Addis Ababa University,

School of Graduate Studies

MSc Research Thesis

Comparative Analysis of Performance of PREVI Fluo TB Method by Using LED-Microscopy in TB Suspected Patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

By:

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A thesis submitted to the Department of Medical Microbiology, Immunology and Parasitology, School of Medicine, Addis Ababa University.

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ABREVIATIONS

AAU	Addis Ababa University
AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
AO	Auramine O
ART	Antiretroviral therapy
AST	Antibiotic Susceptibility Testing
BCG	Bacille Calmette-Guerin
CBC	Complete Blood Cell Count
CD	Cluster of differentiation
DC	Dendritic cells
DNA	Deoxyribonucleic Acid
DOTS	Directly Observed Therapy -short course
EFV	Efavirenz
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme Linked Immuno Spot
E-MTD	Enhanced Microbacterium Tuberculosis Direct Test
EPTB	Extra pulmonary tuberculosis
FDA	Food and Drug Administration
Fl	Fluorochrome
FM	Fluorescent Microscopy
FMOH	Federal ministry of health
HBC	High Burden Countries
HIV	Human Immunodeficiency Virus
ID	Identification
IPT	Isonized Preventive therapy
IUATLD	International Union Against Tuberculosis and Lung Disease

LED	Light Emitting Diod
LJ	Lowenstein-Jensen
MC	Mast cell
MGIT	Mycobacterial Growth Indicator Tube
MHC	Major Histocompatibility Complex
MTB	Mycobacterium tuberculosis
MV	Mercury Vapour
NAAT	Nucleic Acid Amplification Test
OI	Opportunistic Infections
PCR	Polymerase Chain Reaction
PI	Principal Investigator
QC	Quality control
RNA	Ribonucleic Acid
TB	Tuberculosis
TST	Tuberculin Skin Test
UNAIDS	United Nations program for AIDS
UV	Ultra Violet
VCT	Voluntary counseling and testing
WHO	World Health Organization
ZDV	Zidovudine
ZN	Ziehl-Neelsen

ABSTRACT

Background: Tuberculosis is a prominent problem in developing countries. Hence, there is a need for rapid, practical and accurate diagnostic tools that are adapted to resource-poor settings in order to ensure that those affected receive proper and timely treatment. Light-Emitting-Diode microscopy (LED) has recently been endorsed by the WHO for diagnosis of TB in these countries. A much recent smear diagnosis method, PREVI FLUO TB by using LED microscopy, has been made available. However, due to the novelty of the method, there is no information available on the specificity and sensitivity when compared to established methods such as the ordinary LED -Auramine O or the classical widely used Ziehl-Neelsen (ZN) in TB and TB-HIV co-infected persons.

Objective: The study was undertaken to compare the sensitivity, specificity, PPV and NPV of PREVI Fluo TB stain with ZN method and Auramine O- LED microscopy.

Methods: A prospective cross sectional study was conducted in St. Paul's Millennium Medical College, Addis Ababa, Ethiopia from October/2011-April/2012. Spot-Morning-Spot sputum samples from 248 TB suspected study participants were collected. A total of 2,232 slides were made of which 744 slides were subject to be stained by each method. Confirmation was made by Lowenstein-Jenssen (LJ) culture which was used as a reference standard.

Results: The smear detection rate of PREVI Fluo TB, ZN, Auramine O and culture were found to be 35 (14.1%), 24(9.7%), 44(17.7%) and 30(12.1%) respectively.The sensitivity of the PREVI Fluo TB method was 76.67%, better than ZN and slightly less than Auramine O which were 59.07% and 78.13% respectively. Nevertheless, the specificity (94.5%) was lower than ZN (96.79%) and higher than Auramine O (91.2%). The negative likelihood ratio of PREVI Fluo TB, ZN, and Auramine O methods were 0.25, 0.45, and 0.24 respectively. The PREVI Fluo TB method had a PPV of 65.71% which is higher than the Auramine O (56.82%) but with a comparable NPV (96.71% and 96.57% respectively). The respective agreements of the ZN, Auramine O and PREVI Fluo TB methods with the gold standard were K=0.585, K=0.621 and K=0.664. There was a substantial agreement of PREVI Fluo TB result with ZN (k=0.636) and Auramine O methods (K=0.745).

Conclusion: The PREVI Fluo TB fluorescent staining method had nearly identical sensitivity compared to the conventional Auramine O and significantly higher sensitivity than ZN staining method. Given the practical benefits of PREVI Fluo TB for TB diagnosis, and comparable accuracy to the current standard of Auramine O fluorescence method and the gold standard culture, PREVI Fluo TB should be considered by TB diagnostic laboratories, as an alternative diagnostic tool for conventional Auramine O fluorescent stain.

Key words: Tuberculosis, ZN, Auramine O, PREVI Fluo TB

1. INTRODUCTION

1.1 General Introduction

Tuberculosis (TB), one of the oldest known human diseases, is still one of the major causes of mortality and a major public health problem particularly in low-income countries. It is estimated that nearly one billion people will be infected with TB, 200 million develop the disease, and 35 million will die from TB during 2000- 2020 (Floyd *et al*., 2002).

TB has many manifestations, affecting the bone, the central nervous system, and many other organ systems, but it is primarily a pulmonary disease that is initiated by the deposition of *Mycobacterium tuberculosis*, contained in aerosol droplets, onto lung alveolar surfaces. From this point, the progression of the disease can have several outcomes, determined largely by the response of the host immune system (Cole *et al*., 1998).

The prevalence of tuberculosis is continuing to increase because of the increased number of patients infected with human immunodeficiency virus, bacterial resistance to medications and increased international travel (Goldrick, 2004). Due to the high incidence of both HIV and MTB infection in the developing countries, TB has emerged as the most common opportunistic infection (OI) in HIV-infected patients worldwide (WHO, 2006a).

The “gold standard” of TB diagnosis is identification of the bacilli after culturing it and growing sufficient quantities for analysis. However, culturing the TB bacteria is expensive and slow (taking weeks), and requires technical facilities that are often unavailable in developing countries. Thus, diagnosis is often based on a test for “acid- fast” bacteria in a sputum smear (made by smearing sputum on a microscope slide). In patients with active pulmonary TB, only an estimated 45% of infections are detected by sputum microscopy (Dye *et al*., 2005a). This test [Ziehl-Neelsen], first developed in the 1880s and basically unchanged today, has the advantage of being simple but is hampered by very low sensitivity; it may only detect half of all cases with active infection. It is also very dependent on the skill

of the technician, and a single technician can only process a relatively small number of slides per day (Perkins *et al*., 2006).

Numerous reports have confirmed the superior diagnostic performance of fluorescence microscopy, compared with Ziehl-Neelsen (ZN) staining and light microscopy. However, the main reason that fluorescence microscopy is not used more widely is the need for a more complex and expensive fluorescent microscope, the limited lifespan (typically 200– 300 hrs) and the high cost of the short-arc mercury vapor lamp (MVP), which has traditionally been used as the excitatory light source. Repeated on-and-off switching, as may occur with unreliable local power supply, shortens the lifespan even further (Anthony *et al*., 2006). In addition, MVPs are energy inefficient and require an extensive power supply; they may also fail catastrophically and release toxic mercury into the environment (Anthony *et al.,* 2006). Consequently, fluorescence microscopes provided by donor agencies often fall into disuse because of high maintenance costs (Hung *et al*., 2007) .

New generation of fluorescence microscopes has now been developed based on LED technology. Considerable research and development have subsequently resulted in inexpensive, robust LED microscopes or LED attachments aimed at routine use in resource-limited settings. A leading microscope manufacturer (Zeiss MicroImaging, Göttingen, Germany), in a joint development agreement with FIND, has developed a fluorescence microscope (Primo Star iLED). Adequate evidence is available to recommend the use of auramine stains for LED microscopy (WHO, 2010a).

Further research is required on patient important outcomes of LED microscopy, as well as research into combining LED microscopy with novel approaches for early case detection and/or sputum processing (WHO, 2010a).

This study was designed to compare three methods of microscopy stains; the Ziehl-Neelsen, Auramine O and PREVI fluo TB by using well characterized clinical specimens from patients who were assessed according to standard parameters for *M. tuberculosis.*

1.2 Statement of the Problem

The backbone of TB diagnosis worldwide continues to be smear microscopy. Thus, increasing the sensitivity of smear microscopy could have a large impact on global TB case detection rates. As a result there have been several initiatives to optimize smear microscopy including changes in specimen collection procedures, specimen processing, and microscopy techniques (Steingart *et al.*, 2006b);(Mase *et al*., 2007b);(WHO, 2007a)

Conventional light microscopy using Ziehl-Neelsen (ZN) stained smears prepared directly from sputum specimens is the most widely available test for diagnosis of tuberculosis (TB) in resource-limited settings. Specificity of ZN microscopy is high but sensitivity is variable (20- 80%) and significantly reduced in extra-pulmonary TB and in HIV-infected TB patients (WHO, 2007a). Besides being labour-intensive, direct sputum smear microscopy may have considerable patient costs and inconvenience associated with the need to submit multiple sputum specimens over a period of up to three days. A number of TB control programs have reported high rates of initial patient default as a result (WHO, 2009).

Conventional fluorescent microscopy has documented higher sensitivity than ZN and takes less time, but uptake has been hampered by high cost due to expensive mercury vapour light sources, the need for regular microscopy maintenance, and the requirement for a dark room. Light emitting diode (LED) technology has been developed over recent years to allow the benefits of fluorescent microscopy without the associated costs (WHO, 2010a). Light emitting diode (LED) microscopy is a novel diagnostic tool developed primarily to allow resource-poor parts of the world access to the benefits of FM (Hanscheid, 2008). Compared to conventional mercury vapour fluorescence microscopes, LED microscopes are less expensive and have lower maintenance requirements. The diodes are very durable, do not require warm-up time, and do not contain toxic products (Minion *et al*., 2009). Importantly, they are reported to perform equally well without a darkroom. These qualities make them attractive for use in low- and middle-income countries, and they have performed well in evaluations in these settings (Shenai *et al*., 2011).

For microscopic detection of acid fast bacilli (AFB), fluorescence microscopy using auramine staining has been shown to have 10% higher sensitivity compared to routine light microscopy used with ZN staining, without compromising specificity (Steingart *et al*., 2006c).In 2009, the evidence base for LED microscopy was assessed by the World Health Organization (WHO) following standards appropriate for evaluating both the accuracy and patient/public health impact of new TB diagnostics. Results showed equivalent accuracy of LED microscopy to international reference standards, improved sensitivity over conventional ZN microscopy, and qualitative, operational and cost advantages of LED relative to both conventional fluorescent and ZN microscopy. Based on these findings, WHO recommend that conventional fluorescent microscopy must be replaced by LED microscopy, and that LED microscopy be phased in as an alternative for conventional ZN light microscopy. Sensitivity is largely determined by the duration of microscopic examination. Where workloads are high and the amount of time spent examining smears is low, sensitivity is correspondingly low (Cambanis *et al*., 2007). FM is also more time efficient, with one large study reporting FM to take only 25% of the time required for ZN examinations (Bennedsen and Larsen, 1966). Countries implementing LED microscopy should address laboratory staff training, country validation, introduction of appropriate quality assurance, and monitoring of impact on TB case detection and treatment outcome (WHO, 2010a).

Therefore, this study aims to assess the performance of a new PREVI Fluo TB method with LED-FM for the diagnosis of TB compared to ZN microscopy in a referral hospital.

1.3 Literature Review

1.3.1 Mycobacterium tuberculosis

Mycobacterium tuberculosis belongs to the family Mycobacteriaceae, order Actinomycetales and genus Mycobacterium. There are three species under this genus and these include M. tuberculosis complex, the non-tuberculosis mycobacteria and *M. leprae* (Shinnick and Good, 1994). On the basis of growth rate, catalase and niacin production, and pigmentation in light or dark, mycobacteria are classified into members of the Mycobacterium tuberculosis complex (*M. tuberculosis*, *M. bovis, M. africanum, M. microtii, M.caprae, M.caneti and M.pinnepedi*) and non tuberculous species. Gene probe technology now facilitates this distinction (David, 2000). These are genetically closely related sub-species where repetitive DNA elements such as insertion sequence IS 6110 and direct repeat have been found to be restricted to the *M. tuberculosis* complex. Of the pathogenic species belonging to the *M. tuberculosis* complex, the most frequent and important agent of human disease is *M. tuberculosis* (vanSoolingen *et al*., 1997).

M. tuberculosis is rod-shaped, non-motile and non-sporulated. It has high lipid content in the wall, probably the highest in proportion among all bacteria. The complete genome of the mycobacterial strain H37Rv has been sequenced and is known to contain 4,411,529 base pairs, about 4000 genes with a G+C content of 65.6% (Cole *et al*., 1998). Mycobacteria are primarily intracellular pathogens, have slow growth rates, are obligate aerobes, and produce a granulomatous reaction in normal hosts. In cultures, *M. tuberculosis* does not produce significant amounts of pigment, has a buff-colored, smooth surface appearance, and biochemically produces niacin. These characteristics are useful in differentiating *M. tuberculosis* from nontuberculous mycobacteria (Habeenzu *et al*., 1998). One characteristic but not distinctive morphologic property of *M. tuberculosis* is the tendency to form cords, or dense clusters of bacilli, aligned in parallel. The biochemical background of cording is called cord factor (a trehalose dimycolate), and its contribution to bacterial virulence is still unclear (Mehta *et al*., 1996).The main defining characteristic of the genus Mycobacterium is the property called acid-fastness, which is the ability to withstand decolorization with an

acid-alcohol mixture after staining with carbolfuchsin or auramine-rhodamine (Mehta *et al*.,1996).

Mycobacteria, including *M. tuberculosis*, are often neutral on Gram's staining. However, once stained, the bacilli cannot be decolorized by acid alcohol, a characteristic justifying their classification as acid-fast bacilli. Acid fastness is due mainly to the organisms' high content of mycolic acids, long-chain cross-linked fatty acids, and other cell-wall lipids. In the mycobacterial cell wall, lipids (e.g., mycolic acids) are linked to underlying arabinogalactan and peptidoglycan. This structure confers very low permeability of the cell wall, thus reducing effectiveness of most antibiotics (Mario *et al*., 1995).

More recently, HIV has brought about an enormous growth in the incidence of tuberculosis especially in sub-Saharan Africa (Corbett *et al*., 2003). For most of the world tuberculosis has remained a common threat with more than 9 million people still developing active TB each year and nearly 2 million die (WHO, 2011a). In immunocompetent individuals infected with *M. tuberculosis*, there is a 5–10% risk of developing active tuberculosis within 2 years of primary disease. Later there is a 5–10% lifetime risk of developing acute disease – reactivation, while in countries with high prevalence the disease is found in the young and very young adults (Rieder, 1999). However, the interaction between HIV and tuberculosis serves to facilitate the spread of the disease (Corbett *et al*., 2003). HIV makes the individual more susceptible to infection, and HIV positives also have a 10% risk of reactivation of the disease per year (Glynn, 1998).

1.3.2 Virulence and Pathogenesis

Virulence

The lipid composition and quantity of the cell wall components affect the bacteria's virulence and growth rate (Lee *et al*., 2005). The peptidoglycan polymer confers cell wall rigidity and is just external to the bacterial cell membrane, another contributor to the permeability barrier of mycobacteria. Another important component of the cell wall is lipoarabinomannan, a carbohydrate structural antigen on the outside of the organism that is immunogenic and facilitates the survival of mycobacteria within macrophages (Lee *et al*., 2005); (Joe *et al*., 2007).

