DIAGNOSIS OF PULMONARY TUBERCULOSIS AMONG HIV POSITIVE AND HIV NEGATIVE PATIENTS IN MBAGATHI DISTRICT HOSPITAL: COMPARISON OF DIRECT SMEAR, CONCENTRATE SMEAR AND CULTURE TECHNIQUE.

A dissertation submitted in partial fulfilment for the award of degree in Master of Science in Medical Microbiology, department of Medical Microbiology, University of Nairobi.

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H56/P/8238/04
DECLARATION

I Sheilla J. Chebore declare that the work contained herein is my original idea and has not been presented at any other place to the best of my knowledge. No part of this document should be reproduced without permission of the author and/or University of Nairobi.

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This dissertation has been submitted for examination with my approval as university supervisor;

Signed........................................ Date November 23, 2007

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DEDICATION

To my beloved daughter Heizel Selesi, Aglean, my brother Korir, Nathan, family members and all my friends.
ACKNOWLEDGEMENT

I hereby extend my sincere gratitude to all those who made it possible for me to start and complete this work.

Prof J.O. Ndinya Achola, Department of Medical Microbiology for his encouragement, supervision guidance, patience and concern throughout the study.

Dr Sitienei, Director NLTP for his supervision, guidance, encouragement and financial support throughout the study.

Prof J Smith, Emeritus Professor Indiana University for his guidance and support.

Wangoi, CRL for assistance in specimen analysis.

Mbagathi TB laboratory and CRL staffs.

Dr N. Buziba, Head Haematology and blood transfusion Department, Moi University for his guidance and support.

Aglean K.Chelimo, Haematology/blood transfusion KNH for her support and care.

All masters students Medical Microbiology 2004 class for their critique during protocol preparation.

Finally, I thank brothers and Sisters in the Lord Jesus Christ for their deep prayers and spiritual nourishments.
ABBREVIATIONS

AIDS  Acquired Immunodeficiency Syndrome
AFB  Acid Fast Bacilli
BCG  Bacille Calmette Guerin
CTL  Cytotoxic T Lymphocytes
CMI-  Cell Mediated Immunity
CDR  Case Detection Rates-TB cases notified divided by the estimated incidence of TB cases
DNA  Deoxyribonucleic Acid
DOTS  Direct Observed Treatment Short Course
TB  Tuberculosis
MTB  Mycobacterium tuberculosis
MAI  Mycobacterium Avium Intracellulare
WHO  World Health Organization
MOTT  Mycobacteria Other Than Tuberculosis
MDR  Multiple Drug Resistance
IUATLD  International Union Against TB and Lung Disease
HI  Human Immunodeficiency Virus
MGIT  Mycobacteria Growth Indicator Tube
LJ  Lowenstein Jensen
NALC  N. Acetyl lysine cytosine
NAOH  Sodium Hydroxide
PTB  Pulmonary Tuberculosis
HPLC  High Performance Liquid Chromatography
GLC  Gas Liquid Chromatography
PCR  Polymerase Chain Reaction
PTB  Pulmonary Tuberculosis
rRNA  ribosomal Ribonucleic Acid
GOK  Government of Kenya
<table>
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<tr>
<td>NLTP</td>
<td>National Leprosy and TB Program</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<tr>
<td>IDH</td>
<td>Infectious Disease Hospital</td>
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<tr>
<td>FCR</td>
<td>Fragment Crystallisable Region</td>
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<tr>
<td>ROI</td>
<td>Reactive Oxygen Intermediates</td>
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<tr>
<td>TNFα</td>
<td>Tumour Necrotic Factor-Alpha</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>HLA Dr</td>
<td>Human Leucocyte Antigen –DR</td>
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<td>IFN</td>
<td>Interferon –Gamma</td>
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<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
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<tr>
<td>MOH</td>
<td>Ministry of Health</td>
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<td>NLTP</td>
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ABSTRACT

Introduction: Tuberculosis (TB) has probably killed more people than other diseases in history. The number of yearly deaths due to TB has continued to increase and is now over two million with a death occurring every 15 seconds. Pulmonary Tuberculosis (PTB) remains a major public health threat and has been fuelled by the concurrent Human immunodeficiency Virus (HIV) epidemic. HIV infection is known to be a potent risk for reactivation of latent infection and also increases the risk of progression to disease of newly acquired infection.

