%, and 97.5%, respectively. In HIV positive and smear negative cases for TB detection the sensitivity, specificity, PPV and NPV of Xpert MTB/RIF assay were 60.0%, 79.7%, 17.6% and 96.5%. While in smear positive cases the sensitivity was 94.4% and PPV was 93.4%. In general, the sensitivity, specificity, PPV and NPV of Xpert MTB/RIF assay in smear negative cases were 75.0%, 81.6%, 31.2% and 96.7%, respectively. Where as in smear positive cases, the sensitivity was 88.2% and PPV was 88.2%. [43].

## **4. Objectives of the Study**

### **4.1. General objective:**

- To determine the performance of Xpert MTB/RIF assay in comparison with LED fluorescence microscopy and ZN light microscopy for detection of Mycobacterium Tuberculosis in sputum sample.

### **4.2. Specific objectives;**

- To assess the incremental yield of MTB/RIF assay over LED fluorescence microscopy and ZN light microscopy for detection of Mycobacterium Tuberculosis in sputum sample.
- To determine the percent agreement of LED fluorescence microscopy with ZN smears microscopy for detection of Mycobacterium Tuberculosis in sputum samples.
- To determine sensitivity and specificity of Xpert MTB/RIF assay, LED fluorescence microscopy and ZN smears microscopy for detection of Mycobacterium Tuberculosis in sputum sample using sub-samples.

## **5. Methodology**

### **5.1. Study Area and study period:**

The study was conducted in Addis Ababa city administration regional health research and laboratory from December 2013 to April 2014. The laboratory is owned by government under Addis Ababa City administration Health Bureau which is situated at central part of Addis Ababa, the capital city of Ethiopia. The Laboratory serves as a referral laboratory for Addis Ababa city government and private health facilities. The Laboratory has Facilities for tuberculosis diagnosis like Xpert MTB/RIF assay, line probe assay and microscopy, as well as HIV molecular tests and other routine ART tests. For this study the sputum were obtained from one mobile TB clinic, six government hospitals and 40 health centers. Addis Ababa was established by Emperor Menilik II in 1886. The city is located between 9 degrees latitude and 38 degrees longitude at an altitude of 2200-2800 meters above sea level. It is divided in to 10 sub cities and 103 Kebeles. According to 1994 national census, Addis Ababa has a projected population of 3,042,956 for 2006/07 of which females account for 51%. It is geographically located at the center of the country. There are about 600 health institutions in the city: 394 private clinics, 106 government factory clinics, 28 NGO clinics, 60 government health centers, 9 government clinics, 32 hospitals (30 private, 13 government owned, and and1 NGO hospital). There are about 3000 health professionals working in these health institutions.

### **5.2. Study Design:**

A cross-sectional prospective study was conducted from December 2013 to April 2014 in Addis Ababa.

### **5.3. Study Population:**

All TB suspects identified during the study period were recruited. Those patients were both new or retreatment cases attending the satellite (mobile TB clinic) and non-satellite sites (those Hospitals and Health centers) with symptoms suggestive of TB (including cough of more or equal to 2 weeks) and MDR suspected in accordance with the country NTP guidelines.

#### **5.4. Study Subjects:**

The Study subjects were those individuals who attended the mobile TB clinic to had TB diagnosis and other individuals who were MDR suspected and referred to Addis Ababa regional health research and laboratory from public health centers and hospitals.

#### **5.5. Inclusion**

- Patients aged 18 and above years, with suspected pulmonary TB, presented to the study sites and gave sputa were recruited into the study.
- Informed consent from relevant authorities. `

#### **5.6. Exclusion criteria**

- Patients those unable to produce sputa or with insufficient volume
- Patients those have sputa with gross blood

#### **5.7. Sample Size and Sampling Procedures:**

##### **5.7.1. Sample Size:**

Convenient sampling technique was used for this study and the sample size calculation was based on the average national TB prevalence survey in general population of 2011 .The prevalence of TB is taken as 30.3 % for convenience. The following single population proportion formula is applied to obtain the actual sample size with 10 % contingency and the total sample size for the whole study, therefore, is 357.

$$N = (z\alpha/2)^2 p (1-P)/d^2$$

N = minimum sample size

z = 1.96 at 95% Confidence Intervals (CI)

d= margin of error 0.05 at 95% CI

P= National TB prevalence

### **5.7.2. Sampling Procedures:**

Sputum was collected from patients suspected of having pulmonary tuberculosis (PTB) and MDR TB attending those health centers and Hospitals then referred to AAHRL and others from the mobile TB clinic. Thus, purposive sample collection were applied until the achievement of the expected sample size within the given study period.

### **5.7.3. Data collection procedure**

TB Coordinators or trained personnel (doctor, nurse and health officer) in the respective study site were responsible for clinical diagnosis and selection of cases using the routine diagnosis of TB patients in accordance with NTP. A clinical and laboratory request form were used to extract medical history and socio demographic information, data on previous TB treatment, HIV status and sputum examination results of the participants. Log book was used to record all information of the patients enrolled in the study in a consecutive order. At the patient registration center effort was made to improve screening of patients to ensure that all eligible suspects are recruited. All eligible patients were enrolled during the study period and the number of patients found at the time of the study used conveniently. The main variables include in the study instrument were as a dependent variable diagnostic yield or percent agreement of Xpert MTB/RIF assay over ZN and LED-FM direct smear microscopy, the independent variables include sex, age, HIV status and previous anti-TB treatments history.

## **5.8. Laboratory methods**

### **5.8.1. Sample collection**

Sputum specimens were collected from patients suspected of having pulmonary tuberculosis (PTB) and MDR-TB and referred to AARHRL from different public health centers and Hospitals, and from the TB mobile clinic in Addis Ababa according to WHO guidelines. One morning sputum sample in two falcon tubes with minimum volume of 2 ml were collected from each patient, one for microscopy and Xpert MTB/RIF assay the other for culture. The samples were collected in clean, sterile, leak-proof, wide-mouth containers (falcon tubes). The sputum samples were stored in cool dark place until processed and in the case of delay were kept refrigerated at 4°C. Triple packaging was used while transporting the sputum [6, 44, and 45].

### **5.8.2. Direct smear preparation and staining**

Two sets of direct smears One for ZN and the other for the auramine-O phenol method were prepared from sputum sample by taking a small portion of the purulent part of the sputum with a wire loop, and smearing it on a microscope slide and dried in the air.

In direct ZN smear staining the prepared smears were placed on the staining rack and heat fixed then stained with 0.3% Carbol fuchsin, heated gently until steam rose, and left for 5 minutes, washed with gentle stream of water and flooded with 3% acid -alcohol for 1 minute, washed and flooded with 1% methylene blue for 1 minute. In LED-FM smear staining the prepared slides by direct methods were placed on a staining rack and heat fixed and stained with auramine-O phenol stain for 20 minutes, then rinsed briefly with gentle stream of water, flooded with 0.5% acid alcohol for 3 minutes then rinsed with water and counter stained with 1% Potassium Permanganate for 1 minute [44].

### **5.8.3. Examination and grading of ZN and fluorescent stained slides**

Fluorescent stained slides were examined using a light emitting diode (LED) fluorescent microscopy(primo star iLED, Zees MicrImaging, Jena,Germany) with a 40× objective under a standard fluorescence UV filter viewing at least 40 fields. The tubercle bacilli seen as yellow luminous organisms in a dark field and the results were graded and recorded in a defined manner as per the guidelines of International Union Against Tuberculosis and lung disease while ZN stained slide were examined for the presence of bacilli by using a 100x oil immersion objective, viewing at least 100 fields . AFB seen as bright pink to red, beaded or barred forms whereas the tissues cells and other organisms will stain blue. The results were graded and recorded in a defined manner as per the guidelines of IUATLD[44] as well, in both cases smears were examined separately by two laboratory technologist without knowing the results of each other and in case of discrepancies smears were re-examined by the Principal investigator .