M. tuberculosis usually enters the alveolar passages of exposed humans in an aerosol droplet, where its first contact is thought to be with resident macrophages, but it is also possible that bacteria can be initially ingested by alveolar epithelial type II pneumocytes. This cell type is found in greater numbers than macrophages in alveoli, and *M. tuberculosis* can infect and grow in these pneumocytes ex vivo (Bermudez and Goodman, 1996, Mehta et al., 1996). In addition, dendritic cells (DCs) play a very important role in the early stages of infection since they are much better antigen presenters than are macrophages (Tascon *et al*., 2000) and presumably play a key role in activating T cells with specific M. tuberculosis antigens (Bodnar *et al*., 2001). Since dendritic cells are migratory, unlike differentiated macrophages, they also may play an important role in dissemination of *M. tuberculosis* (Lipsitch and Sousa, 2002). The bacteria are phagocytosed in a process that is initiated by bacterial contact with macrophage mannose and/or complement receptors (Schlesinger, 1993). Surfactant protein A, a glycoprotein found on alveolar surfaces, can enhance the binding and uptake of *M. tuberculosis* by up regulating mannose receptor activity (Gaynor *et al*., 1995)

The relative ease of working with tissue culture has provided many data on *M. tuberculosis* entrance and trafficking in the macrophage and on other responses of the infected cells, but there is much less information on how the bacterium survives and grows during later stages of infection in the lung. It is known that infected macrophages in the lung, through their

production of chemokines, attract inactivated monocytes, lymphocytes, and neutrophils (Troesch *et al*., 1999), none of which kill the bacteria very efficiently (Fenton and Vermeulen, 1996). Then, granulomatous focal lesions composed of macrophage-derived giant cells and lymphocytes begin to form. This process is generally an effective means of containing the spread of the bacteria. As cellular immunity develops, macrophages loaded with bacilli are killed, and this results in the formation of the caseous center of the granuloma, surrounded by a cellular zone of fibroblasts, lymphocytes, and blood-derived monocytes (Dannenberg and Rook, 1994). Although *M. tuberculosis* bacilli are postulated to be unable to multiply within this caseous tissue due to its acidic pH, the low availability of oxygen, and the presence of toxic fatty acids, some organisms may remain dormant but alive for decades. The strength of the host cellular immune response determines whether an infection is arrested here or progresses to the next stages. This enclosed infection is referred to as latent or persistent TB and can persist throughout a person's life in an asymptomatic and nontransmissible state. In persons with efficient cell-mediated immunity, the infection may be arrested permanently at this point. The granulomas subsequently heal, leaving small fibrous and calcified lesions. However, if an infected person cannot control the initial infection in the lung or if a latently infected person's immune system becomes weakened by immunosuppressive drugs, HIV infection, malnutrition, aging, or other factors, the granuloma center can become liquefied by an unknown process and then serves as a rich medium in which the now revived bacteria can replicate in an uncontrolled manner. At this point, viable *M. tuberculosis* can escape from the granuloma and spread within the lungs (active pulmonary TB) and even to other tissues via the lymphatic system and the blood (miliary or extrapulmonary TB). When this happens, the person becomes infectious and requires antibiotic therapy to survive (Dannenberg and Rook, 1994).

Pathogenesis

Mycobacterium tuberculosis is spread by small airborne droplets, called droplet nuclei, generated by the coughing, sneezing, talking, or singing of a person with pulmonary or laryngeal tuberculosis. These minuscule droplets can remain airborne for minutes to hours after expectoration. The number of bacilli in the droplets, the virulence of the bacilli, exposure of the bacilli to UV light, degree of ventilation, and occasions for aerosolization all influence transmission. Introduction of *M tuberculosis* into the lungs leads to infection of the respiratory system; however, the organisms can spread to other organs, such as the lymphatics, pleura, bones/joints, or meninges, and cause extrapulmonary tuberculosis (CDC, 2000).

Once inhaled, the infectious droplets settle throughout the airways. The majority of the bacilli are trapped in the upper parts of the airways where the mucus-secreting goblet cells exist. The mucus produced catches foreign substances, and the cilia on the surface of the cells constantly beat the mucus and its entrapped particles upward for removal (Frieden *et al*., 2003). This system provides the body with an initial physical defense that prevents infection in most persons exposed to tuberculosis (Jensen *et al*., 2005). The complement system also plays a role in the phagocytosis of the bacteria(Li *et al*., 2002). The complement protein C3 binds to the cell wall and enhances recognition of the mycobacteria by macrophages. Opsonization by C3 is rapid, even in the air spaces of a host with no previous exposure to *M tuberculosis* (Ferguson *et al*., 2004).

1.3.3 TB-HIV Co-infection

Infection with HIV constitutes the strongest risk factor for development of TB in subjects with latent MTB infection. Due to the high incidence of both HIV and MTB infection in the developing countries, TB has emerged as the most common opportunistic infection (OI) in HIV-infected patients worldwide (WHO, 2006a). Thus, the interaction of these two pathogens currently and in the future will potentiate morbidity and mortality associated with either. Globally, more than one-third of HIV positive individuals are co infected with MTB and 12% of AIDS deaths are attributed to TB (Raviglione and O'Brien, 2001); (Corbett *et al*., 2003). In Africa, HIV is the single most important factor determining the increased incidence of TB in the past 10 years (WHO, 2006b). HIV/AIDS accounted for 32% of the estimated 141,000 cases of tuberculosis in Ethiopia in 2005 (MOH/HAPCO, 2005).

1.3.4 Clinical Manifestations

Most TB infections are initiated by the respiratory route of exposure, now that milk products are generally pasteurized, at least in the developed world. One study in 1978, prior to the AIDS epidemic, showed that 85% of new TB cases were pulmonary (Hopewell, 1994). Thus, the different forms of the disease discussed usually arise from dissemination of the bacilli from infected lungs.

TB in many cases follows a general pattern as described by Wallgren, who divided the progression and resolution of the disease into four stages (Wallgren, 1948). In the first stage, dating from 3 to 8 weeks after *M. tuberculosis* contained in inhaled aerosols becomes implanted in alveoli, the bacteria are disseminated by the lymphatic circulation to regional lymph nodes in the lung, forming the so-called primary or Ghon complex. At this time, conversion to tuberculin or reactivity occurs. The second stage, lasting about 3 months, is marked by hematogenous circulation of bacteria to many organs including other parts of the lung; at this time in some individuals, acute and sometimes fatal disease can occur in the form of tuberculosis meningitis or miliary (disseminated) tuberculosis. Pleurisy or inflammation of the pleural surfaces can occur during the third stage, lasting 3 to 7 months and causing severe chest pain, but this stage can be delayed for up to 2 years. It is thought

that this condition is caused by either hematogenous dissemination or the release of bacteria into the pleural space from subpleural concentrations of bacteria in the lung. The free bacteria or their components are thought to interact with sensitized CD4 T lymphocytes that are attracted and then proliferate and release inflammatory cytokines. The last stage or resolution of the primary complex, where the disease does not progress, may take up to 3 years. In this stage, more slowly developing extrapulmonary lesions, e.g, those in bones and joints, frequently presenting as chronic back pain, can appear in some individuals. However, most humans who are infected with TB do not exhibit progression of the disease. One-third of exposed HIV-negative individuals become infected, and of this number 3 to 5% develop TB in the first year. An additional 3 to 5% of those infected develop TB later in their lives (Kamholz, 1996).

1.3.5 Epidemiology

M tuberculosis is contagious, but only 5-10 percent of infected normal individuals develop active disease. Tuberculosis is most common among the elderly, poor, malnourished, or immunocompromised, especially persons infected with human immunodeficiency virus (HIV). Persistent infection may reactivate after decades owing to deterioration of immune status; exogenous reinfection also occurs (David, 2000).

The epidemiology of tuberculosis is quite different from most other infectious diseases. This is due to the relatively long incubation period and the presence of both primary and reactivation forms of disease. Mathematical modeling of the epidemiology suggests that epidemics of tuberculosis are slow to develop peaking between 50 and 200 years after introduction of the disease (Blower *et al*., 1995).

In 2009, there were an estimated 9.4 million incident cases (range, 8.9 million–9.9 million) of TB globally (equivalent to 137 cases per 100 000 population). The absolute number of cases continues to increase slightly from year to year (WHO, 2009). Most of the estimated number of cases in 2009 occurred in Asia (55%) and Africa (30%). The 22 HBCs that have received particular attention at the global level since 2000 accounts for 81% of all estimated cases worldwide (WHO, 2010a). The five countries with the largest number of incident

cases in 2009 were India (1.6–2.4 million), China (1.1–1.5 million), South Africa (0.40–0.59 million), Nigeria (0.37– 0.55 million) and Indonesia (0.35–0.52 million). India alone accounts for an estimated one fifth (21%) of all TB cases worldwide, and China and India combined account for 35%.Of the 9.4 million incident cases in 2009, an estimated 1.0–1.2 million (11–13%) were HIV-positive, with a best estimate of 1.1 million (12%). Of these HIV-positive TB cases, approximately 80% were in the African Region (WHO, 2010a). When survey data are not available, it is difficult to estimate its absolute level and trend. There were an estimated 440 000 cases of multi-drug resistant TB (MDR-TB) in 2008 (range, 390 000– 510 000) (WHO, 2010c).The four countries that had the largest number of estimated cases of MDR-TB in absolute terms in 2008 were China (100 000; range, 79 000–120 000), India (99 000; range, 79 000–120 000), the Russian Federation (38 000; range, 30 000–45 000) and South Africa (13 000; range 10 000–16 000). By July 2010, 58 countries and territories had reported at least one case of extensively drug-resistant TB (WHO, 2010c).

Thus in total, approximately 1.7 million people died of TB in 2009. The number of TB deaths per 100 000 population among HIV-negative people plus the estimated number of TB deaths among HIV-positive people equates to a best estimate of 26 deaths per 100 000 population (WHO, 2010c). Tuberculosis remains a major public health problem worldwide. Almost one third of the world's population is infected with *M. tuberculosis*. It is the foremost cause of death from a single infectious agent in adults, which kills a person in the world at every 15 seconds (Dye *et al*., 2005b).

With the current available information, with a population estimate of 82,825,000, Ethiopia stands on the 7th rank of the world's top 22 TB high-burden countries with an estimated annual incidence of 379 cases and prevalence of 643 cases per 100,000 populations (WHO, 2008a) .The high burden of TB in Ethiopia might in part be attributed to the rapid increase of HIV infection, because available data indicate that HIV/ AIDS accounted for an estimated 32% or 141,000 total TB cases in 2005 (MOH, 2007). Ethiopia is one of the top three in

Africa, with regard to the number of TB patients. Over a third of the population has been exposed to TB (WHO, 2004b) .

According to the Ministry of Health hospital statistics data, tuberculosis is one of the leading causes of morbidity, the fourth cause of hospital admission, and the second cause of hospital death in Ethiopia. Nearly a third of all TB cases are fatal, killing over 42,000 people in Ethiopia in 2004, excluding those who had HIV/AIDS. Social and biological factors that have aggravated the problem in Ethiopia include recurrent famine and widespread poverty that leads to severe malnutrition (MOH, 2004a).The incidence rate of tuberculosis is increasing in Ethiopia at a rate of 5 new TB cases per 100,000 populations per year. Masculine gender was also disproportionately affected by tuberculosis during the ten-year study period. On the other hand case detection rate and treatment success rate are found to be increasing at a rate of 0.5% per year (GlobalFund, 2004). The prevalence of smear-positive pulmonary TB in 2010 in Ethiopia was estimated to be 200 per 100 000 in the total population (i.e. including children) (WHO, 2011b) .

The first Ethiopian National TB Prevalence survey was undertaken from 2010-2011. The prevalence of smear positive TB among people aged 15 and above was found to be 108/100,000. Whereas prevalence of bacteriological confirmed TB within the same age group was 277/100,000. This survey found three times lower prevalence of smear positive TB from 2008 estimation (284/100,000) and routine surveillance report of TB cases is close to the true prevalence of smear positive TB in the community (http://ehnri.gov.et/, 2012).

Although much work is currently being conducted in order to develop new diagnostics, in most resource-limited countries, sputum smear microscopy remains the primary means for the diagnosis of TB. Given the known limitations of smear microscopy, considerable research has been conducted to identify methods that can increase the sensitivity and optimize its yield. A series of recent systematic reviews has demonstrated that microscopy can be optimized using at least three different approaches: chemical and physical processing (e.g., treatment with bleach or centrifugation), fluorescence microscopy (FM) and the examination of two (rather than three) sputum specimens (Mase *et al*., 2007a).

There are several reports indicate that fluorochrome staining (either auramin phenol or auramin rhodamine) of smears significantly increases the sensitivity of direct microscopy (Murray *et al.,* 2003). The higher sensitivity of this method is attributed to the ease of the detection of a fluorescent rod against a darker background. This allows the examiner to scan the slide at a lower magnification and thus observe a larger area than with carbol fuchsine-stained smears. These factors reduce the time for screening and lead to greater sensitivity. Therefore, it is generally accepted that the fluorescent method should be given preference over the ZN and Kinyoun methods (Tenover *et al*., 1993).

Fluorescence microscopy has several advantages over light microscopy using ZN staining and is widely used in most developed nations. First, the fluorochrome staining procedure used with FM is simpler than that of ZN staining. Second, it has been estimated, using meta-analysis, that FM has approximately 10% greater sensitivity for detecting acid-fast bacilli in patient specimens. Third, and possibly most important, since FM can be examined at a lower magnification than ZN (20–40 vs 100×), slides are read more quickly and efficiently with FM. It has been estimated that using FM may take up to 75% less time than ZN. This advantage would be of tremendous benefit for overburdened laboratory systems in many low-resource settings (Steingart *et al*., 2006d).

A study was under taken in India to compare the efficacy of fluorochrome stain (Fl) with conventional Ziehl Neelsen (ZN) stain in the diagnosis of pulmonary tuberculosis. Two hundred cases of pulmonary tuberculosis were included in the study. Sputum smears were screened for acid fast bacilli (AFB) by ZN and Fl methods and blood samples were screened for HIV. Sputum positive cases detected by Fl stain were higher in number (69%) when compared to ZN stain (50%). Of the total cases studied 15.5% were HIV seropositive. Fluorochrome staining was found to be more efficient (45%) when compared to ZN staining (29%) in detecting cases associated with HIV seropositivity, especially paucibacillary cases (Prasanthi and Kumari, 2005).

In a similar study done in India, from January 2004 to June 2005, out of 102 patients, 44.1%, 71.6% and 70% were found positive by ZN, AO and culture respectively. AO was found to be superior to ZN on several aspects. The difference in their case detection rates was statistically significant. AO was also able to detect more pauci-bacillary cases than ZN. There was more agreement between culture and fluorescence microscopy (95.1%) than with ZN microscopy (69.6%). The percentage of false negative by AO staining was only 2.78% which was in sharp contrast to that of ZN (40.27%). The study concluded that the better case detection rates of Auramine O over ZN were comparable to those found by several studies. Since screening was done under lower power of magnification (40x), fluorescence microscopy has been found to be less time consuming as compared to ZN method (100x) in the diagnosis of tuberculosis. The tubercle bacilli stood out as bright objects against a dark background in fluorescence microscopy which makes them easily identifiable hence causing less eye-strain. The efficacy of fluorescence microscopy proved to be much higher than conventional light microscopy and comparable to that of culture (Laifangbam *et al*., 2009).

Recently, it has been demonstrated that low-cost, ultra-bright light-emitting diodes (LEDs) could be a viable alternative to MV lamps used in FM. LEDs can produce very narrow spectrum light and are able to excite Auramine and other commonly used fluorescent stains without the production of UV light. LEDs have an expected bulb life of up to 50,000 h (compared with 200 h for a conventional mercury bulb), produce minimal heat and contain no hazardous materials. Power consumption is much lower, to the point where portable battery operation or solar power is feasible. Finally, it has been reported that image quality remains good outside of a darkroom, a significant advantage where space constraints and lack of air conditioning are important barriers to user acceptance (WHO, 2007b).

Three hundred sputum samples from patients in Cameroon were studied by using the CyScope, a new light-emitting, diode-based, fluorescence microscope, to compare auramine-rhodamine fluorescence with the conventional Ziehl-Neelsen staining method. The smear positivity for acid-fast bacilli with the Ziehl-Neelsen staining method was 27.7% (83 of 300) compared with 33.3% (100 of 300) with the fluorescent method. Staining time with the modified fluorescence protocol could be reduced from 21 minutes to 10 minutes. This

study confirmed that the fluorescence staining method is more sensitive than the Ziehl-Neelsen staining method. In this study total 66 (44%) out of 150 sputum specimens were positive for Mycobacterium Tuberculosis by culture. Sensitivity and specificity documented for the different modalities were 95.38% and 94.11%, respectively, for the LED assessment; 68.18% and 90.47%, respectively, for the CFM assessment; and 56.06% and 97.61%, respectively, for brightfield microscopy by ZN stain. The difference in their case detection rate was statistically significant (X^2=119.38, p<0.001) (Khatun *et al*., 2011).