### **5.8.4. Xpert MTB/RIF assay laboratory procedure**

In Xpert MTB/RIF assay first sputum liquefaction and inactivation with the reagent buffer was done by mixing in 1:2 ratios of sample to reagent then shaken and remain still for 10 minutes until concentrated sediments appear at the bottom again shake and wait for 5 minutes. Then after,

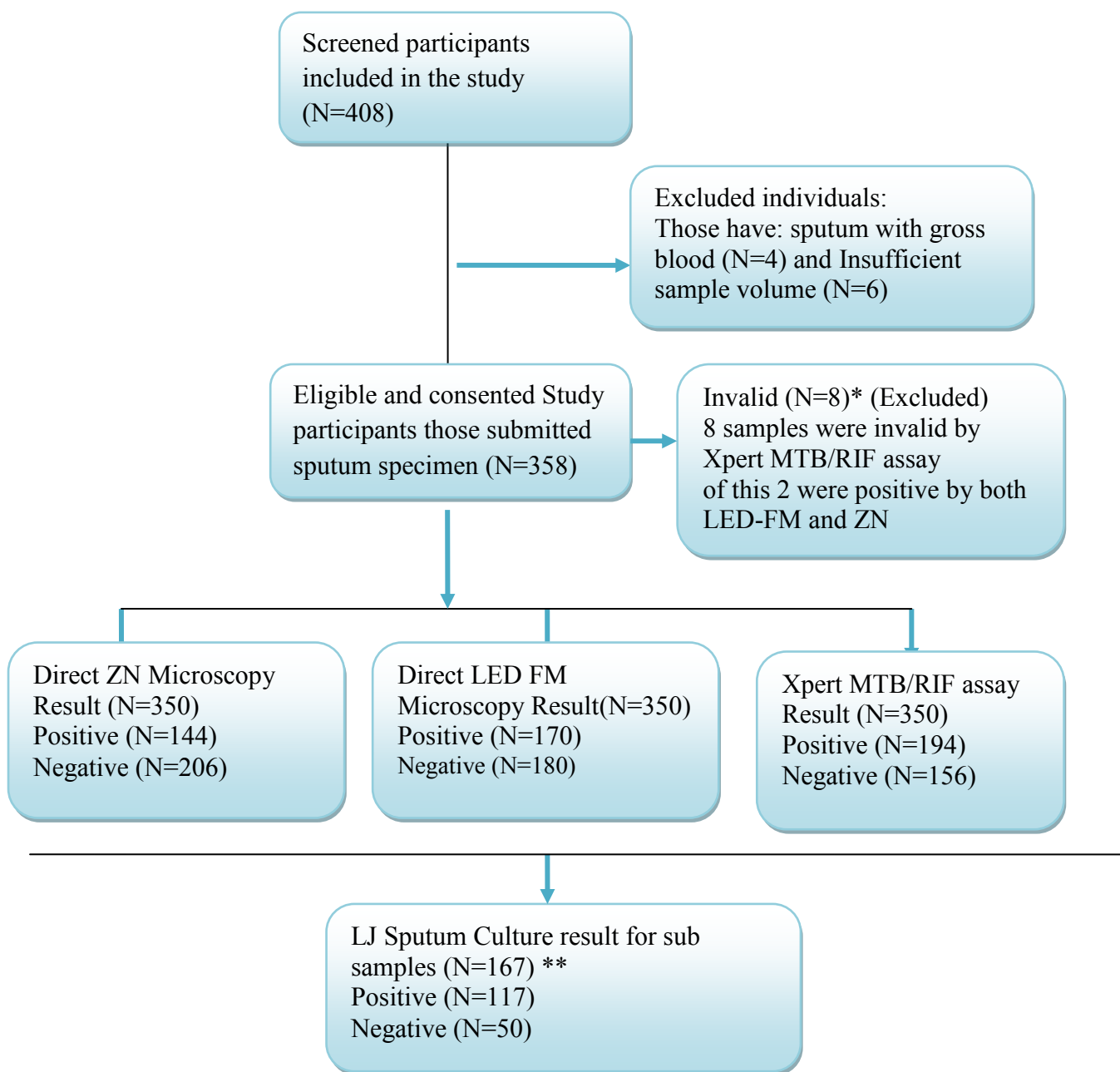


2 ml of processed sample was transferred into test cartridge in a careful manner then inserted into the MTB/RIF automated test platform after appropriate patient data and profile filled into the software. Once inserted, the operations will start automatically by which samples would be filtered and washed then mixed with dry PCR reagents which would finally continue with semi-nested real time amplification and detection that would take place in integrated reaction tubes. Upon finalizing the reaction, after on average of 2 hours, results were displayed in the software then auto saved by the machine [7, 8].

#### **5.8.5. Laboratory procedure for sputum culture and identification**

Specimens were homogenized and decontaminated by the Nalc-4%NaOH-2.9% citrate method (final concentration of NaOH 1%). After vortexing and incubation at room temperature for 15 min, specimens were concentrated at 3000 g for 15 min, decanted completely and re-suspended using 2 ml (pH 6.8) phosphate buffer. Approximately 100µl or 3-4 drops of the suspension was used to inoculate two LJ slants and the specimens inoculated into L-J media were incubated at 37°C for 8 weeks in a vertical position for the better development of individual colonies. MTB complex isolates were identified using time to visible growth, growth at 37°C, colony pigmentation and morphology, AFB smear and Capilia assay test (Capilia TB-Neo kit, TAUNS Laboratories, shizuoka, Japan). When small and buff colored colonies grew on LJ medium, the sample was considered as positive. Contaminated cultures (e.g. growth of molds, and also those in which the medium had liquefied or turned dark green) were discarded [45, 46].

Capilia TB Assay test was performed directly from positive cultures after confirmed the presence of cording Acid fast bacilli (AFB) by using Ziehl–Neelsen method. By dispensing 200 µl of the extraction buffer into a tube and mixed with 1 µL of bacteria or a 1mm-diameter micro-loop bacterial colony that has grown on the solid medium and suspend the collected bacteria in the buffer solution then by placing 100 µl of the suspension onto the specimen placement area of the Capilia TB cartridge, allowing a maximum of 15 min incubation and observing a purple–reddish color change in the test area and the test provided results in 15 minute. [47].



\*8 participants excluded from the study since the Xpert MTB/RIF assay result is invalid.

\*\*47% (167/350) of study participant's sputum sample was diagnosed using LJ culture medium

Fig. 1 Flow chart for the recruitment and findings of study participants in Addis Ababa, 2014

### **5.8.6. Safety Precautions**

Even if the procedure categorized under low risk biosafety level Sputum processing, decontamination, transferring to the Xpert MTB/RIF assay cartridge, and slide preparation was performed in a suitable biosafety level III laboratories using class II BSC dedicated for mycobacterium work [6]. Accesses to the room were restricted when work was in progress, and proper protective gowns, gloves and respirator masks were used while handling specimens. For culture the pellet was prepared in AAHRL and inoculated and incubated at AHRI TB lab.

### **5.8.7. Quality control**

Built in internal Quality control was performed continuously to ensure precise and reliable operation by the Xpert MTB/RIF assay instrument. Regarding the AFB smear microscopy at regular intervals and always when a new batch of staining solution was started two sputum smears of known high and low AFB positivity and one smear negative sputum smear were stained with routine smears to check that the reagent, staining method and the microscopic examination of smears are satisfactory. And also cross checking of slides were done to enhance the quality of microscopic examination of slides. The quality of the LJ medium to grow *Mycobacteria* was checked by inoculating media with *Mycobacterium gordoneae*, a rapidly growing species. Growth of this organism was checked for 3-4 days. The color of the media was checked before using it for culture and only those media with appropriate color (light green) were chosen. Media with a color between deep green to blue were frequently observed to give negative cultures with further color changes during incubation. This was believed to be due to the acidic pH of the media. Therefore careful adjustment of the inoculum pH (7.2- 7.4) was mandatory to avoid overwhelming the already diminished buffering capacity of the media.

### **5.8.8. Data Management, handling and analysis**

All laboratory and clinical data was recorded on a logbook during the study period. All data was entered into an excel spreadsheet, and then transfer for statistical analysis in SPSS version 20. Descriptive statistics was performed and data presented in tables. Kappa and McNamara's statistics was used for the percent agreement and proportion analysis. A 2x2 table was used for sensitivity and specificity analysis. All the paper based data was indexed and filed in box files. For electronic records, password protected databases was created and maintained throughout the study period and back up was picked using compact discs.

#### **5.8.9. Ethical considerations**

The protocol was approved by the Department Research and Ethics Review Committee (DRERC) Department of Medical Laboratory Science, College of Health Sciences, Addis Ababa University. Approval and Consent letters were also obtained from the Addis Ababa City Administration Health Bureau. The purpose of the study was clearly explained for each study participant. Written and oral consent was obtained from the study participants prior to enrolment. The results of the study were communicated to the responsible physicians as soon as they had been confirmed of having tuberculosis and treatments were given according to Ethiopian national policy for tuberculosis management and all results were kept confidentially.

## 6. Results

### 6.1. Socio-demographic characteristics of the study population

In this study, a total of 358 pulmonary tuberculosis suspected patients were enrolled from December, 2013 to April, 2014 in Addis Ababa City Administration Health bureau Health and research laboratory. The patients were mainly young adults and the median age was found to be 30.5. The minimum and maximum age was 18 years and 90 years respectively. There were 219 (61.2%) males, and 139 (38.8%) females with a ratio of 1.6:1. (Table 1) Regarding the HIV status of the patients 143(40.9%) know their HIV status. Among these 69(48.3%) are HIV positive (Table 2).