Adults with cough ≥2 wk were enrolled consecutively in Ethiopia, Nepal, Nigeria, and Yemen. Sputum specimens were examined by ZN smear microscopy and LED-FM and compared with culture as the reference standard. In total, 529 (21.6%) culture-positive and 1,826 (74.6%) culture-negative patients were enrolled, of which 1,156 (49%) submitted SSM specimens and 1,199 (51%) submitted SMS specimens. Single LED-FM smears had higher sensitivity but lower specificity than single ZN smears. Using two LED-FM or two ZN smears per patient was 72.8% (385/529, 95% CI 68.8%–76.5%) and 65.8% (348/529, 95% CI 61.6%–69.8%) sensitive (P=0.001) and 90.9% (1,660/1,826, 95% CI 89.5% 92.2%) and 98% (1,790/1,826, 95% CI 97.3%–98.6%) specific (P=0.001). Using three LED-FM or three ZN smears per patient was 77% (408/529, 95% CI 73.3%–80.6%) and 70.5% (373/529, 95% CI 66.4%–74.4%) sensitive and 88.1% (95% CI 86.5%–89.6%) and 96.5% (95% CI 96.8%–98.2%, (P=0.001) specific. The sensitivity/specificity of ZN smear microscopy and LED-FM did not vary between SMS and SSM (Luis *et al*., 2011a).

In conclusion, despite the generally archaic system of diagnosis for TB globally, progress is indeed being made. The application of high-powered LEDs for use in diagnostic fluorescent microscopy is an excellent example of using existing technology to fill a practical need, and one that may prove to have an important impact on global TB-diagnostic programs.

1.3.6 Diagnosis of Pulmonary Tuberculosis

Clinical diagnosis

The medical history includes obtaining the symptoms of pulmonary TB: productive, prolonged cough of three or more weeks, chest pain, and hemoptysis. Systemic symptoms include low grade remittent fever, chills, night sweats, appetite loss, weight loss, easy fatiguability, and production of sputum that starts out mucoid but changes to purulent. Other parts of the medical history include prior TB exposure, infection or disease; past TB treatment; demographic risk factors for TB; and medical conditions that increase risk for TB disease such as HIV infection. Depending on the sort of patient population surveyed, as few as 20%, or as many as 75% of pulmonary tuberculosis cases may be without symptoms. Tuberculosis should be suspected when a pneumonia-like illness has persisted longer than three weeks, or when a respiratory illness in an otherwise healthy individual does not respond to regular antibiotics. A physical examination is done to assess the patient's general health and find other factors which may affect the TB treatment plan. It cannot be used to confirm or rule out TB (Burke and Parnell, 1948).

Laboratory Diagnosis of Tuberculosis

I) Microscopy

One of the most successful tests for the diagnosis of mycobacterial disease is the direct microscopic examination of specimens for the presence of acid-fast bacteria. Although it requires a staff who is experienced in reading smears, it has the advantage of being rapid and inexpensive. It has a lower limit of detection of approximately 10,000 organisms per milliliter (Hobby *et al*., 1973). This is 100 times less sensitive than culture, and consequently a negative smear does not preclude the diagnosis of tuberculosis (Cruickshank, 1952). There are two main staining methods: the Ziehl–Neelsen and related methods and fluorochrome techniques that use auramine or auramine– rhodamine dyes. Smears are semi quantitative and may provide the clinician an impression of the severity of the infection or progress of a patient on treatment (Anon, 2000). However, the excretion of bacteria into the sputum can be intermittent, and organisms that have been killed by therapy remain in the

sputum for several months. Thus at least three good quality samples of sputum should be examined before the diagnosis is rejected (Anon, 2000).

Presumptive diagnosis of TB is commonly based on the finding of acid fast bacillus (AFB) on microscopic examination of a diagnostic specimen such as a smear of expectorated sputum or of tissue (for example, a lymph node biopsy or fine needle aspiration). The lower limit of detection of ZN staining is 5×10^3 organisms/mL, whereas rhodamine-auramine fluorochrome staining tends to be more sensitive. In children, *M. tuberculosis* can be recovered from gastric aspirates, with yields varying from 30% to 50% in older children to 70% in infants for three consecutive specimens. The role of induced sputum or bronchoscopy in diagnosing TB is well established in patients unable to provide good-quality sputum specimens (Hudson *et al*., 2000); (Johnson and JJ., 2006). Definitive diagnosis depends on the isolation and identification of MTB from a diagnostic specimen (in most cases a sputum) using Mycobacterial Culture on Lowenstein-Jensen or Middle brook 7H10 media by incubating at 37^0C under 5% CO_2 (Stephen, 2006).

In developing countries, direct sputum smear examination should be provided at all district hospital laboratories. At least a proportion of all isolates should be sent to reference centers for confirmation of identification and susceptibility testing. With the increasing burden of HIV infection and MD RTB, it is desirable that more specimens are cultured and susceptibility testing is performed (Banner, 1979).

II) Culture

Culture of mycobacteria remains the cornerstone of microbiological diagnosis of tuberculosis, as it is still the most sensitive diagnostic techniques available, and the isolation of the organisms allows definitive identification, susceptibility and molecular epidemiological tests to be performed (Stephen, 2006). The slow growth of *M. tuberculosis* is a challenge for the diagnostic laboratory to produce a result in as timely a fashion as possible. There are three main types of isolation media available for primary diagnosis: egg based such as Lowenstein–Jenssen, agar based such as Middlebrook 7H10 and finally liquid

growth media, e.g. Middlebrook 7H9 reviewed by Collins, Grange and Yates (1997) (Ådjers-Koskelat and Katila, 2003). In today's laboratories, the use of liquid media with radiometric growth detection (e.g., BACTEC-460) and mycobacterial growth indicator tubes (MGIT) have replaced the traditional methods of isolation on solid media. These new methods have decreased the time required for isolation to 2 to 3 weeks compared to the 8 weeks required for the traditional culture methods.

III) Molecular Diagnosis

Molecular diagnostics in tuberculosis has enabled rapid detection of Mycobacterium tuberculosis complex in clinical specimens, identification of mycobacterial species, detection of drug resistance, and typing for epidemiological investigation. In the laboratory diagnosis of tuberculosis, the nucleic acid amplification (NAA) test is rapid and specific but not as sensitive as culture of mycobacteria NAA methods allow for detection of mycobacterial DNA or RNA directly from the specimens before the culture results are available (Tevere *et al*., 1996).

Advances in knowledge about genetic structure of tubercle bacillus helped develop gene probes and gene amplification methods for identification and detection of tubercle bacillus, from culture or directly from clinical specimens and molecular detection of drug resistance. While the gene probes can help in rapid identification of isolates, gene amplification methods (E.g. PCR) developed for diagnosis of TB is demonstrably highly sensitive and detection can be done within hours (Katoch, 2004).The Food and Drug Administration (FDA) has approved two NAA tests for direct detection of *M. tuberculosis* from clinical specimens. These are the Enhanced *Mycobacterium tuberculosis* Direct Test (E-MTD; Gen-Probe, San Diego, CA) and the Amplicor *Mycobacterium tuberculosis* Test (Amplicor; Roche Diagnostic Systems, Inc., Branchburg, NJ) (Bergman and Woods, 1996) (Stauffer *et al*., 1995).

Molecular methods offer many advantages over conventional methods in the identification of mycobacterial species. The results are obtained rapidly, are reliable and reproducible, and even mixed or contaminated cultures can be analyzed. The probes are already widely used in clinical laboratories for the identification of the most common mycobacterial species. Because automatic DNA sequencers and the programs used for analyzing sequence data have become technically simpler, the PCR-based sequencing method is now being used in many mycobacterial reference laboratories as the routine method for species assignment. The DNA microarray method holds great promise for the future because it is easy to perform, it can be readily automated, and it allows for identification of a large number of mycobacterial species in one reaction (Tevere *et al*., 1996); (Chin *et al*., 1995).

IV) Tuberculin Skin Test

Skin testing with Tuberculin Skin Test (TST) is used globally in screening for MTB infection. The test is of limited value in the diagnosis of active TB because of its low sensitivity and specificity (Johnson and JJ., 2006). The skin test works by injecting Purified Protein Derivative (PPD) into the skin. PPD is a collection of mixed proteins and other materials filtered from killed *M. tuberculosis* cultures. The test works on the basis that if the body has been exposed to infection with TB it will recognize the proteins and mount an immune response to it. This response would take the form of a lump, swelling or blister at the site of injection. If there is a lump (called an induration) then this may mean that the person is infected. Unfortunately, false-negative reactions are common in immunosuppressed patients. Positive reactions are obtained when patients have been infected with MTB but do not have active disease and when persons have been sensitized by non-tuberculous mycobacteria or Bacilli Calmette-Guerin (BCG) vaccination (Johnson and JJ., 2006). In the past years, new diagnostic methods like Enzyme-linked immunospot (ELISPOT) and Enzyme-linked immunosorbent assay (ELISA) for the diagnosis of infection with MTB have been developed. The ELISPOT and ELISA detect the secretion of γ-interferon by mononuclear cells in venous blood, specific for MTB peptides, ESAT-6 and CFP-10. ESAT-6 and CFP-10 are peptides that mediate MTB virulence (Brodin *et al*., 2005). These tests are more sensitive and specific for the diagnosis of MTB infection and

are superior to the tuberculin skin test (TST) in patients with immunosuppression (Ferrara *et al*., 2006)

Radiographic Procedures

Radiographic procedures and clinical sign and symptoms can also be used in the process of diagnosing TB. The initial suspicion of pulmonary TB is often based on abnormal chest radiographic findings in a patient with respiratory symptoms (Johnson and JJ., 2006). Radiographic findings suggesting TB include upper lobe infiltrates, cavitary lesions, and hilar or paratracheal lymphadenopathy. In many patients with primary progressive disease and in HIV patients, radiographic findings can be subtle and include lower lobe opacities, a miliary pattern, or both. Of the clinical features, cough is reported less frequently in HIV patients, probably because of weak cough reflex due to debilitated condition of the patients in advanced disease, absence of cavitations, and less endobronchial irritation (MOH/HAPCO, 2005).

1.3.7 Treatment of *M. tuberculosis*

All patients (including those with HIV infection) who have not been treated previously should receive an internationally accepted first-line treatment regimen using drugs of known bioavailability. The initial phase should consist of two months of isoniazid, rifampicin, pyrazinamide, and ethambutol (Hawken *et al*., 1997). The preferred continuation phase consists of isoniazid and rifampicin given for four months. Isoniazid and ethambutol given for six months is an alternative continuation phase regimen that may be used when adherence cannot be assessed, but it is associated with a higher rate of failure and relapse, especially in patients with HIV infection. The doses of antituberculosis drugs used should conform to international recommendations. Fixed-dose combinations of two (isoniazid and rifampicin), three (isoniazid, rifampicin, and pyrazinamide), and four (isoniazid, rifampicin, pyrazinamide, and ethambutol) drugs are highly recommended, especially when medication ingestion is not observed (WHO, 2003).

Several countries with good TB control programmes have shown that cure of XDR TB is possible for up to 50–60% of affected people. But successful outcomes also depend greatly on the extent of the drug resistance, the severity of the disease and whether the patient's immune system is compromised (Glenn, 2007).

1.3.8 Prevention

Tuberculosis is highly contagious and may even spread to other organs in the body. Therefore, TB prevention and control are very important to stop this disease from spreading rapidly (Hadley and Maher, 2000). If an infected person sneezes or coughs, it is very easy for another person to get infected by simply breathing in those infected air droplets. Tuberculosis remains inactive in most infected people but may become active again within a few weeks or months after the primary infection. People who are at high risk of active tuberculosis infections include infants, elderly people and people who have weak immunity such as in the case of AIDS, diabetes and chemotherapy patients (WHO, 2004a).

Tuberculosis prevention programs are very important so as to educate people about this disease. This will help people seek treatment at the earliest and avoid further spread of this infection. As a part of these pulmonary tuberculosis prevention programs, people are screened for latent tuberculosis infections and preventive treatments are also provided (Hadley and Maher, 2000). BCG vaccinations are given to infants and adults as a part of tuberculosis prevention programs. By following the guidelines of tuberculosis prevention and control, this disease can be prevented and controlled even in people who have been exposed to this infection. Vaccinations, early detection and preventive therapies are therefore of prime importance in tuberculosis prevention and treatment (WHO, 2001).

All people living with HIV, wherever they receive care, should be regularly screened for TB using a clinical algorithm at every visit to a health facility or contact with a health worker. Screening for TB is important, regardless of whether they have received or are receiving IPT

or ART. This recommendation is applicable for those living with HIV irrespective of the degree of immunosuppression, and for those on ART, those who have previously been treated for TB and pregnant women (Harries, 2004).Adults and adolescents living with HIV who have any one of the four symptoms (current cough, fever, weight loss or night sweats) may have active TB and should be evaluated for TB and other diseases. The diagnostic work-up for TB should be done in accordance with national guidelines and sound clinical practice to identify either active TB or an alternative diagnosis (WHO, 2003).

The WHO guideline reviewed studies of the drug combinations used for prevention including INH, rifampicin, pyrazinamide and rifapentine. A number of studies compared INH alone with other regimens, and found that regimens that included pyrazinamide, rifampicin and rifapentine were as efficacious as INH alone, but were associated with higher rates of toxicity. The WHO Guidelines Group concluded that INH at 300 mg/ day remains the drug of choice for chemotherapy to prevent TB in adults living with HIV (WHO, 2006b).

1.4 Significance of the Study

Ongoing analysis of test types and algorithm in a country or regional level is important for detecting prevalence, unexpected increase and decrease in disease occurrence, monitoring disease trends, and to evaluate the effectiveness of disease control programs and policies. This information is also needed to determine the most appropriate and efficient allocation of public health resource and personnel.

Factors such as TB- and HIV-prevalence, disease severity, proportion of non-tuberculous mycobacteria, and type of specimens received will affect the external validity of any TB diagnostic evaluation. For laboratory managers considering the implementation of LED microscopy with different kinds of staining methods in either a low- or high-income setting, the choice of the appropriate kind is important. There are several commercial manufacturers now marketing the different stains of the light microscope and the LED microscopes, but there aren't many studies comparing their head-to-head performance. Given the wide variety of methods available, each with different benefits claimed and potential roles, it is important to compare them with respect to a specific setting or situation. For instance, in Low income, High-incidence laboratories, increased sensitivity, increased diagnostic accuracy, higher negative predictive value and lower positive predictive value, portability and the ability to save the technician's time, minimal effort needed for reagent preparation, including a less toxic reagent are all important considerations. The result of the study could be applicable for better diagnosis of TB and algorithm improvement.

2. OBJECTIVES

2.1. General Objective

To assess the comparative performance of PERVI Fluo TB stain by using LED-Microscopy among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

2.2. Specific objectives

- To compare the performance of PREVI Fluo TB stain sensitivity, specificity, PPV and NPV with Auramine-O conventional LED method and the classical ZN method.
- To evaluate the diagnostic advantage of PREVI Fluo TB stain in HIV positive and HIV negative PTB suspected patients
- To assess the smear positivity of Tuberculosis at St. Paul's Hospital Millennium Medical College in TB suspected patients

3. METHODS AND MATERIALS

3.1. Study Design and Study Area

A hospital based comparative cross sectional study was conducted at St. Paul's hospital, Millennium Medical College, Addis Ababa, Ethiopia. There are 149 governmental and 1,788 private hospitals in the country. The altitude of Addis Ababa is about 2400 m above sea level and has a population of about 4 million as it divides in to ten sub-cites. The principal investigator was conducting the study in a governmental health facility, at St. Paul's Hospital Millennium Medical College, which is the second largest referral hospital in the country compared to other hospitals. St. Paul's Hospital was built in 1969 during Emperor Haile Selassie. It currently has 370 beds, with an annual average of 200,000 patients and a catchment population of more than 5 million. There are 1600 clinical and non-clinical staffs in over 13 departments. The hospital became a medical college in 2007. The hospital receives referred patients from urban and rural areas of the country and provides local emergency service.

3.2. Study Period

The study was conducted from October 2011 to April 2012.

3.3. Study population

All patients who were suspected for pulmonary tuberculosis during the data collection period were included. In this study 246 study participants were included. The sample size was calculated using the formula required for determination of sample size for estimating single proportions (Daniel, 1987). An estimated 20% prevalence of HIV-TB co infection for Ethiopia (WHO, 2011), 95% confidence interval and a precision of 5% was used in sample size determination. All the patients that give sputum samples were included by convenience sampling. Their sputum was smeared for ZN method, conventional LED-FM and for PREVI Fluo TB staining.

$$n = \frac{(Z\alpha/2)^2 pq}{d^2}$$

Where, n = sample size that will be calculated

Z = confidence interval

P = proportion

q = 1-P

By using 95% confidence interval i.e. Z = 1.96

P = 0.2, an estimated 20% prevalence of HIV-TB co infection for Ethiopia (WHO, 2011)

d = 0.05 since 95% C.I is taken

$$\text{Then } n = \frac{Z^{\alpha}/_{2}{}^{2}\ pq}{d^2} \quad \frac{(1.96)^2 \times (0.20)(0.80)}{(0.05)^2} = 246$$

3.4 Inclusion and exclusion criteria

3.4.1 Inclusion criteria

- Suspected to pulmonary tuberculosis by clinician
- Patients who were able to produce sputum
- Those who signed the consent form
- Patients who were greater than 14 years old

3.4.2 Exclusion criteria

- Patients who were less than 15 years old
- All patients who were unable to produce sputum
- Patients who were on follow up

3.5 Study variables

3.5.1 Dependent variable

-Smear positivity

- Colony of *Mycobacterium tuberculosis*

3.5.2 Independent variable

- Age
- Sex
-Type of method
-Type of sputum
-Close contact
-Imprisonment
-HIV

3.6. Sample Collection, Handling and Transport

Socio demographic and Clinical data were collected using well structured questionnaire. Sputum samples were collected after obtaining informed consent from study participants. Based on IUATLD recommendation three sputum samples were collected from each participant, "on the SPOT – early MORNING – on the SPOT", preferably within two days. Specimen was collected using a rigid wide-mouthed screw capped container made of unbreakable transparent plastic which can be sealed to prevent desiccation of the sample and leakage. Sputum samples were taken using standard methods by the collaborating laboratory attendants and technologists who gave instructions on how to cough so that the expectoration is produced from as deep down in the chest as possible with sufficient volume (3 to 5 ml) and that it contains solid or purulent material.

For every sample taken 3 smears were made for ZN, Auramine-O and PREVI Fluo TB stains. Hence for every study participants a total of 9 slides were made, 3 for every sample. ZN smears were examined at 1,000× magnification, and fluorescent smears (in accordance with the International Union Against Tuberculosis and Lung Disease Working Group on Smear Microscopy) at 200×, with confirmation of positive smears at 400× magnification. Each of the microscopy readings were made without knowledge of the results of each slide and all microscopic readings were made without knowledge of the culture result. Smears were classified as positive when ≥1 AFB was detected per 100 fields, and patients were

considered smear-positive if they had ≥1 positive smear (WHO, 2007b). The main outcomes for comparison were the sensitivity and specificity of PREVI Fluo compared with the sensitivity and specificity of ZN and Auramine O stains using LED microscope for the fluorescent stains and light microscopy for ZN. Culture was used as the reference standard.

The early morning sputum was transported with ice, in an ice box to EHNRI for culture. For culturing, the specimens reached the laboratory within 7 days and were refrigerated while waiting for transport. In this study, Petroff's method was used to process and decontaminate clinical specimens (Bartelt, 2000). For all samples the same protocol for digestion and decontamination, followed by centrifugation under the above standard conditions were used. For culture, 0.1 ml of sediment was placed on to Löwenstein-Jensen (LJ) medium prepared in each laboratory according to international standards. Each batch of cultures was performed in parallel with cultures of negative controls. For Lo¨wenstein-Jensen culture, 200µl of sample were inoculated onto each slope, incubated at 37°C, and examined once every week for 8 weeks before being reported as negative. Colony formation was checked every week, preferably twice within the first week, to allow rapid detection of contamination. Contaminated cultures were removed. The patient sample was kept for one week for re-decontamination and re-inoculation procedures if one culture is found to be contaminated. *M. tuberculosis* colonies develop within 3–4 weeks if they are present.

3.7. Quality Assurance

The quality of the test was maintained by:

- The sample was collected using sterile wide mouth cup.
- For the staining procedure staining of known positive and negative smears were performed.
- The re-reading of positive smears by another technologist was performed for the smears
- Performing AFB-smear microscopy against a quality control for AFB staining in a routine series daily by using known positive and known negative samples.
- In the culturing process, verification that the rotor reached and maintains the required RCF of 3000*g* for 15 minutes in order to obtain good recovery of the mycobacteria

- Incubating tubes of the new batch of medium prior to inoculation to perform batch checks
- Inoculation of the specimen on appropriately prepared LJ media
- Inoculation of one sample on two LJ culture tubes
- For every batch a control of distilled water was inoculated as a start and end control
- Random culturing one sample twice to check for concordance of result
- Maintaining the incubation temperature of the media at 35–37°C range without fluctuation.
- Placing thermometers at different and easily visible places in the incubator to ascertain temperature uniformity
- Reading of cultures every week of incubation to detect and record early contamination

3.8. Statistical Analysis

The data was entered in to Epi data version 3.1 and analyzed by SPSS version 16. Statistical significance was considered when p value is less than 0.05. Sensitivity, specificity, positive and negative predictive values and likelihood ratios were calculated by using Open Epi Version 2.3 for the PREVI Fluo LED, Auramine O LED and conventional ZN microscopy using mycobacterial culture as the reference standard. The agreement of PREVI Fluo TB method with the reference culture and its agreement with the ZN and Auramin O was calculated using Kappa statistics.

3.9. Ethical considerations

Ethical clearance was obtained from Addis Ababa University Medical School, Department of Microbiology, Immunology and Parasitology Ethical Review Committee. The study was conducted after getting official permission from the college's Institutional review board. PTB suspected patients were approached by asking their willingness and their permission to participate in the study. Informed consent form was obtained from the participants.

4. RESULTS

Socio-demographic characteristics of study population are presented in Table 4.1. The majority (53.6%, 133/248) of enrolled participants were males. The highest proportion of study participants were in the age range 25-34(30.6%, 76/248) years, followed by the age range 35-44(21%, 52/248) years (Table 4.1). The higher majority of the participants 66.1% (164/248) were married. 35.1% (87/248) of the study participants' educational level was illiterate followed by 32.7% (81/248) primary. In regards to occupational level, 28.8% (64/248) participants are unemployed, while 15.3% (38/248) have a private business or are employed by an NGO.

Table 4.1 Socio demographic characteristics of study participants investigated for PREVI luo TB stain by using LED-Microscopy among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

Variables	Frequency	Percent
Sex		
Male	133	53.6
Female	115	46.4
Age category		
15-24 years	46	18.5
25-34 years	76	30.6
35-44years	52	21
45-54years	35	14.1
55-64years	17	6.9
≥65years	22	8.9

Residence		
Urban	119	47.9
Rural	129	52.1
Marital Status		
Married	164	66.1
Single	72	29
Divorced	10	4
Widowed	2	8
Educational status		
Illiterate	87	35.1
primary	81	32.7
Secondary	55	22.2
Higher education	25	10.1
Occupation		
Governmental	23	9.3
Private or NGO	38	15.3
Daily laborer	15	6
Unemployed	64	25.8
Own business	12	4.8
Other	96	38.7

Out of the 248 participants there were a 25.4% (63/248) of previous pulmonary tuberculosis history contact, while the remaining 74.6% had no history of previous pulmonary tuberculosis contact (Table 4.2). Of the 248, 39 participants (15.7%) had exposure to tuberculosis cases with in the past two years, 66.1% (164/248) had no exposure within the past two years and 18.1% (45/248) do not remember (Table 4.2). In the current study 9.3% (23/248) participants had a family history of tuberculosis while the remaining 90.7% had no history of family pulmonary tuberculosis. As well from the total 248 participants, 10.3% (26/248) were imprisoned and the remaining 89.5% had no history of imprisonment (Table 4. 2). Of the 39 exposed participants 53.8% (21/39) had contact with tuberculosis patients at their residence, 23.1% (9/39) had contact in prison, 20.5% (8/39) at their work place and while only 2.6% had contact in a hospital or health center (Table 4.3)

Table 4.2 History of previous PTB contact and imprisonment among study participants investigated for PREVI Fluo TB stain by using LED-Microscopy among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

	Previous TB history No (%)	Exposure to PTB within the past 2 yrs No (%)	Family PTB History No (%)	Imprisonment No (%)
No	185(74.6)	164(66.1)	225(90.7)	222(89.5)
Yes	63(25.4)	39(15.7)	23(9.3)	26(10.3)
Don't remember		45(18.1)		

Table 4.3 Place of exposure of TB among participants investigated for PREVI Fluo TB stain by using LED-Microscopy among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

Place of Exposure	Frequency	Percent
Residence	21	53.8
Work place	8	20.5
Prison	9	23.1
Hospital/Health Center	1	2.6
Total	39	100

The percentage distribution of study participants who had previously defaulted from PTB medication is shown below in Table 4.4. Among the total 248 study participants, 8(3.2%) had stopped PTB medication. The remaining 96.8% had not stopped TB medication or did not have previous PTB history. From these 28.6% stopped after two months, another 28.6% after four months and the rest after six months and above.

Table 4.4 Previous TB medications of study participants investigated for PREVI Fluo TB stain by using LED-Microscopy among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

Defaulted from PTB	**Frequency**	**Percent**
No	240	96.8
Yes	8	3.2
Total	**248**	**100**

Sero status of participants involved is presented in Table 4.5. Forty three (17.3 %) of the participants were HIV positive while the remaining 51.2% (127/248) were found to be HIV negative. Seventhly eight (31.5%) did not know their HIV sero status. From the 43 HIV cases, 69.8% (30/48) have started using ART, while the remaining 30.2% (13/248) were naïve for Anti Retro Viral Therapy (ART) (Table 4.5).

Table 4.5 HIV sero statuses and ART usage of participants investigated for PREVI Fluo TB stain by using LED-Microscopy among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

	Frequency	Percent
HIV sero status		
Yes	43	17.3
No	127	51.2
Do not know	78	31.5
ART Usage		
Yes	30	69.8
No	13	30.2

Laboratory Findings

Laboratory investigation of every collected sputum samples of the study participants were done by the three methods and confirmed with the forth gold standard method. A total of 2,232 slides were made for ZN, Auramine O and PREVI Fluo methods, 744 for each type of method and 248 slides for each Spot-Morning-spot sample .By ZN method out of the total study participants 24(9.7%) were positive, while 224 (90.3%) had negative smear result. In Auramine O 44(17.7%) were positive and the remaining 204 (82.3%) had a negative result while in PREVI Fluo TB method, 35(14.1%) participants were positive and the rest 213 (85.9%) were found to have a negative smear result. From the sputum of the participants cultured on LJ culture media, 30(12.1%) of the participants became positive for TB bacilli where as the remaining 213(85.9%) were negative (Table4.6).

Table 4.6 Smear results of the ZN, Auramine O and PREVI Fluo TB methods in a comparative study of PREVI Fluo TB method among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

Methods	PTB Lab result Positive No. (%)	Negative No. (%)	Total No. (%)
ZN	24(9.7)	224(90.3)	248(100)
Auramine O	44(17.7)	204(82.3)	248(100)
PREVI Fluo	35(14.1)	213(85.9)	248(100)
Culture	30(12.1)	218(87.9)	248(100)

Grading scale (Scanty, 1+, 2+ and 3+) of the bacilli in different methods is shown below in table 4.7. In ZN method of the three sputum samples collected, in 'slide a'(1st spot), 8 of the smears (3.2%) had a 1+ result, 2 (0.8%) had 2+, 6 (2.4%) had a 3+ and 3 (1.2%) had a scanty result. In 'slide b' (morning), 6 of the smears (2.4%) had a 1+ result, 4 (1.6%) had 2+, 4 (1.6%) had a 3+ and 3 (1.2%) had a scanty result. In 'slide c' (2nd spot) of the ZN stain, 4 of the smears (1.6%) had a 1+ result, 4(1.6%) had 2+, 8 (3.2%) had a 3+ and 14 (5.6%) had a scanty result. In the Auramine O staining method, in 'slide a' 4 of the smears (13.3%) had a 1+ result, 4 (13.3%) had 2+, 8 (26.7%) had a 3+ and 14 (46.7%) had a scanty result. In 'slide b' 6 of the smears (2.4%) had a 1+ result, 4 (1.6%) had 2+, 8 (3.2%) had a 3+ and 12 (4.8%) had a scanty result. And finally, in the PREVI Fluo method, in 'slide a' 3 of the smears (1.2%) had a 1+ result, 3 (1.2%) had 2+, 10 (4%) had a 3+ and 10 (4%) had a scanty result. In 'slide b' 4 of the smears (1.6%) had a 1+ result, 4 (1.6%) had 2+, 10 (4%) had a 3+ and 10 (4%) had a scanty result. And at last, in 'slide c' 6 of the smears (2.4%) had a 1+ result, 3 (1.2%) had 2+, 8 (3.2%) had a 3+ and 14 (5.6%) had a scanty result. The bacilli detected in culture from positive finding were graded as 23.3% (7/30), 10% (3/30), 33.3% (10/30) and 33.3% (10/30) for 1+, 2+, 3+ and scanty respectively.

Table 4.7 Grading results of PTB positive results in ZN, Auramine O & PREVI Fluo TB methods based on WHO grading system, 2012.

WHO grading scale	ZN Freq (%)	Auramine O Freq (%)	PREVI Fluo Freq (%)
Slide A(1st spot)			
Scanty	3(1.2)	14(46.7)	10(4)
1+	8(3.2)	4(13.3)	3(1.2)
2+	2(0.8)	4(13.3)	3(1.2)
3+	6(2.4)	8(26.7)	10(4)
Slide B(morning)			
Scanty	3(1.2)	12(4.8)	10(4)
1+	6(2.4)	6(2.4)	4(1.6)
2+	4(1.6)	4(1.6)	4(1.6)
3+	4(1.6)	8(3.2)	10(4)
Slide C (2nd spot)			
Scanty	14(5.6)	12(4.8)	14(5.6)
1+	4(1.6)	4(1.6)	6(2.4)
2+	4(1.6)	6(2.4)	3(1.2)
3+	8(3.2)	7(2.8)	8(3.2)

The distribution of the different socio demographic characteristics in association with PTB is revealed below in Table 4.8. Fifteen (11.3%) of male and 15 (13%) of female participants were positive for PTB. Although PTB case in female participants was slightly higher in percentage than male participants, statistical significance difference between male and female study participants was not found in respect to PTB (p=0.67). In the present study most of PTB positive study groups were in the age of 35-44 years which accounted 15.4% followed by 13.6%, 13.2%, 13% ,5.9% and 5.7% of ≥65 years, 25-34 years, 15-24 years, 55-

64 years and 45-54 years respectively. Statistically significant association was not found in each age category in respect of PTB ($P>0.05$). Among the study participants with PTB, 17 (13.2%) are from rural areas and 13 (10.9%) are from urban. There was no statistical significant association between the urban and rural residents with regards to PTB ($P=0.59$). Again in participants with PTB groups, 16.7%, 10% and 9.8% are married, divorced and single respectively. There was no significant association between these groups with relation to PTB ($P>0.05$). In regards to occupation with relation to PTB, 18.4% , 16.7%, 13.3% and 8.7% are in private, own their own business, daily laborers, unemployed and employed by the government respectively. There was no significant association with in each group ($p>0.05$). In regards to association between PTB and previous TB contact (7.8%), travelling with in the past two years (10.6%), family PTB history (4.3%), and imprisonment (7.7%) none of these had significant association ($P>0.05$). Defaulters from TB medication in relation to PTB was 12.5% wich had no significant association as $P=0.93$. HIV sero positivity with PTB was 4.7% and 13.4% for HIV negative with a P value of 0.13 and 0.89 showing no significant association with PTB. Still in association with ART usage of 3.3% had PTB and showed no association with PTB ($P=0.54$)

Table 4.8 PTB related to different variable for the assessment participants investigated for PREVI Fluo TB stain by using LED-Microscopy among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

Variables		TB Culture result Positive No (%)	Negative No (%)	0R(95%CI)	P-value
Sex					
	Male	15(11.3)	118(88)	1.18(0.55-2.53)	0.67
	Female	15(13)	100(86.1)	1.00	
Age (yrs)					
	15-24	6(13)	40(87)	1.05(0.23-4.66)	0.946
	25-34	10(13.2)	66(85.5)	1.042(0.26-4.17)	0.954
	35-44	8(15.4)	44(84.6)	0.87(0.207-3.64)	0.847
	45-54	2(5.7)	33(91.4)	2.61(0.31-17.01)	0.317
	55-64	1(5.9)	16(94.1)	2.51(0.23-26.71)	0.441
	>+65	3(13.6)	19(86.4)	1.00	
Residence					
	Urban	13(10.9)	106(89.1)	1.24(0.57-2.67)	0.59
	Rural	17(13.2)	112(86.8)	1.00	
Marital status					
	Married	16(9.8)	148(90.2)	9.25(0.55-15.09)	0.122
	Single	12(16.7)	60(83.3)	5 (0.29-85.60)	0.267
	Divorced	1(10)	9(90)	9 (0.28-285.5)	0.213
	Widowed	1(50)	1(50)	1.00	