Table 1. Distribution of study subjects attending at Addis Ababa regional health and research laboratory according to age and sex (n = 358) in Addis Ababa, 2014.

| Age group (yrs) | Male<br>n (%)    | Female<br>n (%)  | Total<br>n (%)  |
|-----------------|------------------|------------------|-----------------|
| 18-27           | 57(49.6)         | 58(50.4)         | 115(100)        |
| 28-37           | 51(38.9)         | 80(61.1)         | 131(100)        |
| 38-47           | 19(31.1)         | 42(68.9)         | 61(100)         |
| 48-57           | 5(20)            | 20(80)           | 25(100)         |
| >57             | 7(26.9)          | 19(73.1)         | 26(100)         |
| <b>Total</b>    | <b>139(38.8)</b> | <b>219(61.2)</b> | <b>358(100)</b> |

Table 2. Frequency distribution for HIV status of the participants with positive MTB detection pattern in Addis Ababa, 2014.

|              |                 | MTB Detection(positive) |                  |                |
|--------------|-----------------|-------------------------|------------------|----------------|
| HIV result   | n(%)            | ZN (n %)                | LED-FM (n %)     | MTB/RIF (n %)  |
| Negative     | 74(21.1)        | 51(14.6)                | 55(15.7)         | 58(16.6)       |
| Positive     | 69(19.7)        | 26(7.4)                 | 35(10)           | 38(10.7)       |
| Unkown       | 207(59.1)       | 67(19.1)                | 80 (22.9)        | 98(28)         |
| <b>Total</b> | <b>350(100)</b> | <b>144(41.1)</b>        | <b>170(48.6)</b> | <b>196(56)</b> |

## 6.2. Comparison of results of direct ZN microscopy, direct LED-fluorescent microscopy and Xpert MTB/RIF assay for MTB Detection

Out of 358 pulmonary tuberculosis suspected patients 170(48.6%) were positive for direct LED-fluorescent microscopy, 144 (41.1%) were ZN microscopy positive and 194(56%) were Xpert MTB/RIF assay positive, a total of 8 samples were invalid by Xpert MTB/RIF assay and excluded, among these invalid samples by Xpert MTB/RIF assay 2 were positive and the remaining 6 samples were negative both by ZN and LED-FM smear microscopy. Kappa agreement test values among the method were obtained between 0.73 and 0.82 indicate good agreement between any two tests (Table 3).

Cohen's kappa was run to determine if there was agreement between LED-FM microscopy and ZN smear microscopy, Xpert MTB/RIF assay and LED-FM microscopy, Xpert MTB/RIF assay and ZN smear microscopy on 350 individuals for MTB detection and the result showed that there was moderate agreement in three combination with  $k=75.6\%$ (95%CI,67% to 81%), $p<.0005$ ,  $k=81.8\%$ (95%CI, 70% to 84%), $p<.0005$  and  $k=72.6\%$ (95%CI,60%to74%), $p<.0005$ , respectively. Among the three combinations better agreement seen in Xpert MTB/RIF assay and LED-FM microscopy. The percent agreement of the three tests showed that there were an overall percent agreement of 87.4%, 84.6%, and 90.9% between LED-FM and ZN microscopy, Xpert MTB/RIF assay and ZN microscopy, LED-FM and Xpert MTB/RIF assay respectively these are summarized in (Table 3).

Table 3. Percent Comparison between direct ZN and LED-FM microscopy, ZN microscopy and Xpert MTB/RIF assay, LED-FM microscopy and Xpert MTB/RIF assay for MTB detection in Addis Ababa, 2014.

| Xpert MTB/RIF assay result (count) (n=350) |               |               |             |            |       |
|--------------------------------------------|---------------|---------------|-------------|------------|-------|
|                                            | Positive n(%) | Negative n(%) | Total n (%) | %agreement | Kappa |
| Direct ZN Microscopy                       |               |               |             |            |       |
| Positive                                   | 142(40.6)     | 2(0.6)        | 144(41.2)   | 73.2%      | 0.726 |
| Negative                                   | 52 (14.9)     | 154 (44)      | 206(58.9)   | 98.7%      |       |
| Total                                      | 194(55.5)     | 156(44.5)     | 350(100)    | 84.6%      |       |
| LED-FM Microscopy                          |               |               |             |            |       |

Table 3 continued

|                 |                  |                  |                 |              |              |
|-----------------|------------------|------------------|-----------------|--------------|--------------|
| <b>Positive</b> | 166(47.4)        | 4(1.1)           | 170(48.5)       | 97.6%        |              |
| <b>Negative</b> | 28(8)            | 152(43.4)        | 180(41.4)       | 97.4%        |              |
| <b>Total</b>    | <b>194(55.5)</b> | <b>156(44.5)</b> | <b>350(100)</b> | <b>90.9%</b> | <b>0.818</b> |

**Direct LED-FM microscopy**

|                             |                  |                  |                 |              |              |
|-----------------------------|------------------|------------------|-----------------|--------------|--------------|
| <b>Direct ZN microscopy</b> |                  |                  |                 |              |              |
| <b>Positive</b>             | 135(38.6)        | 9(2.6)           | 144(41.2)       | 93.8%        |              |
| <b>Negative</b>             | 35(10)           | 171(48.9)        | 206(58.9)       | 95%          |              |
| <b>Total</b>                | <b>170(48.6)</b> | <b>180(51.5)</b> | <b>350(100)</b> | <b>87.4%</b> | <b>0.756</b> |

Table 4. Test characteristics of the direct ZN microscopy, Xpert MTB/RIF assay and LED fluorescent Microscopy against LJ culture results (n=167) in Addis Ababa, 2014.

|                                   | <b>LJ Culture results</b> |                     |                  |
|-----------------------------------|---------------------------|---------------------|------------------|
|                                   | <b>Positive (%)</b>       | <b>Negative (%)</b> | <b>Total (%)</b> |
| <b>Direct ZN Microscopy</b>       |                           |                     |                  |
| <b>Positive</b>                   | 90 (53.9)                 | 2 (1.2)             | 92(55)           |
| <b>Negative</b>                   | 27 (16)                   | 48(28.7)            | 75 (44.9)        |
| <b>Total</b>                      | <b>117 (70)</b>           | <b>50(30)</b>       | <b>167(100)</b>  |
| <b>LED Fluorescent Microscopy</b> |                           |                     |                  |
| <b>Positive</b>                   | 95 (56.9)                 | 5 (3)               | 100 (73.2)       |
| <b>Negative</b>                   | 22 (13.2)                 | 45(26.9)            | 67(26.8)         |
| <b>Total</b>                      | <b>117 (70)</b>           | <b>50 (30)</b>      | <b>167(100)</b>  |
| <b>Xpert MTB/RIF assay</b>        |                           |                     |                  |
| <b>Positive</b>                   | 105 (62.9)                | 4 (2.4)             | 109(65.3)        |
| <b>Negative</b>                   | 12 (7.2)                  | 46(27.5)            | 58(34.7)         |
| <b>Total</b>                      | <b>117 (70)</b>           | <b>50(30)</b>       | <b>167(100)</b>  |

Of 350 sputum sample, 167 were tested using LJ culture medium, of these The ZN stained smear results showed that 92(55%) were both positive for culture and direct ZN microscopy but 27(16%) were culture positive and ZN microscopy negative. The direct fluorochrome stained smears results showed that 100(73.2%) were both positive for culture and LED-FM microscopy but 22(13.2%) were culture positive and LED-FM microscopy negative. Of Xpert MTB/RIF assay tested 109 (65.3%) were positive for culture and Xpert MTB/RIF. 12(7.2%) specimens

were culture positive, but Xpert MTB/RIF negative and 4(2.4%) samples were Xpert MTB/RIF positive but culture negative (Table 4)

### 6.3. Comparison of results direct ZN microscopy, direct LED-FM and Xpert MTB/RIF assay with culture method for MTB detection.

In the sub group study the sensitivity, specificity, PPV and NPV achieved with direct ZN microscopy, LED-FM and Xpert MTB/RIF assay using culture as a gold standard were (76.9%,96%,97.8% and 64%), (81.2%, 90%, 95%, 67.2%) and (89.7%,92%,96.3%,86.2%) respectively (Table 5).