Education status				
Illiterate	10(11.5)	77(88.5)	9.25(0.55-15.09)	0.122
Primary	11(13.6)	70(86.4)	5(0.29-85.6)	0.267
Secondary	7(12.3)	48(87.3)	9(0.28-285.5)	0.213
Higher Education	2(8)	23(92)	1.00	
Occupation				
Governmental	2(8.7)	21(91.3)	1.359(0.28-6.6)	0.704
Private	7(18.4)	31(86.7)	0.573(0.20-1.6)	0.291
Daily Laborer	2(13.3)	13(86.7)	0.841(0.16-4.23)	0.834
Unemployed	6(9.4)	58(90.6)	1.251(0.44-3.57)	0.676
Own business	2(16.7)	10(83.3)	0.65(0.125-3.35)	0.604
Other	11(11.5)	85(88.5)	1.00	
Previous TB history				
Yes	5(7.8)	59(92.2)	1.812(0.66-4.95)	0.25
No	25(13.6)	159(86.4)	1.00	
Traveled in the past two years				
Yes	5(10.6)	42(89.4)	1.46(0.483-4.42)	0.502
No	25(12.4)	176(87.6)	1.00	
Family TB History				
Yes	1(4.3)	22(95.7)	3.23(0.42-25.07)	0.26
No	29(12.9)	196(87.1)	1.00	
Imprisonment				
Yes	2(7.7)	24(92.3)	1.74 (0.39-7.77)	0.47
No	28(12.6)	194(87.4)	1.00	

Stopped TB medication				
Yes	1(12.5)	7(87.5)	1.105(0.13-9.15)	0.93
No	29(12.1)	211(87.9)	1.00	
HIV sero positivity				
Yes	2(4.7)	41(95.3)	3.36(0.71-15.95)	0.13
No	17(13.4)	110(86.6)	1.06(0.46-2.405)	0.89
Do not know	11(14.1)	67(85.9)	1.00	
ART usage				
Yes	1(3.3)	29(96.7)	2.42(0.14-41.87)	0.54
No	1(7.7)	12(92.3)		

In a cross tabulation made between PTB culture result and the different methods, of the total 24 Zn positive slides 17(70.8%) were positive with LJ TB culture while 7(29.2%) were negative with culture and 13(5.8%) of the total culture positives were negative with ZN. Among the total 34 Auramine O positive slides, 25(56.8%) were positive with culture, the rest 19(43.2%) were negative with culture and 5(2.5%) of the total culture positives gave negative results with Auramine O. Within the total 35 PREVI Fluo positive slides, 23(65.7%) were also positive with culture, 12(34.3%) were negative and 7(3.3%) of the culture positives, gave negative result with PREVI fluo method. Concerning the agreement of the three methods with the gold standard (LJ culture), ZN has a kappa agreement result of K= 0.585, for Auramine O K=0.621 and PREVI Fluo TB K=0.664.

Table 4.9 PTB culture result related to ZN, Auramine O and PREVI Fluo for comparative analysis of PREVI Fluo among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

Variables	Culture confirmed TB result		Agreement with Kappa
	Positive *Yes (%)*	Negative *No (%)*	
ZN TB result			
Positive	17(70.8)	7(29.2)	0.585
Negative	13(5.8)	211(94.2)	
Auramine O TB result			
Positive	25(56.8)	19(43.2)	0.621
Negative	5(2.5)	199(97.5)	
PREVI Fluo TB result			
Positive	23(65.7)	12(34.3)	0.664
Negative	7(3.3)	206(96.7)	

The PREVI Fluo TB method has an agreement with the kappa scale of 0.745 with the Auramine o method and K=0.636 with regards to its agreement with the ZN method (Table 4.10).

Table 4.10 Agreement of Kappa statistics of PREVI Fluo TB with Auramine O for comparative analysis of PREVI Fluo among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

PREVI Fluo TB result	Auramine O TB result		Agreement with Kappa
	Positive *Yes (%)*	Negative *No (%)*	
PREVI Fluo TB result			
Positive Count	31(88.6)	4(11.4)	0.745
Negative Count	13(6.1)	200(93.9)	
	ZN TB result		
PREVI Fluo TB result			
Positive Count	20(57.1)	15(42.90	0.636
Negative Count	4(1.9)	209(98.1)	

PTB positive results with the different stains related to HIV positive study participants is shown in the Table 4.11. Four (9.3%) of the total 43 HIV positives, were also positive for ZN smear, six (14%) were positive with Auramine O and 5 (11.6%) were positive with PREVI Flue TB.

Table 4.11 PTB positive results with the stains related to HIV sero status for comparative analysis of PREVI Fluo among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

Variables	TB ZN Slide Result		0R(95%CI)
	Positive *Yes (%)*	Negative *No (%)*	
HIV sero status			
Positive	4(9.3)	39(90.7)	0.961(0.27-3.49)
Negative	13(10.2)	114(89.8)	0.87(0.329-2.270)
Do not know	7(9)	71(91)	1.00
	TB Auramine O Slide Result		
HIV sero status			
Positive	6(14)	37(86)	1.35(0.478-3.81)
Negative	24(18.9)	103(81.1)	0.94(0.453-1.95)
Do not know	14(17.9)	64(82.1)	1.00
	PREVI Fluo TB Slide Result		
HIV sero status			
Positive	5(11.6)	38(88.4)	1.38 (0.45-24.2)
Negative	18(14.2)	109(85.8)	1.1(0.499-2.43)
Do not know	12(15.4)	66(84.6)	1.00

The sensitivity and Specificity of ZN, Auramine O and PREVI Fluo are 56.67% & 96.79%, 78.13 & 91.2% and 76.67% & 94.5% respectively. The different tests gave a PPV and NPV results of 70.83% & 94.2% (ZN), 56.82% & 96.57% (Auramine O) and 65.71% & 96.71% PREVI Fluo methods. In addition, the diagnostic accuracy for the different tests were 91.94,

89.52 and 92.34% respectively for ZN, Auramine O and PREVI Fluo TB. As well the likely hood ratio for a negative test was calculated to be 0.44, 0.23 and 0.24 for these tests.

Table 4.12 The sensitivity, specificity, PPV, NPV, diagnostic accuracy and likely hood ratio, for the assessment for comparative analysis of PREVI Fluo among TB suspected patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2012.

	ZN % (95%CI)	Auramine O% (95%CI)	PREVI Fluo% (95%CI)
Sensitivity	56.67 (39.2-72.62)	78.13 (61.24-88.98)	76.67(59.07-88.21)
Specificity	96.79 (93.52-98.44)	91.28(6.67-94.3)	94.5(90.63-96.82)
Positive Predictive value	70.83(50.83-85.09)	56.82(42.22-70.32)	65.71(49.15-79.17)
Negative predictive value	94.2(90.33-96.58)	96.57 (93.03-98.33)	96.71 (993.37-98.4)
Diagnostic Accuracy	91.94 (87.87-94.72)	89.52 (85.08-0.92)	92.34 (88.34-95.04)
Likely hood ratio of a Negative test	0.44 (0.38-0.52)	0.23 (0.18-0.31)	0.24 (0.19-0.33)

5. DISCUSSION

Each year, approximately 2 million persons worldwide die of tuberculosis and 9 million become infected (CDC, 2007). The occurrence of HIV has also fueled the Tuberculosis burden. WHO reported in 2007 that the African region accounted for most HIV positive Tuberculosis cases (79%) and that HIV infection among patients with Tuberculosis ranges from 50%- 80% in sub-Saharan Africa (WHO, 2007b).Although advances in diagnostics are leading to the introduction of new tests , the backbone of TB diagnosis worldwide continues to be smear microscopy (Wallis *et al*., 2010).

Based on a small number of studies by comparing different fluorescent staining techniques, there seems to be little difference in sensitivity or specificity between smears stained with auramine O and other stains (Torrea *et al*., 2008). This research tries to fill this gap by studying three different types of staining methods head on, the PREVI Fluo stain and Auramine O by LED-FM and ZN using light microscopy. Culture was used as the reference standard.

The current study comprised a total 248 study participants from which 53.6% were males and the rest 46.4% were female participants. The age range of the participants was 15-87 years and most of the participants were between 25-34 years and followed by age ranges 35-44 and 15-24. With regards to HIV sero status, small number of HIV positive study participants were enrolled which was only 17.3% where as the vast majority of patients did not know their HIV sero statu. This is in agreement with the study by Umeh *et al*. (2007) recorded a lower TB/HIV prevalence rate of 12.6% in a referral chest clinic in Nasarawa State. On the other hand in a study in Nigeria the study demonstrated a TB/HIV co-infection rate of 27.5% (Onubogu *et al.,* 2012). In a study done in Harare, Zimbabwe, where provider initiative HIV testing and counseling was given in the study, 63.3% of participants enrolled in the study were HIV positive. This difference is due to the fact that in the current study only patients who volunteered this information and knew their sero status were categorized as HIV positive or negative. Also in this study of HIV positive study participants 69.8% were on ART where as the rest 30.2% were naïve to ART. While in a study performed in Harare only 14.7% of all HIV positive participants had started ART. Their low finding

might be that in their study they considered participants who were started on ART only during their 12 month cohort study (Munyaradzi *et al.,* 2010).

In this study though significance different association was not found between male and female participants in respect to culture confirmed pulmonary tuberculosis cases (P>0.05), the number of men participants is still a little bit higher. This is in agreement with different studies where men have a significant higher number than women (Ndugga *et al*., 2005). Similar to the findings from various studies throughout the world the finding in this study showed that tuberculosis is affecting every one regardless of age, but higher number of infected study participants were in the age range of 35-44 years though significant association was not seen. This is in agreement with the study from India where most common age groups were from 31-40 years (Ajay *et al*., 2011). Although significant association was not found regarding to residence the rate of culture confirmed pulmonary tuberculosis was slightly higher in the participants who came from the rural area (13.2%) than urban residents (10.9%) (P=0.59). This could be the participants who were from urban area had better awareness to PTB. It could also be due to the fact that in this study there were more participants from rural areas than urban.

The overall culture confirmed Pulmonary Tuberculosis cases accounted for 12.1%.This is in contrast with the study done in Turkey, 23.1% were diagnosed as having TB by culture (Mustafa *et al*., 2000). The higher finding could be attributed to the fact that in their study both PTB and EPTB cases were included while this study only included PTB suspected participants. A TB burden of 40.3% was recorded in a study in Nigeria (Onubogu *et al.*, 2012). This appears higher than reported in this study. Reason for high TB rate in their study may be because of the category of patients that visited their study center, patients that visited their study center were mostly confirmed TB cases who were referred not necessarily for TB diagnosis but for Drug susceptibility testing. In the current study from the total 12.1 % culture positives 16.67% had a previously history contact. Similarly in a study in Kenya 18.8% had a past history of TB (Bonnet *et al*., 2007), their slight increment might be due to their inclusion of participants who had received broad-spectrum antibiotics in the 2 weeks before sputum investigation. This is again in agreement with the study done in San

Francisco where the previous history of contact was 11%. The slight difference might be attributed to the fact that San Francisco has a lower prevalence of PTB where accordingly the previous history of contact could also be less (Alka *et al*., 2012). Three percent of culture confirmed PTB cases had a family history of PTB and of the total PTB positives, 3% had defaulted from anti tuberculosis drugs. Among the total PTB positive study participants 6.67% were HIV positive.

The smear positivity rate of PTB by ZN, Auramine O and PREVI Fluo TB methods were investigated. In ZN method, out of the total study participants, 9.7%, in Auramine O 17.7% and in PREVI Fluo TB method 14.1% participants were positive for at least one slide. This has a similar finding from Vietnam where the prevalence of smear-positive TB by ZN method was 7% (Duc, 2011) The sensitivity and specificity of ZN, PREVI Fluo and Auoramine O were 56.67% (39.2-72.62) and 96.79% (93.52-98.44), 76.67% (59.07-88.21) and 94.5% (90.63-96.82) and 78.13% (61.24-88.98) and 91.2% (86.67-94.3) respectively. ZN method was the least sensitive with highest specificity compared to the two methods. This is in line with Studies using Auramine O for comparison with conventional microscopy where sensitivity of conventional microscopy ranged from 0·48 to 0·93, and from 0·57 to 0·93 for fluorescence microscopy (Karen *et al*., 2006). The decreased magnification used during LED microscopy, compared with light microscopy may also have contributed, particularly toward the sensitivity differences noted. This is similar to the study in Iran where the ZN sensitivity was found to be 51% lower than the sensitivity of Auramine O (57%) (Masood *et al*., 2008). Again the finding of the current study is in agreement with the study conducted in Ethiopia, Kenya and Tanzania which used three spot-morning-spot sputum sample for the comparison of LED-FM and ZN with findings of 77% and 70.5% of sensitivity and 88% and 96.5% specificity respectively (Luis *et al*., 2011b).It is also in agreement with the study done in Turkey where the ZN sensitivity was much lower than the FM. The ZN sensitivity was found to be 67.6% and the FM was 85.2% (Mustafa *et al*., 2000). Still Another study done in Lagos, Nigeria on the sensitivity of direct smear microscopy gave a sensitivity of 53.8% by the ZN method and gave a much lower sensitivity of ZN for HIV positive individuals (Onubogu *et al*., 2012).

Moreover, this study is in agreement with the study by Torrea *et al, (*2008) who reported FM detected on average 18% more positives than ZN. In addition, there was a substantial agreement with ZN TB smear positivity result and PREVI Fluo TB methods (K=0.636). Out of the total 35 PREVI Fluo positive cases, 20 (57.1%) had also a positive result with the Auramine O, while 14 had a discordant result. Out of 9.7% of the ZN total cases 1.9% were negative with PREVI Fluo.

The LJ culture was inoculated twice for a single specimen and read weekly for 8 weeks. If both the culture tubes were contaminated then the sample was re decontaminated and inoculated again. Culture confirmed TB positive case was 12.1%. It was found that for three patient samples, there was discordance between the smear results and the culture finding. In all the three types of methods the results gave a positive slide reading while the culture gave a negative reading. This could be that these participants were infected by non-tuberculous mycobacteria. It could also be due to the fact that the bacilli might be non viable bacteria. This is unfortunately an inherent weakness of solid culture (and, to a lesser extent, liquid culture) as a diagnostic reference standard. Judgments on the performance of new diagnostic tests must be done with considerations of these limitations.

The smear negativity rate of this study was seen in ZN stained slides from which 24(9.7%) were positive by ZN and 30(12.1%) were confirmed culture positive. Out of these 6 of the slides which were missed by ZN were positive by culture. Therefore the ZN miss diagnosed as a negative 2.4% of the confirmed positive cases. In a similar study in Vietnam the smear negativity was reported to be 2% (Duc, 2011). For those patients with a high clinical suspicion, clinicians must face the dilemma of empirically treating or waiting for up to 8 weeks for the final culture results. These newer fluorescent diagnostic tests are most suitable in identifying these smear negative cases, as they did not miss any of these cases in this study.

The smear detection rate of the bacilli was lower in ZN compared to other methods which was 9.7%. This is similar to the finding with the 5 year study done in Agaro which revealed a smear positivity of 10.9% (Hussen *et al*., 2012). The smear positivity rate in this study is a little bit higher than the finding from Jimma University Specialized hospital, (8.5%) (Gebreselassie, 2003).This difference might be due to the increased awareness of people about the disease through health information dissemination programs (MDG, 2010). However, it is very high when compared with a study from Southern part of Ethiopia which found a prevalence rate of 3% (Shargie *et al*., 2006). This could be attributed to differences in awareness level of the patients resulting in lower detection in those who had sign and symptoms of the disease. This study revealed a finding lower than that of Nigeria, which had a prevalence of 14.7% (Imam and Oyeyi, 2008). This might be that Ethiopia has tremendously increased health service coverage to 89.6% in 2009/10 and recent data shows significant possible progress in relation to TB (MDG, 2010) . Therefore, the decrease in the number of patients examined in the subsequent years could be the cumulative outcome of the various strategies. It is also in contrast with the report by Khatun *et al* .(2011) who reported the smear positivity of ZN and LED was 22.7% and 33.3% and by Prasanthi and Kumari, (2005) that showed the smear detection rate of FM and ZN was 69% and 50% respectively. Also Laifangbam *et al*. (2009), reported the detection rate of ZN was lower than FM which was 44.1% and 71.6% respectively. The decreasing smear positive detection rate could be attributed to involved comparisons using individual patients while other studies used individual specimens, and thus the sample unit differed and may have had an impact on the precision of the accuracy estimates. The health extension program designed to achieve significant basic health care coverage in Ethiopia might have had an impact through the provision of a staffed health post where they assist individuals with signs and symptoms of tuberculosis infection to get timely treatment and educate and follow up TB patients on precautions they have to take to reduce the spread of the infection (MOH, 2004b). Still this difference could be the study participants’ severity of the disease, living condition or the difference in the identifying ability of the physician such as the screening method of patients with tuberculosis and other chronic pulmonary diseases. Moreover, in

Ethiopia the percentage of smear positive pulmonary tuberculosis cases showed gradual decrease from 2005/6 to 2009/10(Hussen *et al*., 2012).

The first Ethiopian National population based TB prevalence survey indicates the exact prevalence of smear positive TB is lower than the WHO anticipated prevalence. This survey found three times lower prevalence of smear positive TB from 2008 estimation (284/100,000) and routine surveillance report of TB cases is close to the true prevalence of smear positive TB in the community (http://ehnri.gov.et/, 2012), this is in agreement with the current study.

The detection rate in ZN was also lower than the new PREVI Fluo methods with a smear detection rate of 9.7% and 14.1% respectively. The detection rate of bacilli by ZN method is lower in HIV positive individuals (Maryline *et al*., 2011) similarly in the current study ZN is slightly lower than Auramine O and PREVI Fluo. However statistical significance difference was not shown in each method in respect to detection rate of the bacilli in HIV positive study participants.

The sensitivity and specificity of ZN method were 56.7% and 96.8% respectively with culture taken as a gold standard and PPV, NPV and diagnostic accuracy were found to be 70.8%, 94.2% and 91.9% correspondingly. Sensitivity of ZN was lower than any other methods. This is in line with the report by Khatun *et al*., (2011) found that the sensitivity of and specificity of ZN were lower than LED microscope which were 56.06% and 97.61% respectively, also in a similar study in the Netherlands with regards to ZN, 61.1% and 98.9% sensitivity and specificity were found respectively (Ben *et al*., 2008). The sensitivity of ZN in this study was lower than that found in Tanzania (61.8%) (Matee *et al.*, 2008). Reasons for this could be that the concentrated smear method was used for the study in Tanzania and all study participants were HIV positive. However, the finding is in contrast with the report by Maryline *et al*. (2011) who found a sensitivity of 72%, this might be due to whether the Ziehl Neelsen staining method is always done first, as might be required in a diagnostic setting. A factor that may contribute to variability in the findings between these studies is of

study participant difference. Again in their study all the study participants were only HIV positives, but in the current study only 43 HIV positive individuals were included. Similar finding to this study was also reported from India which was 55.55% sensitivity of ZN and 71.85% sensitivity of Auramine O. Direct fluorescent microscopy detected 9.29% paucibacillary sputum samples that were missed on ZN staining (Saroj *et al*., 2011).

The PPV and NPV of ZN gave a value of 70.8% and 94.2% respectively while the Auramine O gave 56.82% and 96.57%. This is in agreement with the report from Turkey where the NPV of ZN was lower than the fluorescent staining (Mustafa *et al*., 2000).The test agreement of ZN with Culture gave a value of 0.585 Kappa which was lower than the Auoramine O and PREVI Fluo result. It had a moderate agreement with culture, however there was a substantial agreement of ZN with PREVI Fluo (kappa=0.636). Although there was substantial agreement between these two methods, still ZN had lower sensitivity.

The rate of false negativity in ZN was higher than PREVI Fluo and Auramine O which means the method had high probability to loss true positive cases. However, the false positivity of ZN was lower than the two methods this suggested that the probability of ZN to detect non bacilli artifacts was lower than the two methods. The likelihood ratio of ZN was slightly higher than PREVI Fluo and Auramine O methods which were 0.45, 0.25 and 0.24 respectively. Compare to these two methods ZN was not a good diagnostic method since its negative likelihood ratio was far from zero.

A total of 744 slides were run with Auramine O, spot-morning-spot samples were smeared and stained and from which 17.7% were positive from the total 248 patients. In a similar study in Kenya (Torrea *et al*., 2008) overall 23% of FM smears were positive which was higher than the current study with Auramine O. This could be as result that they used higher sample size in their study (21,104 smears were included in their study). In another study, sensitivity and specificity documented for the different modalities were 84.7% and 98.9% (Ben *et al*., 2008), respectively, for the LED assessment with Auramine O. The increased sensitivity of Auramine O is greatest in low grade positives. The proportion of low grade positives in the population served may thus determine the relative sensitivity of the method

over LM in any setting. This becomes more clear with the finding that the PPV for the Auramine O is 56.82% while for the ZN it is 70.83% (as the PPV gives lower result, it could be an indication that the test could be giving more false positives).One disadvantage of the fluorescent staining technique is that it may sometimes yield false positive results. However, most of these can be prevented by restaining the smear by the ZN method for bright-light microscopy (Heifets, 1997).

Apart from these studies, in a study done in kenya, Nairobi, there was no difference in sensitivity and specificity between LED-FM and ZN microscopy (Maryline *et al*., 2011). This could be explained by the great experience of study technicians with the ZN method and the fact that they never used FM before. Also they did not process the culture reference standard for each participant used for microscopy as they were requesting a 4th sample for culture confirmation in which case not all patients were able to produce a 4th specimen for culture and among those who did; the macroscopic appearance of the 4th specimen was of poorer quality than those specimens submitted for microscopy. With regards to its agreement with the kappa scale in relation to the gold standard, culture, Auramine O in this study has a kappa of 0.621(substantial agreement) which agrees better than the ZN (0.585, moderate agreement) but still a little less than the PREVI Fluo method (0.664, substantial agreement). In addition the Auramine O and the PREVI Fluo had a substantial agreement of K= 0.745. Auramine O had a smear positivity of 14% in HIV positive which was higher than the ZN (9.3%) but with no significant difference where as in PREVI Fluo it had a case detection rate of 11.6%.

Concerning the sensitivity and specificity of Auramine O stain, it had a sensitivity of 78.13% and a specificity of 91.2%, which is significantly higher in sensitivity than ZN and lower than the ZN finding in specificity (56.7% and 96.79% respectively for ZN). This is in agreement with the study in Ethiopia, Nepal, Nigeria, and Yemen (Luis *et al*., 2011b) using three LED-FM smears per patient resulted in sensitivity of 77.1% (73.3%–80.6%). The lower specificity may thus be the impact of the larger amount of operational data collected in this study, at the cost of a less-than-ideal reference standard.

In this study smears were made within a three-specimen set from the same patient and for processed by different techniques. This will ensure that bias associated with which sputum sample is used for which slide, that is morning sputum sample being used for one method alone kind of bias is eliminated. The negative likelihood ratio of Auramine O was smaller than ZN and PREVI Fluo methods, which were 0.24, 0.45, and 0.25 respectively. Compared to these two methods Auramine O was a very good diagnostic method since its negative likelihood ratio was nearest to zero.

PREVI Fluo TB is the new and promising method for the diagnosis of PTB which is developed by Biomerieux SA. The method has similar principle with the conventional LED fluorescent microscope except that it has a fixative, decolorizer ethanol than the more toxic methanol, a red Thiazine Red counter staining dye. The fixative helps to fix the smear to the slide so that there would not be cross contamination and environmental contaminant.

Moreover, the counter staining dye makes the back ground of the smear bright red color which creates a good contrast between the fluorescing bacilli and back ground. The back ground helps the stain to have fewer artifacts. Consequently, even if epithelial and other WBCs are present, the stain can use that as an advantage to give a better background contrast. Furthermore, it helps the technician better to focus and find the field and it becomes easier to differentiate when the objective is out of focus. Subsequently, this could be ideal for the inexperienced technician.

In the current study the smear detection rate of PREVI Fluo was found to be 14.1% while the culture confirmed cases were 12.1%.This is more close to culture results compared to the other two methods (ZN and Auramine O). There was a substantial agreement with PREVI Fluo TB result and TB Auramine O methods (K=0.745). Out of the total 34 PREVI Fluo positive cases, 88.6% had also a positive result with the Auramine O, while only 4 had a discordant result. Furthermore, while 6.1% of the Auramine O totals (17.7%) were negative with PREVI Fluo.

The sensitivity of the PREVI Fluo method was 76.67% better than ZN and slightly less than Auramine O which were 59.07% and 78.13% respectively. Nevertheless, the specificity (94.5%) was lower than ZN (96.79%) and higher than Auramine O (91.2%). The likely hood Ratio of a negative test of the PREVI Fluo method (0.24) was lower than ZN (0.44) which indicated PREVI Fluo is a better diagnostic test than ZN since it value is closer to zero.

The PREVI Fluo TB method has a PPV of 65.71% which is lower than the Auramine O (56.82%) but with a comparable NPV (96.71% and 96.57% respectively). Hence, in the case of any infectious disease with public health implications, it is important to have a test or rule that has a high sensitivity, negative predictive value and Positive predictive value, so that patients with true disease are treated and those with a low possibility of disease can be discharged from the hospital safely without treatment. The rate of false positivity is lower as well than the Auramine O method. There is not any previously done research on PREVI Fluo TB.

LIMITATION OF THE STUDY

- The study was limited by small numbers of HIV positives and failed to demonstrate statistically significant differences among this groups. Analysis of the results by HIV status might be underpowered, subject to self-selection bias, and confounded by the duration since last test.
- The current research could not differentiate Tuberculoid and non-tuberculous mycobacteria
- Drug susceptibility was not performed due to shortage of time but samples were kept in a deep freezer for further susceptibility and identification.

CONCLUSION AND RECOMMENDATION

Conclusion

The PREVI Fluo TB fluorescent staining method had nearly identical sensitivity compared to the conventional Auramine O fluorescent staining method for the detection of AFB in patient specimens. It had a significant higher sensitivity than ZN staining method, but the specificity was less than ZN. Auramine O also had significantly higher sensitivity than ZN where as the specificity was lower.

Recommendations

- PREVI Fluo can be used as an alternative laboratory diagnostic method for PTB
- All staff who are to examine sputum samples should become familiar or be trained with fluorescent staining in order to prevent false positive and/or negative results.
- Further studies have to be conducted to see the performance of PREVI Fluo in the comparison of other methods
- Upgrading any light microscope to a LED microscope is import

REFERENCES

Adjers-Koskelat K and Katila ML (2003) Susceptibility testing with the manual Mycobateria Growth Indicator Tube (MGIT) and the MGIT 960 system provides rapid and reliable verification of multidrug-resistant tuberculosis. *J. Clin. Microbiol,* 41**,** 1235-1239.

Ajay J, Rajeev R, Malay S and Ashok S (2011) Manifestation of Tuberculosis in HIV/AIDS patients and its relationship with CD4 count. *Lung India,* 28(4).

Alka MK, David V and Chambers GAH (2012) Identifying Pulmonary Tuberculosis in Patients With Negative Sputum Smear Results. *Chest 2001,* 120**,** 349-355.

Anon (2000) Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med,* 161**,** 1376-1395.

Anthony R, Kolk Ah, Kuijper S & Klatser Pr (2006) Light emitting diodes for auramine O fluorescence microscopic screening of Mycobacterium tuberculosis. *Int J Tuberc Lung Dis* 10**,** 1060-1062.

Banner A. (1979) Tuberculosis. Clincal aspects and diagnosis. *Arch Intern Med,* 139**,** 1387-1390.

Ben J, Marais, Wendy B, Katrien P, Anneke C, Hesseling, Nulda B, Elizabeth W, Dick VS, Rob M and Warren (2008) Use of Light-Emitting Diode Fluorescence Microscopy to Detect Acid-Fast Bacilli in Sputum. *Clinical Infectious Dis,* 47**,** 203-7.

Bennedsen J and Larsen S (1966) Examination for tubercle bacili by fluorescence microscopy. *Scand J Respir Dis* 47**,** 114-120.

Bergman J. And Woods G (1996) Clinical evaluation of the Roche Amplicor PCR Mycobacterium tuberculosis test for detection of M. tuberculosis in respiratory specimens *J Clin Microbiol,* 34**,** 1083-1085.

Bermudez L and Goodman J (1996) Mycobacterium tuberculosis invades and replicates within type II alveolar cells. Infect. *Immun,* 64**,** 1400-1406.

Blower M, Mclean R & Porco T (1995) The intrinsic transmission dynamics of tuberculosis epidemics. *Nat Med,* 1 815-821.

Bodnar K, Serbina N & Flynn J (2001) Fate of Mycobacterium tuberculosis within murine dendritic cells. . *Infect. Immun,* 69**,** 800-809.

Bonnet M, Ramsay A, Gagnidze L, Githui W, Guerin PJ & Varaine F (2007) Reducing the number of sputum samples examined and thresholds for positivity: an opportunity to optimise smear microscopy. *INT J TUBERC LUNG DIS* 11**,** 953-958.

Brodin P, Jonge M, Majlessi L, Leclerc C, Nilges M, Cole S & Brosch R (2005) Functional Analysis of Early Secreted Antigenic Target-6, the Dominant T-cell Antigen of Mycobacterium tuberculosis, Reveals Key Residues Involved in Secretion, Complex Formation, Virulence, and Immunogenicity. *J Biol Chem* 280**,** 33953-33959.

Burke & Parnell (1948) Minimal Pulmonary Tuberculosis. *Canadian Medical Association Journal,* 59**,** 348.

Cambanis A, Ramsay A, Wirkom V, Tata E & Cuevas L (2007) Investing time in microscopy: An opportunity to optimize smear-based case detection of tuberculosis. *Int J Tuber Lung Dis,* 11 40-45.

CDC (2007) World TB day-March 24, 2007. *MMWR Morb Mortal Wkly Rep,* 56(11)**,** 245.

CDC(2000) Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med,* 161**,** 1376-1395.

Chin D, Yajko D, Hadley W, Sanders C, Nassos P, Mandej J & Hopewel P (1995) Clinical utility of a commercial test based on the polymerase chain reaction for detecting Mycobacterium tuberculosis in respiratory specimens. *Am J Respir Crit Care Med* 151**,** 1872-1877.

Cole S, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon S, Eiglmeier K, Hornsb T & Jagels K (1998) Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. *Nature* 393**,** 537-44.

Corbett L, Watt J & Walker N (2003) The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* 163 1009-1021.

Cruickshank B (1952) Bacteriology, In Modern Practice in Tuberculosis. *Butterworth; London***,** 53.

Dannenberg AJ & Rook A (1994) Pathogenesis of pulmonary tuberculosis: an interplay of tissue-damaging and macrophage-activating immune responses. Dual mechanisms that control bacillary multiplication. 459-483.

David NM. (2000) Mycobacteria and Nocardia. *Medical Microbiology Baron's*, 552 4th edition.

Duc TMN. (2011) Tuberculosis in HIV-infected individuals in Ho Chi Minh City, Vietnam. *Texas Medical Center Dissertations,* AAI3459881.

Dye C, Watt C & Bleed D (2005a) Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. *JAMA,* 293**,** 2767-2775.

Dye C, Watt C, Bleed D, Hossein S & Raviglione M (2005b) Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. *JAMA,* 293**,** 2767-75.

Fenton M & Vermeulen W (1996) Immunopathology of tuberculosis: roles of macrophages and monocytes. *Infect. Immun,* 64**,** 683-690.

Ferguson J, Weis J, Martin J & Schlesinger L (2004) Complement protein C3 binding to Mycobacterium tuberculosis is initiated by the classical pathway in human bronchoalveolar lavage fluid. *Infect Immun,* 72**,** 2564-2573.

Ferrara G, Losi M, D'amico R, Roversi P, Piro R, Meacci M, Meccugni B, Dori I, Andreani A, Bergamini B, Mussini C, Rumpianesi F, Fabbri L & Richeldi L (2006) Use in routine clinical practice of two commercial blood tests for diagnosis of infection with Mycobacterium tuberculosis: a prospective study. *Lancet,* 367(9519)**,** 1328-34.

Floyd K, Blanc L, Raviglione M & Lee J (2002) Resources required for global tuberculosis control. *Sci,* 295**,** 2040-41.

Frieden T, Sterling T, Munsiff S &Watt C (2003) Tuberculosis. Lancet. 362**,** 887-899.

Gaynor D, Mccormack F, Voelker D, Mcgowan S & Schlesinger L (1995) Pulmonary surfactant protein A mediates enhanced phagocytosis of Mycobacterium tuberculosis by a direct interaction with human macrophages. *J. Immunol,* 155**,** 5343-5351.

Gebreselassie S (2003) Evaluation of the concentration sputum smear technique for the lab diagnosis of PTB. *Ethiop Med J* 33**,** 160-162.

Glenn T (2007) XDR TB Fact Sheet. *TB Anywhere is Everywhere***,** 3.