Table 5. Performance parameters of direct LED fluorescent microscopy, direct ZN smear microscopy and MTB/RIF assay against culture results. (n=167) in Addis Ababa, 2014.

|                    | LED-FM (%)<br>95%CI | ZN (%) 95%CI    | Xpert MTB/RIF assay (%)<br>95%CI |
|--------------------|---------------------|-----------------|----------------------------------|
| <b>Sensitivity</b> | 81.2(77.5-84.9)     | 76.9(74.5-79.5) | 89.7(88.6-90.8)                  |
| <b>Specificity</b> | 90(89-91)           | 96(94.4-97.6)   | 92(91.1-92.9)                    |
| <b>PPV</b>         | 95(94.4-95.6)       | 97.8(97.5-98.1) | 96.3(95.9-96.7)                  |
| <b>NPV</b>         | 67.2(64.5-69.9)     | 64.0(61.2-66.8) | 86.2(84.6-87.6)                  |
| <b>TE</b>          | 87(85.6-88.4)       | 83.5(81.8-85.2) | 90.6(89.6-91.6)                  |

LED-FM = light emitted diode Fluorescent Microscopy PPV= positive predictive value NPV= Negative predictive value ZN=Zeihl-Neelson, TE=Test Efficiency

### 6.4. AFB quantification reported by direct smear technique and XpertMTB/RIF assay and sputum consistency

Among 350 specimens 144(41.1%) were found to be AFB positive and 206 (58.9%) were found AFB negative by direct ZN AFB smear. Among the positive specimens, 19 (13.2%) were scanty positive, 92 (63.9%) were 1+, 33(23%) were 2+ and above. In contrast, 170 (48.6%) were found to be AFB positive and 180 (51.4%) were AFB negative when smear prepared from Auramine-O stain. Among the positive specimens 21 (11.8%), 79 (46.5%), 62 (36.5%), 7 (4.1%) were scanty positive, 1+, 2+ and 3+ respectively. (Table 6)



Table 6. Distribution of quantified reported results of LED-FM, ZN microscopy technique and Xpert MTB/RIF assay in Addis Ababa, 2014.

| Quantitative result Percentage by test methods |               |                |                                        |           |
|------------------------------------------------|---------------|----------------|----------------------------------------|-----------|
| Grading                                        | DZNM(n = 144) | DLFM (n = 170) | Xpert MTB/RIF assay (N=194)<br>Grading |           |
| <b>Scanty</b>                                  | 20 (13.9%)    | 22 (12.9%)     | Very low                               | 20(10. %) |
| <b>+1</b>                                      | 91 (63.2%)    | 81 (47.6%)     | Low                                    | 54(27.8%) |
| <b>+2</b>                                      | 26 (18.1%)    | 61 (35.9%)     | Medium                                 | 89(45.9%) |
| <b>+3</b>                                      | 7 (4.8%)      | 7 (4.1%)       | High                                   | 31(16%)   |

DLFM= Direct LED fluorescence microscopy DZNM= Direct Ziehl—Neelsen microscopy

Among sputum specimens included in the study, 39.5% were salivary (contained non mucous elements), and 49.1% muco-purulent and 11.4% were purulent. Strongly positive (2+ and 3+) smears made up only 22.9% of direct ZN smear microscopy, but 40% of direct LED fluorescent smears. Smears with 1+ made up 63.2% for direct ZN smear microscopy and 47.6% for direct LED fluorescent smears. Smears with scanty AFB were 12.9% for direct ZN smear microscopy and 13.9% for direct LED fluorescent smears. Of the 194 Xpert MTB/RIF assay positive results semi-quantification pattern showed 20% very low, 27.8% low, 45.9% medium and 16% high detection value (Table 6).

Of the 350 specimen's sputum appearance versus positive yield showed that of the purulent sample the yield was 25.7%, 21.8%, 20.6% for ZN, LED-FM and Xpert MTB/RIF assay respectively and of the muco-purulent sample the yield was 57.6%, 58.2%, and 58.2% for ZN microscopy, LED-FM and Xpert MTB/RIF assay respectively. Of the saliva sample the yield was 16.7%, 20.0%, and 21.1% for ZN, LED-FM and Xpert MTB/RIF assay respectively these are summarized in (Table 7).

Table 7. Distribution of reported results of LED-FM, ZN microscopy technique and Xpert MTB/RIF assay against consistency of sputum in Addis Ababa, 2014

| <b>Sputum consistency (n=350)</b>      |                |                 |                |               |
|----------------------------------------|----------------|-----------------|----------------|---------------|
| <b>Result of test methods</b>          | <b>Pru (%)</b> | <b>Mpru (%)</b> | <b>Sal (%)</b> | <b>Total%</b> |
| <b>GenXpert(MTB/RIF assay)</b>         |                |                 |                |               |
| <b>Negative</b>                        | 1(0.6%)        | 59(37.8%)       | 96(61.5%)      | 156(100%)     |
| <b>Positive</b>                        | 40(20.6%)      | 113(58.2%)      | 41(21.1%)      | 194(100%)     |
| <b>Total</b>                           | 41(11.7%)      | 172(49.1%)      | 137(39.1%)     | 350(100%)     |
| <b>Zeihi-Neelsen smears microscopy</b> |                |                 |                |               |
| <b>Negative</b>                        | 4(1.9%)        | 89(43.2%)       | 113(54.9%)     | 206(100%)     |
| <b>Positive</b>                        | 37(25.7%)      | 83(57.6%)       | 24(16.7%)      | 144(100%)     |
| <b>Total</b>                           | 41(11.7%)      | 172(49.1%)      | 137(39.1%)     | 350(100%)     |
| <b>LED-FM smear microscopy</b>         |                |                 |                |               |
| <b>Negative</b>                        | 4(2.2%)        | 73(40.6%)       | 103(57.2%)     | 180(100%)     |
| <b>Positive</b>                        | 37(21.8%)      | 99(58.2%)       | 34(20.0%)      | 170(100%)     |
| <b>Total</b>                           | 41(11.7%)      | 172(49.1%)      | 137(39.1%)     | 350(100%)     |

Pru=prulent, Mpru= muco-prulent, Sal= saliva

### 6.5. Test characteristics of the direct ZN microscopy, LED fluorescent Microscopy and GenXpert test according to sputum consistency

Among sputum specimens included in the study, 39.5% were salivary, and 49.1% muco-purulent and 11.4% were purulent. Strongly positive (2+ and 3+) smears made up only 22.9% of direct ZN smear microscopy, but 40% of direct LED fluorescent smears. Smears with 1+ made up 63.2% for direct ZN smear microscopy and 47.6% for direct LED fluorescent smears. Smears with scanty AFB were 12.9% for direct ZN smear microscopy and 13.9% for direct LED fluorescent smears. Of the 194 Xpert MTB/RIF positive results semi-quantification pattern showed 20% very low, 27.8% low, 45.9% medium and 16% high detection value.

Of the 350 specimen's sputum appearance versus positive yield showed that of the purulent sample the yield was 25.7%, 21.8%, 20.6% for ZN, LED-FM and MTB/RIF assay respectively

and of the muco-prulent sample the yield was 57.6%, 58.2%, and 58.2 for% ZN,LED-FM and Xpert MTB/RIF respectively. Of the saliva sample the yield was 16.7%, 20.0%, and 21.1% for ZN, LED-FM and Xpert MTB/RIF respectively these are summarized in (Table 6 & Table 7). The unusual increase of positivity rate in saliva sputum of this study may be due to the high number of retreatment and HIV positive cases of study participants.

Among the study participants 167 sputum samples were confirmed by using LJ culture medium as a gold standard and of those samples 117(70.1%), 92(55.1%), 100(59.9%), 109(65.3%) are positive by LJ culture medium, ZN smear microscopy, LED-FM smear microscopy, and Xpert MTB/RIF assay. Of 117 positive samples by culture 76(65%) are pretreated cases and among 109 positive samples by Xpert MTB/RIF assay 36(33%) are detected as RIF resistance positive of these samples treatment history of the participants showed that 2,7,18,1 and 8 are defaulter, relapse, treatment failure, MDR contact, and new cases (Table 8).