Globalfund (2004) Tuberculosis and Malaria, Ethiopia Proposal, Fourth round

Glynn R (1998) Resurgence of tuberculosis and the impact of HIV Infection. *Br Med Bull* 54 579-593.

Goldrick B (2004) Once dismissed, still rampant: tuberculosis, the second deadliest infectious disease worldwide. *Am J Nurs,* 104(9)**,** 68-70.

Habeenzu C, Lubasi D & Fleming A (1998) Improved sensitivity of direct microscopy for detection of acid-fast bacilli in sputum in developing countries. *Trans R Soc Trop Med Hyg,* 92**,** 415-16.

Hadley M & Maher D (2000) Community involvement in tuberculosis control: lessons from other health care programmes. *Int J Tuberc Lung Dis,* 4(5)**,** 401-408.

Hanscheid T (2008) The future looks bright: low-cost fluorescent microscopes for detection of Mycobacterium tuberculosis and Coccidiae. *Trans R Soc Trop Med Hyg* 102 520-521.

Harries A (2004) How does treatment of tuberculosis differ in persons infected with HIV? In: Frieden TR, ed. Toman's tuberculosis. Case detection, treatment and monitoring, 2nd Edition. Geneva: World Health Organization. 169-172.

Hawken M, Meme H, Elliott L, Chakaya J, Morris J, Githui W, Juma E, Odhiambo J, Thiong'o L, Kimari J, Ngugi E, Bwayo J, Gilks C, Fa Plummer, Porter J, Nunn P & Mcadam K (1997) Isoniazid preventive therapy for tuberculosis in HIV-1-infected adults: results of a randomized controlled trial. *AIDS,* 11**,** 875-882.

Heifets L (1997) Mycobacteriology laboratory. *ClinChest Med,* 18**,** 35-41.

Hobby L, Holman P, Iseman D & Jones M (1973) Enumeration of tubercle bacilli in sputum of patients with pulmonary tuberculosis. *Antimicrob Agents Chemother* 4 94-104.

Hopewell P (1994) Overview of clinical tuberculosis:Tuberculosis: pathogenesis, protection and control Washington, D.C. *American Society for Microbiology***,** 25-46.

Http://EHNRI.gov.et/ (2012) Ethiopian National population-based TB Prevalence survey. *Abstract of research findings presented on the 2nd National Science conference of EHNRI***,** 7.

Hudson C, Wood R & Maartens G (2000) Diagnosing HIV-associated tuberculosis:Reducing costs and diagnostic delay. *Int J Tuberc Lung Dis* 4(3)**,** 240-245.

Hung N, Sy D, Anthony R, Cobelens F & Soolingen DV (2007) Lancet Infect Dis *Fluorescence microscopy for tuberculosis diagnosis,* 7**,** 238-9.

Hussen A, Ahmed Z, Abiyu M, Solomon A & Solomon A (2012) Smear posetive pulmonary tuberculosis (ptb) prevalence amongst patients at agaro teaching health center, south west ethiopia *Ethiop J Health Sci.,* 22(1).

Imam T & Oyeyi T (2008) Retrospective study of PTB prevalence among patients attending in infectious disease hospital. *Bayero Journal of pure and applied science,* 1(1)**,** 10-15.

Jensen P, Lambert L, Iademarco M & Ridzon R (2005) Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings. *Centers for Disease Control and Prevention. ,* 54(RR-17)**,** 1-141.

Joe M, Bai Y, Nacario R & Lowary T (2007) Synthesis of the docosanasaccharide arabinan domain of mycobacterial arabinogalactan and a proposed octadecasaccharide biosynthetic precursor. *J Am Chem Soc,* 129 (32)**,** 9885-9901.

Johnson J & Ellner JJ (2006) Tuberculosis and atypical mycobacterial infection,In: Tropical infectious disease, principles, pathogenesis and practice, Guerrant RL, Walker

DH Weller PF (eds); 2nd edn, Churchill living stone. 394.

Kamholz S (1996) Pleural tuberculosis In W. N. Rom and S. Garay (ed.), Tuberculosis. Little, Brown and Co., Boston, Mass. 483-491.

Karen RS, Megan H, Vivienne N, Philip CH, Andrew R, Jane C, Richard U, Perkins Mark , Mohamed AA & Madhukar P (2006) Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis,* 6**,** 570-81.

Katoch V (2004) Newer diagnostic techniques for tuberculosis *Indian J Med Res,* 120(4)**,** 418-428.

Khatun Z, Kamal M, Roy C, Sultana T, Rahman M, Azad M & Ahmed A (2011) Usefulness of light emitting diode (LED) fluorescent microscopy as a tool for rapid and effective method for the diagnosis of pulmonary tuberculosis. *Pubmed,* 37(1)**,** 7-10.

Laifangbam S, Singh H, Singh N, Devi K & Singh N (2009) A comparative study of fl uorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmonary tuberculosis. *Kathmandu University Medical Journal* 7**,** 226-230.

Lee R, Li W, Chatterjee D & Lee R (2005) Rapid structural characterization of the arabinogalactan and lipoarabinomannan in live mycobacterial cells using 2D and 3D HR-MAS NMR: structural changes in the arabinan due to ethambutol treatment and gene mutation are observed. *Glycobiology,* 15**,** 139-151.

Li Y, Petrofsky M & Bermudez L (2002) Mycobacterium tuberculosis uptake by recipient host macrophages is influenced by environmental conditions in the granuloma of the infectious individual and is associated with impaired production of interleukin-12 and tumor necrosis factor alpha. *Infect Immun,* 70**,** 6223-6230.

Lipsitch M & Sousa O (2002) Historical intensity of natural selection for resistance to tuberculosis. *Genetics,* 161**,** 1599-1607.

Luis EC, Najla AS, Lovett L, Melkamsew A & Abraham A (2011a) LED Fluorescence Microscopy for the Diagnosis of Pulmonary Tuberculosis: A Multi-Country Cross-Sectional Evaluation. *Plos Medicine,* 8**,** 1.

Luis EC, Najla AS, Lovett L, Mohammed AY, Isabel A, NasherAA, Jeevan BS, Amin AA, Emmanuel NE, Yared M, Mosis IO, Juliana OO, Melkamsew A, Abraham A, Greg H, Rachel MAD, Sally JT, Carl-Michael N, Jean J, Brian F, Stephen BS & Andrew R (2011b) LED Fluorescence Microscopy for the Diagnosis of Pulmonary Tuberculosis: A Multi-Country Cross Sectional Evaluation. *Plosmedicine,* 8(7).

Mario C, Richard J, Anthony S, Eugene B & Kurt J (1995) Tuberculosis 966 -972 in In J. Larry Jameson, ed. Harrison's Principles of Internal Medicine, 14th ed. McGraw-Hil, New York.

Maryline B, Laramie G, Willie G, Philippe JGR, Laurence B, Francis V & Andrew R (2011) Performance of LED-Based Fluorescence Microscopy to Diagnose Tuberculosis in a Peripheral Health Centre in Nairobi. *Plos ONE,* 6.

Mase S, Ramsay A & Alng VE (2007a) Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. *Int. J. Tuberc. Lung Dis.,* 11**,** 485-495.

Mase S, Ramsay A, Ng V, Henry M & Hopewell P (2007b) Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. *Int J Tuberc Lung Dis* 11**,** 485-495.

Masood Z, Mohammad N, Majid K & Ghodsieh A (2008) Comparison of the value of two different sputum staining for diagnosis of acid-fast bacilli. *Iranian Journal of Clinical Infectious Diseases,* 3**,** 99-102.

Matee M, Mtei L, Lounasvaara T, Wieland-Alter W, Waddell R, Lyimo J, Kari M, Pallangyo K & Reyn CV (2008) Sputum microscopy for the diagnosis of HIV-associated pulmonary tuberculosis in Tanzania. *BMC. Infect. Dis.,* 8**,** 68.

Mdg R (2010) Trends and prospects for meeting MDGs by 2015.

Mehta K, King H, White H, Murtagh J & Quinn D (1996) Comparison of in vitro models for the study of Mycobacterium tuberculosis invasion and intracellular replication *Infect. Immun,* 64 2673-2679.

Minion J, Sohn H & Pai M (2009) Light-emitting diode technologies for TB diagnosis: what is on the market? *Expert Rev Med Devices* 6**,** 341-345.

MOH (2004a) The Global Fund to Fight Aids, Tuberculosis and Malaria, Ethiopia Proposal, Fourth round

MOH (2004b) HIV/AIDS and Tuberculosis Prevention and Control Extension Package. 39.

MOH (2007) Sixth report. *Aids in Ethiopia***,** 6.

MOH/HAPCO (2005) AIDS in Ethiopia 6th report. Addis Ababa.

Munyaradzi D, Peter M, Tsitsi B, Abbas Z, Shungu S, Munyati, Anthony E & Corbett (2010) The Risk and Timing of Tuberculosis Diagnosed in Smear-Negative TB Suspects: A 12 Month Cohort Study in Harare, Zimbabwe. *Plos one,* 5(7).

Murray S, Barrett A, Magee J, Freeman R & Pathol C (2003) Optimization of acid fast smears for the direct detection of mycobacteria in clinical samples. 56**,** 613-15.

Mustafa U, Gonul A & Sami T (2000) A Comparative Study on the Different Staining Methods and Number of Specimens for the Detection of Acid Fast Bacilli. *Mem Inst Oswaldo Cruz, Rio de Janeiro,* 95(6)**,** 855-858.

Ndugga LK, Cleeff MV, Ng'ang'a L, Meme H, Odhiambo J & Klatser P (2005) Sex-specific performance of routine TB diagnostic tests. *INT J TUBERC LUNG DIS,* 9(3)**,** 294-300.

Onubogu CC, Nwokoye NN, Kunle-Ope CN & Raheem TY (2012) Sensitivity of direct smear microscopy for the diagnosis of TB in high HIV prevalent population *Scientific Research and Essays,* 7(5)**,** 593-597.

Perkins M, Roscigno G & Zumla A (2006) Progress towards countries. *Lancet,* 367**,** 942-943.

Prasanthi K & Kumari A (2005) Efficacy of fluorochrome stain in the diagnosis of pulmonary tuberculosis co-infected with HIV. *Indian J Med Microbiol* 23**,** 179-85.

Raviglione M & O'brien R (2001) Mycobacterial diseaseTuberculosis. In: Harrison's principle of internal medicine ,Wald EB, Hauser SL, Fauci AS, Long DL, Jameson JL, Kasper DL(eds); 15th edn, McGraw-Hill, New York.

RIEDER, L. (1999) Epidemiologic Basis of Tuberculosis Control. *International Union Against Tuberculosis and Lung Diseases, Paris.*

Saroj H, Nita P, Bharti M, Sumit G, Vipin K & Leela V (2011) comparison of ziehl neelsen & auramine o staining methods on direct and concentrated smears in clinical specimens. *Indian J Tuberc,* 58**,** 72-76.

Schlesinger S (1993) Macrophage phagocytosis of virulent but not attenuated strains of Mycobacterium tuberculosis is mediated by mannose receptors in addition to complement receptors. *J. Immunol* 150**,** 2920-2930.

Shargie E, Yassin M & Lintjorn B (2006) Smear positive pulmonary TB in rural district of Ethiopia. *Int J tuber Lung Dis* 10(1)**,** 87-97.

Shenai S, Minion J, Vadwai V, Tipnis T & Shetty S (2011) Evaluation of light emitting diode-based fluorescence microscopy for the detection of mycobacteria in a tuberculosis-endemic region. *Int J Tuberc Lung Dis* 15**,** 483-488.

Shinnick T & Good R (1994) Mycobacterial taxonomy. *Eur J Clin Microbiol Infect Dis,* 13**,** 884-901.

Stauffer F, Mutschlechne R, Hasenberger P, Stadlbauer S & Schinko H (1995) Detection of Mycobacterium tuberculosis complex in clinical specimens by a commercial polymerase chain reaction kit. *Eur J Clin Microbiol Infect Dis,* 14**,** 1046-1051.

Steingart K, Henry M, Ng V, Hopewell P, Ramsay A & Cunningham J (2006b) Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review *Lancet Infect Dis,* 6**,** 570-581.

Stephen H (2006) Principles and Practice of Clinical Bacteriology Second Edition,Hampstead, London. *Gillespie Centre for Medical Microbiology, Royal Free and University College Medical School*.

Tascon R, Soares C, Ragno S, Stavropoulos E, Hirst EM & Colston MJ (2000) Mycobacterium tuberculosis-activated dendritic cells induce protective immunity in mice. *Immunology* 99**,** 473-480.

Tenover F, Crawford J, Huebner R, Geiter L, Horsburgh C & Good R (1993) The resurgence of tuberculosis: is your laboratory ready? *J Clin Microbiol,* 31**,** 767-70.

Tevere V, Hewitt P, Dare A, Hocknell P, Keen A, Spadoro J & Young K (1996) Detection of Mycobacterium tuberculosis by PCR amplification with pan-Mycobacterium primers and hybridization to an M. tuberculosis-specific probe. *J Clin Microbiol* 34**,** 918-923.

Torrea G, Chakaya J, Mayabi M & Deun AV (2008) Evaluation of the Fluoreslent and fluorescence microscopy blinded rechecking trial, Nairobi, Kenya. *INT J TUBERC LUNG DIS* 12**,** 658-663.

Troesch A, Nguyen H, Miyadac G, Desvarenne S, Gingeras RT, Kaplan MP, Cros P & Mabilat C (1999) Mycobacterium species identification and rifampin resistance testing with high-density DNA probe arrays *J. Clin. Microbiol,* 37**,** 49-55.

Vansoolingen D, Hoogenboezem T, Haas PD, Hermans P, Koedam M, Teppemaksbrennan P, Besra G, Portaels F, Top J, Schouls L & Vanembden J(1997) A novel pathogenic taxon of the Mycobacterium tuberculosis complex, Canetti: characterization of an exceptional isolate from Africa. *Int J Syst Bacteriol,* 47**,** 1236-45.

Wallgren A (1948) The time table of tuberculosis *Tubercle,* 29**,** 245-251.

Wallis R, Pai M, Menzies D, Doherty T & Walzl G. (2010) Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. *Lancet* 375**,** 1920-1937.

WHO (2001) The Global Plan to Stop Tuberculosis. Geneva: World Health Organization.

WHO(2003) Treatment of tuberculosis. Guidelines for national programmes. Geneva:swezerland

WHO (2004a) TB/HIV: A clinical manual. Geneva: World Health Organization.

WHO (2004b) Global TB Report.

WHO (2006a) Global tuberculosis control report. Geneva, Switzerland.

WHO (2006b) Guidelines for intensified tuberculosis case-finding and isoniazid preventivetherapy for people living with HIV in resource-constrained settings,World Health Organization, Geneva, Switzerland.

WHO (2007a) The use of liquid medium for culture and DST.

WHO (2007b) Definition of Smear Positive TB. Geneva: World Health Organization. *New WHO policies*.

WHO (2008a) Stop TB Partnership Retooling Task Force New Laboratory Diagnostic Tools for Tuberculosis. Geneva, Switzerland.

WHO (2009) Approaches to improve sputum smear microscopy for tuberculosis diagnosis, expert group meeting.

WHO (2010a) fluorescent light emitting diode (led) microscopy for diagnosis of tuberculosis policy statement.

WHO (2010c) Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response.

WHO (2011a) The Global Plan to Stop TB 2011-2015.

WHO (2011b) Tuberculosis Prevalence survey: A hand book.

ANNEXES

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

You are kindly invited to participate in this study, which involves TB suspected individuals. The aim of this study is to perform a comparative analysis of a new PREVI Fluo TB method with the conventional methods. TB can infect different part of the body and develop Tuberculosis; Pulmonary Tuberculosis and Extra pulmonary Tuberculosis, but Pulmonary Tuberculosis is the focus of this study. Therefore this study will compare the microscopic methods and as well do prevalence of TB at St Paul's Hospital Millennium Medical College

a. Purpose: the purpose of this research study is to assess the comparative performance of PERVI Fluo TB by using LED-Microscopy among TB suspected patients at St.Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

b. Duration: This research study will be conducted from November/2011-February 2012.

c. Procedures to be carried on: the procedure of sample collection is easy; sputum sample will be collected with a wide mouth sterile container.

d. Risk and discomfort: There will be no discomfort during sample collection time.

e. Expected benefits: from this study you are not directly benefited, however, it has significance role on the prevention as well as treatment of tuberculosis.

g. Confidentiality: All your personal information collected for the purpose of the present study will be kept confidential.

h. Compensation: No compensation will be provided by participating in this study.

g. Termination of the study: Participation in the study is voluntary, and refusal to participate involves no penalty or loss of benefits to which you are otherwise entitled. The study participants have a right to

- Keep hold information
- Decline to cooperate in the study
- To refuse provision of specimens

I would also like to inform you that this study has been approved by Microbiology, Immunology and Parasitology Department Ethical and Review Committee, Faculty of Medicine Addis Ababa University. If you have any question about the right of the study participant the address is:

Faculty of Medicine, Addis Ababa University
Office of Associate Dean, Postgraduate Programs and Research
P.O. Box 9086. Addis Ababa, Ethiopia
Tel. 251-011-551-28-765

If you have question about the study the address of the principal investigator is:

Semaria Solomon
Tel: 0911217830
Department of Microbiology, Immunology and Parasitology
Faculty of Medicine, Addis Ababa University
P.O.Box. 25181/1000,
Addis Ababa, Ethiopia

<u>የጥናቱ ተሳታፊዎች የመረጃ ቅጽ</u>

ሀ.<u>የጥናቱ ዓላማ፡-</u> የዚህ ጥናት አላማ አዲስ የሳንባ ነቀርሳ መመርመሪያ ዘዴ ለማጥናትና በቅ/ጳ/ሆ ያለውን የቲቢ ስርጭት ለማውቅ እና ለማዎዳደር ነው፡፡

ለ. <u>የሚፈጀው ጊዜ</u>፡-ይህ ጥናት ከህዳር 2004 ዓ ም እስከ የካተቲት 2004ዓ ም ይካሄዳል፡፡

ሐ. <u>አጠቃቀም</u>፡-በዚህ ጥናት ከሚሳተፉ ሰውች የአክታ ናሙና ይወሰድላቸዋል፡፡

መ. <u>ሊደርስ የሚችል አደጋ</u>፡-በዚህ ጥናት ውስጥ አደጋ የሚያደርስ ድርጊት የለም፡፡

ሠ. <u>የሚገኝበት ጥቅም</u>፡- ይህ ጥናት ቲቢ በሽታን ቀደም ብሎ ለመከላከል እና አንዴ በሽታው ከተከሰተም በኋላ በአግባቡ ለማከም ትልቅ ሚና አለው፡፡

ረ. <u>ሚስጥራዊነት</u>፡-የማንኛውም የጥናቱ ተሳታፊ መረጃ በሚስጥራዊነት ይያዛል፡፡ የእያንዳንዱን ግለሰብ መረጃ ከዋናው ተመራማሪ እና አማካሪው በስተቀር ማንም ሊያገኝ አይችልም፡፡

ሰ. **<u>ፈ</u>ቃደኝነትን ስለማቋረጥ**፡-የጥናቱ ተሳታፊዎች መረጃ ያለመስጠት፣በጥናቱ ለመሳተፍ ፈቃደኝነት ያለማሳየት እንዲሁም ናሙና ያለመስጠት መብታቸው የተጠበቀ ነው፡፡

አድራሻ ማዎቅ ካስፈለግዎ፡-

ህክምና ፋካልቲ ፣ አዲስ አበባ ዩኒቨርስቲ

የድህስ ምረቃ ፕሮግራምና ምርምር የተባባሪ ዲን ቢሮ

የመ.ሳ.ቁ. 9086 ፣አዲስ አበባ

ስልክ. 251-011-551-28-765

የዋናው ተመራማሪ አድራሻ፣ ሰማሪያ ሰለሞን

የመ.ሳ.ቁ. 25181/1000

ስልክ፣ 0911217830

Annex II Consent Form (English and Amharic version)

Name……………………..Card No…………Ward………Serial No ………

I have read the information sheet (or it has been read to me); I have understood that this study is to assess the performance of a new Tuberculosis diagnostic reagent and as well to study the prevalence of Tuberculosis at St. Paul's Generalized Specialized Hospital. I have asked some questions and clarification has been given to me. I have given my consent freely to participate in the study, and I herby to approve my agreement with my signature.

Participant's signature _______________Date_____________________

Investigator's signature ______________ Date_____________________

Witness signature 1. _______________Date_____________________

2. _________________ Date_____________________

Annex III: Sputum smears preparation and Acid Fast Staining procedures

1. Label the slide with patient code/number
2. Make the appropriate thickness and width sputum smear on the slide
3. Lets it air dry by placing on a rack
4. Fix the dried smear by passing 3 times over the top of Bunsen- burner or sprit flame
5. Stain the fixed smear with Ziehl-neelsen hot method

A) Place the slide on the slide rack with the smear upper most, their edges separated. Make sure that the slides do not touch each other.

B) Cover the whole surface of the slide with filtered Ziehl carbol fuchsin

C) Heat very gently until steam appears. Use the flame of a cotton wool in methylated Sprit fixed on end of metal rod or stick for heating.

D) Leave the warm stain for 5 minutes

E) Tilt the slide to drain off excess stains. Rinse each slide individually in a gentile stream of running water until the all free stain is washed away.

F. Decolorize by 3% acid alcohol. Leave until the slides become clear and then wash the slide with a gentile stream of running water.

G. Counter stain the smear by flooding it with 0.3%methyleen blue and leaving it for 1-2 minutes.

H. Pour off methylene blue stain and wash the slide with gentle stream of running water.

I. Tilt and place the slide on the rack to dry in the air

Microscopic slide examination for AFB

1. Select the well-distributed smear area on the slide using 10 times objective
2. Add a drop of oil immersion and switch the objective to 100x (oil immersion objective)
3. Read the slide systematically. Look at least for 100-oil immersion fields.

Reporting of microscopic reading for ZN by grading (Quantitation scale recommended by the World Health Organization)

Report	Grading report
No AFB/100 oil immersion filed	No AFB seen
1-9/100 oil immersion fields	exact count
10-99/100 immersion fields	1+
1-10/ immersion field	2+
> 10/ immersion field	3+

Annex IV: Test Procedure for staining with PREVI Fluo TB

1. Prepare labeled sputum sample
2. Flame the wire loop
3. Take the sample using the wire loop
4. Make a smear on the slide and dry the slide
5. Put in a fixative solution for 10 minutes
6. Rinse with water for 1 minute (filtered or deionized)
7. Add Auramine mix for 20 minutes
8. Rinse in water for 1 minute
9. Add decolorizer for 3 minutes
10. Rinse in water for 1 minute
11. Add thiazine red mix for 5 minutes
12. Rinse in water for 1 minute
13. Air dry and away for light

Laboratory Data Recording Format

1. Date and time of specimen collection ……………………………

2. Type of specimen :

3. Sputum consistency: Clear _______ yellowish _______ Greenish ____

   Bloody ______ Others: Specify _______________

IV. X- ray findings: suggestive of PTB_____________ Not suggestive__________

Comments ____

Name of investigator___________________________________

Signature _________________________ Date_______________

Annex V: Questionnaire (English and Amharic version)

ADDIS ABABA UNIVERSITY

School of Medical Microbiology

Date---------------

The purpose of this questionnaire is to gather information for a study intended to investigate the comparative analysis of PREVI Fluo TB and assess the prevalence of TB at St. Paul's Generalized Specialized Hospital, Addis Ababa, Ethiopia. The study will have a major role in the process of early intervention design, implementation and other tuberculosis programs. Therefore, your frank and sincere response is highly appreciated, for it will enable the researcher to obtain reliable and valid information.

-Be sure to answer every item

-your responses will be kept confidential and writing your name is not needed.

Thank you in advance for your cooperation.

General Information

Read the following items and fill the appropriate words or encircle the letter of your choice.

1. Card no---------------- Sex---------------- Age---------------
2. Ward: ………….
3. Residence: Urban-------------Rural----------------
4. Address---
5. Marital Status
   - 5.1 Married
   - 5.2 Single
   - 5.3 Separated
   - 5.4 Widowed
6. Educational Level
   - 6.1 Literate (reading and writing)
   - 6.2 Illiterate
   - 6.3 Elementary education
   - 6.4 Secondary education

6.5 Higher education

6.6 Other specify------------------------------------

7. What is your current occupation?

7.1 Governmental Organization

7.2 NGO or Private Company Employee

7.3 Daily laborer

7.4 House wife

7.5 Business Man/Woman

7.6 Other specify-------------------------------------

8. Reason for visit to the hospital ………………

9. Was there a previous history of Tuberculosis?

10. From where did you acquire the disease? Any family member with TB (contact)

11. HIV status: Negative-----------Positive-----------------Unknown----------------

12. If your answer for Q. 12 is yes, Are you using ART?

II. Some Medical Information

1. Have you ever been with TB patients in the last two years?

A. Yes B. No C. I do not remember

If yes for Q1, where was the place that you were with him/her?

A .at the village B. at working place C. in prison D. at hospital/health center E. if any other (specify) ----------

2. Have you ever been diadnosed with pulmonary tuberculosis before? A. yes B. No

3. Is there any other person in your family infected with pulmonary tuberculosis?

A. Yes B. No

If yes for Q3, how many of your family members are infected with tuberculosis?

How many of your family members sleep in a single room with you or other TB patient?

4. Have you any history of imprisonment?

A. Yes B. No

If yes for Q4, where was the prison?

Do you remember the correct time you were there? A. Yes B. No

Was there any TB patient with you there? A. Yes B. No

5. Have you ever stopped taking anti TB drug prescribed for you before? A. Yes B. No
If yes for Q5, do you remember the exact time you stopped taking anti TB drugs?
A .after taking for a month B. after taking for two months C. after 5 months D .if any other (specify)----------------
6. Have you ever gone to any other place far from your residence in the past two years?
A. Yes B. No C.I don't remember
If yes for Q6.do you remember the exact time you went there? A. Yes B. No
Do you also remember the place you went? A. Yes B. No
Have you contacted to pulmonary tuberculosis Patient there A. Yes B. No

Clinical Signs and Symptoms Data Recording Format

1. Type of TB diagnosed. A) Pulmonary AFB positive. B) Pulmonary AFB negative.
C) Extra-pulmonary (if possible write type of TB) _________.
2. CD4+ T cell count /mm3___________ CD8+ T cell/mm3______________
CD4+/CD8+Ratio __________________.

3. Signs and symptoms of Tuberculosis
3.1 Fever 1.Yes 2 No
3.2 Chills 1.Yes 2 No
3.3 Chronic cough more than 2 weeks 1.Yes 2 No
3.3 Night sweating 1.Yes 2 No
3.4 Weight loss 1.Yes 2 No
3.5 Indicative chest X ray finding 1.Yes 2No
3.6 Others specify----------------------

አዲስ አባባ ዩኒቨርስቲ

የሜዲካል ማይክሮ ባይሎጂ ትምህርት ክፍል

ቀን፡ ___________________

መጠይቅ፡ የዚህ ጥናት አላማ በቅዱስ ፓውሎስ ሆስፒታል ፒሪቪፍሉ የተባለውን የቲቪ መመርመሪያ ዘዴ ከመደበኛው (ኤኤፍቢ) የመመርመሪ ዘዴ ጋር ለማወዳደር እና በሆስፒታሉ ያለውን የቲቢ በሽታን ሥርጭት ለማጥናት ነው፡፡

ይህ ጥናት ከቲቢ ትምህርታዊ እቅድ አፈፃፀም እንዲሁም ፕሮግራም ጉልህ ሥፍራ ይኖረዋል፡፡ ስለዚህም ከእርሶዎ የሚገኘው ግልጽና ቅንነት የተሞላው መልስ ጥናቱን ለሚያካሔደው ግለሰብ እምነት የሚጣልበት እና አስተማማኝ መረጃ እንዲሰበስብ ስለሚያግዘው እጅግ ያስመሰግንዎታል፡፡

- ሁሉንም ጥያቄዎች መመለስዎን ያረጋግጡ
- መልሶዎ በምስጥር የሚጠበቅ ስለሆነ ስምዎን መፃፍ አያስፈልገትም

ስለትብብሮ በቅድሚያ እናመሰግናለን፡፡

አጠቃላይ መረጃ

የሚከተሉትን አንብበው መልሶን በክፍት ቦታው ላይ በመሙላት ወይም በማክበብ ይመልሱ

1. የካርድ ቁጥር _____________ ጾታ_____________እድሜ_____________
2. ክፍሉ _____________
3. የመኖሪ ቦታ _____________
4. አድራሻ_____________
5. የጋብቻ ሁኔታ
   5.1 ያገባ _____________
   5.2 ያላገባ_____________
   5.3 የተፋታ/የተፋታች _____________
   5.4 ባል/ሚስት የሞተበት/የሞተባት_____________
6. የትምህርት ደረጃ
   6.1 የተማረ፡ (ማንበብና መፃፍ የሚችል)
   6.2 ያልተማረ
   6.3 የመጀመሪያ ደረጃ ትምህርት

6.4 ሁለተኛ ደረጃ ትምህርት

6.5 ከፍተኛ ደረጃ ትምህርት 6.6 ሌሎች

7. የሥራ ሁኔታ

7.1 የመንግስት

7.2 የመንግስት ያልሆነ (የግል)

7.3 የቀን ሠራተኛ

7.4 የቤት እመቤት

7.5 ነጋዴ

7.6 ሌሎች_____________

8. ክፍሉ____________ ድንገተኛ

9. ወደ ሆስፒታሉ የመጡበት ምክንያት

10. ከአሁን በፊት ቲቢ ይዞዎት ያውቃል

11. በሽታው ከየት የያዝዎት ይመስልዎታል? ከቤተሰብዎ መካከል ቲቢ የያዘው ነበር?

12. የኤች አይ ቪ በደሞ ውስጥ አለ? የለም________አለ

__________አይታወቅም____________

13. ለአስራ ሁለተኛ ጥያቄ መልሶ አዎ ከሆነ የፀረ ኤች አይ ቪ መድኃኒት ይጠቀማሉ?

ሀ.አዎ ለ. አይ

<u>የጤናዎት ሁኔታ መጠይቅ</u>

1. ባለፉት ሁለት ዓመታት ከቲቢ በሽተኞች ጋር ነበሩን ?

ሀ. አዎ ለ. አልነበርኩም ሐ አላስታውስም

ለአንደኛው ጥያቄ መልሶዎ አዎን ከሆነ ከበሽተኛው ጋር የነበሩበት ቦታ የት ነበር?

ሀ. መኖሪያ አካባቢ ለ. በስራ ቦታ ሐ. በእስር ቤት

መ. በሆፒታል ጤና ጣቢያ ሠ. ሌላ ከሆነ ይግለጹ

2. ከዚህ በፊት ቲቢ ነበረብዎት?

ሀ. አዎ ለ. አይ

3. በቤተሰብዎ ውስጥ ሌላ ሰው በ ቲቢ የተያዘ ነበሩን ?

ሀ. አዎ ለ. የለም

ለሦስተኛው ጥያቄ መልሶዎ አዎን ከሆነ በቤተሰቦ መካከል ምን ያህሉ ቲቢ ከያዘው የቤተሰብ አባል መኝታ ክፍል ውስጥ አብረው ይተኛሉ?

4. ታስረው ያውቃሉ?

ሀ. አዎ ለ. አላውቅም

ለአራተኛው ጥያቄ መልሶዎ አዎን ከሆነ እስር ቤቱ የት ነበር? እዛ የነበሩበትን ትክክለኛ ጊዜ ያስታውሳሉ?

ከእርሶ ጋር እዛ የ ቲቢ በሽተኞች ነበርን?_______________

5. ቲቢ በሽታ የተዛዘልዎትን መድኃኒት አቋርጠው ያውቃሉ?

ለአምስተኛው ጥያቄ መልሶ አዎን ከሆነ የ ቲቢ መድኃኒት ያቆረጡበት ትክክለኛ ጊዜ ያስታውሳሉ?

ሀ. ከአንድ ወር በኃላ ለ. ከአራት ወር በኃላ ሐ. ከአምስት ወር በኃላ

መ. ሌላ ከሆነ ይግለጹ

6. ባለፉት ሁለት አመታት ከመኖሪያዎ ስፍራ ርቀው ሄደው ያውቃሉ?

ሀ. አዎ ለ. አልሄድኩም ሐ. አላስታውስም

ለስድስተኛው ጥያቄ መልሶዎ አዎ ከሆነ የሄዱበትን ትክክለኛ ጊዜ ያስታውሳሉ?

የሄዱበትን ቦታ ያስታውሳሉ?

የሄዱበት ቦታ ከ ቲቢ በሽታ ጋር ቅርበት ነበርዎ?

ሀ. አዎ ለ. አልነበረኝም

Declaration

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

MSc Candidate

Semaria Solomon

Signature ------------------------

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