Table 8. MTB complex detection by Xpert MTB/RIF assay, ZN smear microscopy and LED-FM smear microscopy among culture confirmed cases against their treatment history pattern in Addis Ababa, 2014 (n=167)

| Treatment History of Culture confirmed cases (n=167) |           |     |             |                   |         |           |
|------------------------------------------------------|-----------|-----|-------------|-------------------|---------|-----------|
| MTB Result                                           | (n %)     | New | MDR contact | Treatment failure | Relapse | Defaulter |
| <b>LJ Culture positive</b>                           | 117(70.1) | 34  | 4           | 38                | 38      | 4         |
| <b>ZN positive</b>                                   | 92(55.1)  | 21  | 2           | 32                | 34      | 3         |
| <b>LED-FM Positive</b>                               | 100(59.9) | 29  | 2           | 30                | 36      | 3         |
| <b>MTB/RIF positive</b>                              | 109(65.3) | 29  | 3           | 35                | 38      | 4         |
| <b>RIF res.positive</b>                              | 36(21.6)  | 2   | 8           | 18                | 7       | 1         |

LED-FM = light emitted diode Fluorescent Microscopy ZN=ZeihlNeelson smear microscopy,  
RIF res. = Rifampicin resistance positive, LJ= Lowsten-Jenson MDR= multidrug resistance  
MTB= Mycobacterium Tuberculosis

## 7. DISCUSSION

In this study, the performance of the MTB/RIF assay with sputum specimens obtained during the study was investigated. Previous studies of the MTB/RIF assay have reported test sensitivities of 66.6 to 75% in cases of smear-negative, culture-positive pulmonary tuberculosis and 88.2 to 100% in cases of smear-positive, culture-positive pulmonary tuberculosis, while the test specificity remained at 96.5% to 100% (31, 32, 37, 38, 39, 40, and 41). In our study the sensitivity of the test with smear- and culture-positive pulmonary specimens was 99.2%, and the specificity was 97.6%, compatible with results presented in previous study. For smear-negative pulmonary specimens, the sensitivity of the test was 63.2%, which was somewhat congruent with the sensitivities of others.

Out of 358 pulmonary tuberculosis suspected patients 170(48.6%) were positive for direct LED-fluorescent microscopy, 144 (41.1%) were ZN microscopy positive and 194(56%) were Xpert MTB/RIF assay positive, a total of 8(2.23%) samples were invalid by Xpert MTB/RIF assay and excluded, among these invalid samples by Xpert MTB/RIF assay 2 were positive and the remaining 6 samples were negative both by ZN and LED-FM smear microscopy. Among the total Xpert MTB/RIF assay positive individuals 51(14.6%) were RIF resistance positive. Among the HIV positive individuals 26(37.7%), 35(50.7%), 38(55.1%) were positive for MTB by ZN microscopy, LED-fluorescent microscopy and Xpert MTB/RIF assay respectively these were indicated in (Table 4). Among 69(48.3%) of HIV positive patients 13 were smear negative by direct ZN smear microscopy of these 4 were smear positive by LED-FM direct smear microscopy and 5 were positive by Xpert MTB/RIF assay and culture this showed the higher detection value of LED-FM direct smear microscopy and Xpert MTB/RIF assay over ZN direct smear which is congruent with the study done in Uganda. Justification for invalid test by Xpert MTB/RIF assay may be due to PCR inhibitors in the sample.

The efficiency of all the assays ranged between 83.5% and 90.6% (Table 5). The various AFB detection level were compared using the kappa agreement test whereby values between 0.73 and 0.82 indicate good agreement between any two tests.

We compared the relative performance of ZN direct smear microscopy, LED based fluorescence microscopy techniques and Xpert MTB/RIF assay from sputum sample in patients with symptoms of pulmonary TB and MDR suspects by using LJ solid culture system as the gold standard for sub group analysis. In this study, the total sputum specimen studied were 350 that is one from each clinically suspected pulmonary tuberculosis patients. Cohen's kappa was run to determine if there was agreement between LED-FM microscopy and ZN smear microscopy, Xpert MTB/RIF assay and LED-FM microscopy, Xpert MTB/RIF assay and ZN smear microscopy on 350 individuals for MTB detection and the result showed that there was moderate agreement in three combination with  $k=75\%$ (95%CI,67% to 81%),  $p<.0005$ ,  $k=81.8\%$ (95%CI, 81% to 82.6%),  $p<.0005$  and  $k=72.6\%$ (95%CI,61.6%to82.6%),  $p<.0005$ , respectively. Among the three combinations better agreement seen in Xpert MTB/RIF assay and LED-FM microscopy. The percent agreement of the three tests showed that there were an overall percent agreement of 87.4%, 84.6%, and 90% between LED-FM and ZN microscopy, Xpert MTB/RIF assay and ZN microscopy, LED-FM and Xpert MTB/RIF assay respectively.

Regarding the comparative sensitivity and specificity of LED-FM and direct ZN microscopy, the WHO meta- analysis study showed that there were a 6% higher sensitivity by LED-FM microscopy than ZN direct smear microscopy without loss of its specificity [12,20] which is congruent with our study by its sensitivity and differ in specificity .In contrast a study done in Indonesia(Chadir L, et,al) showed LED-FM microscopy had a higher sensitivity but lower specificity compared to ZN microscopy which is the same as our study[35].

In our study, we found that 3 (2.4%) specimens were direct LED fluorescent microscopy positive, but culture negative and of those 2(1.6%) were ZN direct smear microscopy positive. This may be due to the following reasons; inorganic material that absorbs fluorochrome stains may on occasion be mistakenly identified as AFB or non-viable bacilli. We also compared the sensitivity, specificity, PPV and NPV of direct ZN versus direct LED fluorochrome staining technique. Direct LED FM sensitivity was slightly higher than direct ZN microscopy 81.2%, (95%CI 77,5-84,9) vs. 76.9%, (95%CI, 74.5-79.5)difference 4.3%,  $P<0.0001$ ). further increased the sensitivity of MTB detection to 89.7% using Xpert MTB/RIF assay, incremental yield of 12.8%, compared with direct ZN microscopy and 8.5 % compared with direct LED-FM microscopy  $P<0.0001$ ). Our results showed that the sensitivity of LED fluorochrome staining method remarkable when compared with the sensitivity of direct microscopy (ZN) in diagnosing pulmonary tuberculosis. This is in agreement with other studies Steingrattet, *et al.* [18].

In our study the sensitivity of Xpert MTB/RIF assay was found to be 89.7%( 95%CI, 88.6-90.8)which is congruent with the study done in Tanzania and south Africa 92%, in West Tanzania 88.4 % USA 89% and in Ethiopia 94.4% [32, 37, 42 and 43] but different from that of the study done in Uganda 79.4%, South Korea 79.5%, German 74.6%[31, 38 and40] the underlying justifications for lower sensitivity may be due to small sample size or different geographical distribution and population diversity. The specificity of this test was 92%( 95%CI, 91.1-92.9) which is different from studies done in South Africa and Tanzania 99%, Egypt 100%, Saudi Arabia 100%,and West Tanzania 99%. The justification for lower specificity in our setting may be due to the presence of mycobacterial DNA from dead bacilli persisting in lung tissue expectorated at the time of an inter current chest infection.

In this study the reduction in detection of quantitative M tuberculosis DNA with Xpert MTB/RIF correlated with smear grades ( $p= 0.52$ ;  $p<0.0001$ ), this is congruent with the study done in Tanzania and South Africa Boehme et ,al [37].

## **7.1. Strengths and limitations of the study**

### **7.1.1. Strength**

- ✚ This study tried to assess the comparative performance of the three tests (ZN smear microscopy, LED-FM smear microscopy, and Xpert MTB/RIF assay) and also try to evaluate 47% of the cases using solid culture as a gold standard with constraint resources and difficult situations.
- ✚ This study was initiated to answer the question, whether Xpert MTB/RIF assay contributed to enhance detection of MTB in TB suspected patients or not.
- ✚ The study is the first to address the performance of Xpert MTB/RIF assay in the case of Addis Ababa next to The EPHI evaluation study.
- ✚ Samples were collected from 30 health centers and 4 Hospitals which provide sufficient representativeness of the sample.

### **7.1.2. Limitation**

- ✚ This study covered only those patients who came to the health facilities during the study period and most of them are MDR suspects this may overestimate findings.
- ✚ Sputum Culture and HIV testing were not done for all participants.

## **8. Conclusion and Recommendation**

### **8.1. Conclusion**

The results of the present study showed that the LED fluorescence microscopy gave more accurate results compared to ZN smear microscopy and Xpert MTB/RIF assay was considered as superior method than LED fluorescence microscopy and ZN smear microscopy especially for those smear negative cases. Direct LED-Fluorescent microscopy positive, but culture negative cases were also reported and these may be due to the inorganic material that absorbs fluorochrome stains and or non-viable bacilli. In both direct ZN smear microscopy and Xpert MTB/RIF assay methods discordant positive result which is negative by culture were reported, these may be due to non-viable bacilli.

This study showed that LED FM has correspondence with culture with decreasing number of bacilli and correlated more with culture than low scanty results found with the Ziehl-Neelsen technique. Treatment history of the patients was highly associated with development of PTB in direct LED-FM smear microscopy and direct ZN smear microscopy as well as Xpert MTB/RIF assay methods.

### **8.2. Recommendations**

According to the present study, combination of Xpert MTB/RIF assay and LED based fluorescent microscopy increase sensitivity with minimal specificity, easy to perform and rapid diagnostic test for pulmonary tuberculosis. This is especially true for laboratories in our countries, where limited resources for usage of culture and other molecular techniques for rapid detection of tuberculosis.

LED fluorescent microscopy might be reasonably expected to improve tuberculosis case-finding by increasing sensitivity and expected decrease in time spent on microscopic examination. This would increase productivity of the laboratory personnel and help to serve more patients as well. The Xpert MTB/RIF test in part takes the great advantage of detecting MTB and RIF resistance at the same time as well as increase detection in smear negatives. It can be used accordingly by considering the cost and the WHO recommendations. As a combination LED-FM and Xpert MTB/RIF assay may give better result and can be used while algorithm designing.



Additional study should be done for smear negative HIV/AIDS patients, Pediatrics and other smear negative cases as well as for RIF resistance (MDR) cases of Xpert MTB/RIF assay.

## 9. References:

1. Das S, Das SC, and Verma R: Occurrence of RD9 region and 500 bp fragment among clinical isolates of *Mycobacterium tuberculosis* and *Mycobacterium bovis*. *Microbiol Immunol* 2007, 51(2):231-234.
2. Van IJ, Rahim Z, Mulder A, Boeree MJ, Simeone R, Brosch R, *et al.*: “characterization of *Mycobacterium orygis* as tuberculosis complex subspecies”. *Emerg Infe Dis* Apr 2012, 18(4). P.653-655. doi:10.3201/eid1804.110888
3. World Health Organization Global Tuberculosis Report 2013, WHO/HTM/TB/2013.6 page1-12. incited from: at [http://www.who.int/tb/publications/global\\_report/20123](http://www.who.int/tb/publications/global_report/20123)
4. Federal Democratic Republic of Ethiopia, Ministry of Health. Health and Health related indicators. Addis Ababa, Ethiopia: Federal Ministry of Health 2010.
5. Guidelines For Clinical And Programmatic Management Of TB, Leprosy And TB/HIV In Ethiopia, 5<sup>th</sup>ed. Addis Ababa, Ethiopia: Federal Ministry of Health (April, 2012)
6. World Health Organization Global tuberculosis control report. World Health Organisation, 2011 Geneva, Switzerland: ([www.who.int/tb/data](http://www.who.int/tb/data).)
7. The stop TB department WHO: Roadmap for rolling out Xpert MTB/RIF for rapid diagnosis of TB and MDR-TB 2010. Incited from [http://www.who.int/tb/laboratory/roadmap\\_xpert\\_mtb-rif.pdf](http://www.who.int/tb/laboratory/roadmap_xpert_mtb-rif.pdf)
8. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. Policy statement. Geneva, World Health Organization, 2011
9. Chegou NN, Hoek KG, Kriel M, Warren RM, Victor TC, and Walzl G: Tuberculosis assays: past, present and future. *Expert review of anti-infective therapy* 2011.9(4):457-469.
10. Dowdy DW, Davis JL, Boon S, Walter ND, and Katamba A: Population-Level Impact of Same-Day Microscopy and Xpert MTB/RIF for Tuberculosis Diagnosis in Africa. *PLoS ONE* 2013 8(8): e70485. doi:10.1371/journal.pone.0070485.
11. The Stop TB Strategy Building on and enhancing DOTS to meet the TB-related Millennium Development Goals. World Health Organisation, 2006 Geneva,

Switzerland;[http://www.stoptb.org/assets/documents/resources/publications/plan\\_strategy/](http://www.stoptb.org/assets/documents/resources/publications/plan_strategy/)

12. Steingart KR, Henry M, Ng V, Hopewell PC, and Ramsay A: Fluorescence versus conventional sputum smear microscopy for tuberculosis: A systematic review. *Lancet Infect Dis* 2006 6: 570–581
13. Same-day diagnosis of tuberculosis by microscopy: WHO policy statement. World Health Organization, Geneva 2011.
14. Kebede AH, Alebachew Z, Tsegaye F, Lemma E, Abebe A, Agonafir M, *et al.*: The first Population-based national Tuberculosis Prevalence survey In Ethiopia, 2010-2011. *Int J Tuberc Lung Dis* 2014, 18(6):635-639
15. World Health Organization. 2008 Report: Global Tuberculosis Control – Surveillance, Planning, Financing ([http://www.who.int/tb/publications/global\\_report/2008/en/](http://www.who.int/tb/publications/global_report/2008/en/))
16. Nakiyingi L., Nankabirwa H, and Lamorde M: Clinical use of GeneXpert in the diagnosis of smear-negative PTB: a case report Uganda. *African Health Sciences* 2013; 13(2): 522 - 524 <http://dx.doi.org/10.4314/ahs.v13i2.46>.
17. Dorak MT: Real-time PCR. Posted date 01/07/09 2006, Cited in: <http://www.dorak.info/g>
18. Steingart KR, Ramsay A, and Pai M: Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis. *Expert Rev Anti Infect Ther*, 2007. 5(3): p. 327-331.
19. Dye C, Scheele S, Dolin P, Pathania V and Raviglione MC: Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. *JAMA* 1999, 282:677-686.
20. Cuevas LE, Al-Sonboli N, and Lawson L: LED fluorescence microscopy for the diagnosis of pulmonary tuberculosis: a multi-country cross-sectional evaluation. *PLOS Med* 2011; 8: e1001057.
21. World Health Organization. Same-day diagnosis of tuberculosis by microscopy: Policy statement. Geneva: WHO 2011
22. Rahman, F, Munshi SK, Kamal SM, Rahman ASM, Rahman MM, and Noor R, *Stamford J. Microbiol.*, 2011, 1, 51–55.

23. Anthony RM, Kolk AH, Kuijper S, and Klatser PR,: Light emitting diodes for auramine O fluorescence microscopic screening of Mycobacterium tuberculosis. *Int. J. Tuberc. Lung Dis.*, 2006, 10, 1060–1062.
24. Hung NV, Sy DN, Anthony RM, Cobelens FG and Van S D, Fluorescence microscopy for tuberculosis diagnosis. *Lancet Infect Dis* 2007; 7: 238-239.
25. Minion J, Pai, M, Ramsay A, Men-zies D, and Greenaway C: Light-emitting diode technologies for TB diagnosis: what is on the market? *Expert Rev Med Devices. PLoS One*, 2011, 6, e22495.
26. Whitelaw A, Peter J, Sohn H, Vilijeon D, Theoron G, Badris M, *et al.*: comparative cost and performance of light emitted diode microscopy in HIV-Tuberculosis Co-infected patients. *Eur. Respir. J.*, 2011, 38, 1393–1397.
27. Chaidir L, Parwati I, Annisa J, Muhsinin S, Meilana I, *et al.*: Implementation of LED Fluorescence Microscopy for Diagnosis of Pulmonary and HIVAssociated Tuberculosis in a Hospital Setting in Indonesia. *PLoS ONE* (2013) 8(4): e61727. doi:10.1371/journal.pone.006172.
28. Marais BJ, Brittle W, Painczyk K, Hesselning AC, and Beyers N: Use of light-emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum.2008; *Clin. Infect. Dis.*, 2008, 47, 203–207.
29. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, *et al.*: Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on demand, near-patient technology. *J ClinMicrobiol* 2010; 48(1): p.229–237.
30. Blakemore, R., Story, E., Helb, D., Kop, J., Banada, P., Jones, M., *et al.* Evaluation of the analytical performance of the Xpert MTB/RIF assay. *J ClinMicrobiol* 2010; 48(7): p.2495–2501.
31. Kwak N, Choi SM, Lee J, Park YS, and Lee C-H: Diagnostic Accuracy and Turnaround Time of the Xpert MTB/RIF Assay in Routine Clinical Practice. (2013) *PLoS ONE* 8(10): e77456. doi:10.1371/journal.pone.0077456
32. Boehme C, Nabeta P, Hillemann D, Nicol M, Shenai S, Krapp F, *et al.*: Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. *N Engl J Med*, 2010. 363(11): p. 1005-15.

33. Al-Darraj HAA, Razak HA, Ng KP, Altice FL, Kamarulzaman A: The Diagnostic Performance of a Single GeneXpert MTB/RIF Assay in an Intensified Tuberculosis Case Finding Survey among HIV-Infected Prisoners in Malaysia. *PLoS ONE* 2013 8(9): e73717. doi:10.1371/journal.pone.0073717
34. Gerardo A, Jose M.A, ManoranjanM, Praveen K, Naik, S. R, and Raghuprakash R: Comparison of LED Fluorescent Microscopy and the GeneXpert MTB/RIF Assay in a District Hospital in India: *Tuberculosis Research and Treatment* Volume 2012, Article ID 932862, doi:10.1155/2012/932862
35. Chaidir L, Parwati I, Annisa J, Muhsinin S, Meilana I, *et al.*: Implementation of LED Fluorescence Microscopy for Diagnosis of Pulmonary and HIV Associated Tuberculosis in a Hospital Setting in Indonesia. *PLoS ONE* 2013 8(4): e61727. doi:10.1371/journal.pone.0061727
36. Rachow A, Zumla A, Heinrich N, Rojas-Ponce G, and Mtafya B: Rapid and Accurate Detection of Mycobacterium tuberculosis in Sputum Samples by Cepheid Xpert MTB/RIF Assay—A Clinical Validation Study. (2011) *PLoS ONE* 6(6): e20458. doi:10.1371/journal.pone.0020458
37. Shah M, Chihota V, Coetzee G, Chirchyard G, and Dorman S : Comparison of laboratory costs of rapid molecular tests and conventional diagnostics for detection of tuberculosis and drug-resistant tuberculosis in South Africa *BMC Infectious Diseases* 2013, 13:352 cited in: <http://www.biomedcentral.com/1471-2334/13/352>
38. Nakiyingi L., Nankabirwa H, Lamorde M: Clinical use of GeneXpert in the diagnosis of smear-negative PTB: a case report Uganda. *African Health Sciences* 2013; 13(2): 522 - 524 <http://dx.doi.org/10.4314/ahs.v13i2.46>
39. Souad M, Maha M, Noura A: Evaluation of direct detection of Mycobacterium tuberculosis complex in respiratory and non-respiratory clinical specimens using the Cepheid Gene Xpert® system. *Saudi Med J* 2012; Vol. 33 (10): 1100-1105
40. Antonenka U, Hofmann-Thiel S, Tuaeov L, Esenalieva A, Abdulloeva M, Sahalchik E, *et al.*: Comparison of Xpert MTB/RIF with ProbeTec ET DTB and COBAS TaqMan MTB for direct detection of MTB complex in respiratory specimens. *BMC Infectious Diseases*; 2013 13:280. doi:10.1186/1471-2334-13-280

41. Manal D, Magda El W, Hazem A: Diagnostic assessment of Xpert MTB/RIF in a sample of Mycobacterium tuberculosis Egyptian patients. *African Journal of Microbiology Research*. November, 2013; Vol. 7(44), pp. 5107-5113, 7. Cited in: <http://www.academicjournals.org/AJMR>
42. Elizabeth M, Susan M, Joven C, Susan E, Michelle A, Anna B, *et al.*: Evaluation of the Cepheid Xpert MTB/RIF Assay for Direct Detection of Mycobacterium tuberculosis Complex in Respiratory Specimens. *Journal of clinical microbiology*, Apr. 2011, p. 1621–1623 Vol. 49, No. 4 0095-1137/11/\$12.00 doi:10.1128/JCM.02214-10
43. Implementation Guideline for GeneXpert MTB/RIF Assay in Ethiopia. April, 2014 ;Addis Ababa
44. Laboratory Services in TB control Part I, II, and III WHO/TB/98.258, 1998
45. Kent PT, Kubica GP, Public health mycobacteriology: a guide for the level III laboratory, p 31–70. U.S. Department of Health and Human Services, Atlanta, GA 1985
46. Allen BW, Baker FJ: Modified Petroff Technique Treatment of specimens, chapter 3 in Mycobacteria Isolation, Identification and Sensitivity Testing. London: Allen BW, Baker FJ Butterworth and Co.; 1968. pp. 9–16.
47. Wang JY, Lee LN, Lai HC *et al.*: Performance assessment of the Capilia TB assay and the BD ProbeTec ET system for rapid culture confirmation of Mycobacterium tuberculosis. *Diagnostic Microbiology and Infectious Disease* (2007) 59, 395–399.

## 10. Annexes

### Annex-I: Request paper

Date \_\_\_/\_\_\_/\_\_\_

Patient Name \_\_\_\_\_ Code No. \_\_\_\_\_ Address \_\_\_\_\_

Hospital No \_\_\_\_\_ (Tele.) \_\_\_\_\_ Age \_\_\_\_\_ Sex \_\_\_\_\_

Living Area: Urban \_\_\_\_\_ Rural \_\_\_\_\_

TB disease Type and Treatment History

Site: Pulmonary ☐ Extrapulmonary ☐

History: New ☐ MDR contact ☐ Relapse ☐ Return after default ☐ Failure ☐

Uncertain ☐

### Annex-II: Laboratory data

Date of sample collection \_\_\_\_\_ day \_\_\_\_\_ Month \_\_\_\_\_ year \_\_\_\_\_

Time of sample collection \_\_\_\_\_

Total no of sample received \_\_\_\_\_

Results:

a) Completed ☐ b) Incomplete ☐ c) Excluded ☐

Action taken for the incomplete data \_\_\_\_\_

Gross appearance of sputum

Bloody ☐ Purulent ☐ Mucopurulent ☐ Saliva ☐

AFB Results

ZN (Direct) positive: scanty 1+ \_\_\_\_\_ 2+ \_\_\_\_\_ 3+ \_\_\_\_\_

FM (Direct) positive: scanty 1+ \_\_\_\_\_ 2+ \_\_\_\_\_ 3+ \_\_\_\_\_

LED- FM: Positive ☐ Negative ☐

ZN: Positive ☐ Negative ☐

Date and signature of laboratory technician \_\_\_\_\_

Comment: \_\_\_\_\_

If you have any question you can ask the following individuals

Daniel Dejene

Addis Ababa University College of Health Sciences,

Department of Medical Laboratory Sciences

Cell phone: +251-91-832293

E.mail:- Daniel.dejene@yahoo.com



**Annex- III: IUATLD/WHO recommended grading of sputum microscopy and Xpert MTB/RIF assay MTB/RIF results.**

| IUATLD/WHO scale<br>(1000x field = HPF) | Microscopy system used                                            |                                                                                |                                                                    | Xpert MTB/RIF assay<br>(MTB/RIF assay                  |
|-----------------------------------------|-------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------|--------------------------------------------------------|
|                                         | Bright field<br>(1000x magnification 1 length<br>= 2 cm = 100 HPF | Conventional fluorescence (200-250x magnification 1 length=30 fields = 300 HPF | iLED fluorescence (400xmagnification; 1 length=40 fields = 200 HPF | Result                                                 |
| Negative                                | Negative Zero AFB/1 length                                        | Negative Zero AFB/1 length                                                     | Negative Zero AFB/1 length                                         | T = MTB detected, rifampicin resistance not detected   |
| Scanty(actual count)                    | 1-9 AFB/1 length or100 HPF                                        | 1-29 AFB/1 length                                                              | 1-19 AFB/1 length                                                  | RR = MTB detected, rifampicin resistance detected      |
| 1+                                      | 10-99 AFB/1 length or100 HPF(1-9 AFB/10field                      | 30-299 AFB/1 lengthth                                                          | 20-199 AFB/1 length                                                | TI = MTB detected, rifampicin resistance indeterminate |
| 2+                                      | 1-10 AFB/1 HPF on average                                         | 10-100 AFB/1 Field on average                                                  | 5-50 AFB/1 Field on average                                        | N = MTB not detected                                   |
| 3+                                      | >10AFB/1 Field on average                                         | >100 AFB/1 Field on average                                                    | >50AFB/1 Field on average                                          | I = invalid / no result / error                        |

Xpert MTB/RIF test result Reporting Format:

Date sample collected: \_\_\_\_\_

*M. tuberculosis*: Detected ☐ Not detected ☐ Invalid / No result / Error ☐

Rifampicin resistance: Detected ☐ Not detected ☐ Indeterminate result ☐

Examined by (name and signature): \_\_\_\_\_ Date \_\_\_\_\_

## **Annex- IV: Ziehl-Neelsen and Fluorochrome Staining Reagent and culture sample decontaminating reagent preparation**

### **Ziehl-Neelsen Staining Reagent Preparation**

#### **CarbolFuchsin (3% ) Quantity per liter**

Basic fuchsin 3.0 g

Denatured alcohol or methanol (95% ethanol) technical grade 100.0 ml

Dissolve basic fuchsin in ethanol Solution 1

#### **Phenol**

Phenol crystals (technical grade) 50 g

Distilled water( purified water 850 ml

Dissolve phenol crystals in distilled water (gentle heat may be required) Solution 2

#### **Working solution**

Combine 10 ml of solution 1 with 90ml of solution 2 and store in an amber bottle. Label bottle with name of reagent as well as preparation and expiry dates. Store at room temperature for six to twelve months and filter before use.

#### **Decolorizing agent: 3% acid-alcohol**

Concentrated hydrochloric acid (technical grade) 30ml

Alcohol, 95% ethanol (technical grade) 970 ml

Carefully add concentrated hydrochloric acid to 95% ethanol. Store in an amber bottle and label bottle with name of reagent and dates of preparation and expiry. Store at room temperature for six to twelve months.

#### **Counterstain: Methylene blue (0.3%)**

Methylene blue chloride 3.0 g

|                 |           |
|-----------------|-----------|
| Distilled water | 1000.0 ml |
|-----------------|-----------|

Dissolve methylene blue chloride in distilled water and store in an amber bottle. Label bottle with name of reagent and dates of preparation and expiry. Store at room temperature for six to twelve month.

### **Fluorochrome Staining Reagent Preparation**

#### **Aura mine O (0.1%)**

Auramine 1.0 g

|                               |        |
|-------------------------------|--------|
| 95% ethanol (technical grade) | 100 ml |
|-------------------------------|--------|

|                              |            |
|------------------------------|------------|
| Dissolve auramine in ethanol | Solution 1 |
|------------------------------|------------|

#### **Phenol**

|                                    |        |
|------------------------------------|--------|
| Phenol crystals (analytical grade) | 30.0 g |
|------------------------------------|--------|

|                 |        |
|-----------------|--------|
| Distilled water | 870 ml |
|-----------------|--------|

|                                   |            |
|-----------------------------------|------------|
| Dissolve phenol crystals in water | Solution 2 |
|-----------------------------------|------------|

Mix solutions 1 and 2 and store in a tightly stoppered amber bottle away from heat and light. Label bottle with the name of the reagent and dates of preparation and expiry. Store at room temperature for three months . Turbidity may develop on standing but this does not affect the staining reaction.

### **Decolorizing solution**

|                                      |      |
|--------------------------------------|------|
| Concentrated hydrochloric acid (37%) | 5 ml |
|--------------------------------------|------|

|                                         |        |
|-----------------------------------------|--------|
| Denatured 95% ethanol (technical grade) | 995 ml |
|-----------------------------------------|--------|

Carefully add concentrated hydrochloric acid to the ethanol. Store in an amber bottle and labeled the bottle with name of reagent and dates of preparation and expiry. Store at room temperature for three months .

### **Counter stains**

#### **Potassium permanganate (0.5%)**

Potassium permanganate (KMnO<sub>4</sub>) certified grade

5.0 g

**Distilled water**

1000ml

Dissolve potassium permanganate in distilled water in a tightly stoppered amber bottle. Label bottle with name of reagent and dates of preparation and expiry. Store at room temperature for one month.

## **Preparation of reagents for digestion and decontamination of sputum**

### **A. NaOH-NALC reagents**

#### **Preparation**

- Prepare 4% NaOH solution by dissolving 4g NaOH pellets into 100 ml distilled/ deionized water. Sterilize by autoclaving. Concentration of NaOH may be varied (3- 6% NaOH solution at the beginning).
- Prepare 2.9% sodium citrate solution by dissolving 2.9 g sodium citrate (21120) in 100 ml distilled/deionized water. Sterilize by autoclaving.

#### **Mixing**

Prior to use, mix equal quantities of NaOH and sodium citrate solution. Prepare only as much volume as can be used in a day. Add NALC powder to achieve a final concentration of 0.5% (for 100 ml NaOH-Na citrate solution, add 0.5 g NALC powder). Mix well and use the same day. NALC activity is lost if left standing for more than 24 hours.

### **B. Sodium hydroxide solution**

Prepare 4% NaOH solution by dissolving 4g of NaOH in 100 ml distilled/deionized water. Sterilize by autoclaving. This solution can be stored and used for decontamination of (nonmucoid) contaminated cultures and specimens.

### **C. Phosphate buffer (pH 6.8, 0.067 M)**

- Dissolve 9.47g of anhydrous disodium phosphate (Na<sub>2</sub>HPO<sub>4</sub>) in 1000 ml (1 liter distilled/deionized water, using a volumetric flask.

- Dissolve 9.07 g mono potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) in 1000 ml (1 liter) distilled/ deionized water, using a volumetric flask.
- Mix equal quantities of the two solutions. Check the pH. Adding more solution A will raise the pH; more solution B will lower the pH. The final pH should be 6.8.
- Sterilize by autoclaving.

## **Annex V: Information sheet for study subjects (English version)**

**Principal Investigator: Daniel dejene**

**Addis Ababa University College of Health Sciences**

**Purpose:** The purpose of this study is to compare a combination of conventional light microscopy and fluorescent microscopy against Xpert MTB/RIF assay for the diagnosis of tuberculosis from sputum samples in Addis Ababa City Administration Health Bauru Health research and laboratory.

**Procedures to be carried on:** you are invited to participate in the study after giving your consent by giving sputum samples

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

**Benefits of the study:** There will be no financial or other direct benefit to you. But the result of the study will play a role in the TB control program. There will be no compensation for using your sputum.

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

**Termination of the study:** We will respect your decision if you later on change your mind. Your withdrawal of consent will not affect your right to receive medication.

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

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Name of Data collector \_\_\_\_\_ Signature \_\_\_\_\_

If you have any question you can ask the principal investigator

Daniel Dejene

Addis Ababa University College of Health Sciences,

Department of Medical Laboratory Sciences

Cell phone: +251-91-832293

E-mail:- [Daniel.dejene@yahoo.com](mailto:Daniel.dejene@yahoo.com)

## **Annex VI: Informed consent form (Amaric version)**

### **የተሳታፊዎች መረጃ ቅጽ**

#### **ጥናቱን የሚያጠናው፣ ዳንኤል ደጅኔ**

በአዲስአበባ የኒሽርሲ ቲጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

#### **የጥናቱ አላማ**

የጥናቱ አላማ ጅንኤ ከስፐርት የተባለው መሳሪያ በአካታውስ ጥቲቢን የመመርመር አቅሙን ለማወቅና በአለም የጤና ድርጅት የተሰጠው የፕራት ደረጃ ከኒኛ ሀገር እንዲገኝ በስራ ላይ ቢውል ጠቀሜታውን ለማሳየትና የመፍትሄ ሀሳብ ለመስጠት ነው፡፡

ጥናቱም በአዲስአበባ ከተማ መስዳድር ጤና ቢሮ የጤናና ምርምርና ላቦራቶሪውስ ጥይካሄዳል፡፡

በጥናቱ ወቅት ከእርስዎ የሚጠበቀው በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ አካታና ሙና መስጠት ነው፡፡

#### **ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም**

በጥናቱ ለሚሳተፉ ፈቃደኛ ተሳታፊዎችም አይነት የገንዘብ ክፍያ የለውም ነገር ግን ከጥናቱ የሚገኘው ጤና ለቲቢ ቁጥጥር ስራ ጤናማ ይሆናል፡፡

#### **በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት**

በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍ ምሊደርስ በሰዎች ላይ አንድም ጉዳት አይኖርም፡፡ በጥናቱም ከንጾች የሚያባከኑት ተጨማሪ ጊዜም አይኖርም፡፡

#### **የመረጃ ሚስጥራዊ አጠባበቅ**

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘው በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡

በጥናቱ ላይ ያለ መሳተፍ መብት አለዎት፡፡ ይህ መረጃ በጥንቃቄ የሚያዝይ ሆኗል፡፡ በመጨረሻም የጥናቱ ጤነኛ መለኮተኛ አካል ለጥናቱ አላማ ብቻ የሚገለፅ ይሆናል፡፡

**ያስታውሱ፤** ስለ ዚህ ጥናት ማንኛውም ጥያቄ ካልዎት በማንኛውም ጊዜ ዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

እኔም የጥናቱ ተሳታፊ ሆኖ ህንጻውን በመገንዘብ የጥናቱ ላይ መሳተፍ ተስማምቼ ያለሁ፡፡

ፊርማ -----

መረጃውን የሰበሰበው ግለሰብ ስም -----

ፊርማ -----

**የዋና ተመራማሪው አድራሻ፤**

📍 ዳንኤሷል ደጃኔ፤ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፤ የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ - አዲስ አበባ፤ ኢትዮጵያ

📧 ኢ-ሜይል፤ danieshah@gmail.com

📞 ስልክ፤ +251911832293

## **Annex VII: Declaration**

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

Name: Daniel Dejene

Signature \_\_\_\_\_

Date of submission \_\_\_\_\_

Place: Addis Ababa

This thesis has been submitted with my approval as University advisor.

1) Name \_\_\_\_\_

Signature \_\_\_\_\_

Date \_\_\_\_\_

Place: \_\_\_\_\_

2) Name \_\_\_\_\_

Signature \_\_\_\_\_

Date \_\_\_\_\_

Place: \_\_\_\_\_