Objectives: To determine the yield of concentrate smear and culture over the yield of direct smear and to determine the prevalence of *Mycobacterium tuberculosis* (MTB) and Mycobacteria Other Than Tuberculosis (MOTT) in culture positive specimens in HIV positive and negative patients. To determine specific socio demographic factors on positive results.

Study design: A cross-sectional descriptive study that was carried between August and November 2006.

Study population: New patients attending Mbagathi District Hospital chest clinic with history of cough over three weeks. A sample of 150 adult patients was drawn for the study by systematic random sampling selecting every third patient who fulfilled the selection criteria.

Methodology: Three smears for direct and concentrate were made from each sputum specimen, stained with Ziel Neelsen and examined microscopically. All concentrate specimens were cultured on LJ and MGIT 960 system and growth identified as MTB or MOTT based on colonial morphology and biochemical tests.

Data analyses: Descriptive statistics were generated from Statistical Package for Social Sciences (SPSS) version 12.0 and Microsoft Excel (MS excel).

Results: Of the 150 patients included in the study, direct microscopy was positive in 53 representing 35.3%. Smears were positive in 43 (47.8%) HIV+ patients and in 10 (16.7%) HIV- patients.

In the concentrate smear microscopy technique, a yield of 79 (52.7%) representing an incremental yield of 26 (17.4%). Among the HIV+ patients smears were positive in 63 (70%) and 16 (26.7%) was obtained from HIV- patients.
Concentration of the sputum samples increased the sensitivity from 62% to 87%. In the culture method a yield of 80% (56.7%) of culture yielded growth representing 32 (21.4%) compared to direct microscopy and 6 (4%) concentrated smear microscopy. The culture contamination rate was 3.3%. Seventy five percent of the culture positive cases were from HIV+ and 40% were from HIV- (p value of <0.0001).

Of the 85 Mycobacteria isolated, 78 (91.8%) were MTB and 7 (8.2%) were MOTT. Among the TB/HIV co-infected patients, 58 (90%) isolates were MTB and 6 (10%) were MOTT. While among the HIV- patients 20 (95.2%) isolates were MTB and 1 (4.8%) was MOTT.

The specific socio demographic characteristics of the study population had a major impact on the infection of tuberculosis. Age group most infected was 23-37 years, males (55%) and married patients had a high rate of infection.

**Conclusion:** The concentration method substantially increased the yield and sensitivity of direct microscopy. LJ culture medium had a considerable advantage for diagnosis, identification and antimicrobial sensitivity, but its relatively long time for isolation makes it a less recommendable option for widespread use in routine district laboratories.
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CHAPTER 1

1.0 INTRODUCTION

1.1 Background information.

The organism that causes Tuberculosis (TB) known as *Mycobacterium tuberculosis* (MTB) was discovered by Dr Robert Koch on March 24th 1882 when he announced in a scientific conference to the world. TB is an infectious disease that spreads from person to person through generation of infected aerosols. At this time TB was being referred to as the "white death" ravaging Europe and North American continents long just before the appearance of settlers and explorers. Skeletal changes have been seen in Egyptian mummies consistent with typical TB infection. This led to telling chronicles of the medical profession at its worst and best. About 60 years later a scientist by the name Dr Salman Waksman discovered the aminoglycoside antibiotic that is effective for the treatment of TB to date.

With the development of chemotherapy, TB appeared to be confined to the annals of medical history. This was however never to be the case because this ancient scourge took a dramatic turn in the late eighties and by 1993 it had emerged to be one of the global health threat having taken a great magnitude that the World Health Organization (WHO) declared TB a "global emergency".

Currently it is estimated that a third of the world population is infected, 8 million are new cases and 3 million deaths occur every year worldwide. The number of TB cases increased by 1.8% annually between 1997 and 2000. During this period the cases in Russia increased by 9% yearly. However in the industrialised countries of western Europe and America, the incidence was much lower and the greatest number of cases was noted among immigrants from high prevalence countries and marginalized populations such as intravenous drug users, Human Immunodeficiency Virus/Acquired Immunodeficiency Disease Syndrome.
(HIV/AIDS) cases, prisoners and aboriginal people. Most of the burden of TB falls on developing countries witnessing a sharp rise accounting for 95% of all TB cases and 98% of all deaths. This may be due to slackening of TB case finding efforts and also high prevalence of HIV infection.

Reider et al reported that, in developing countries, the TB epidemic is large in number having been an opportunistic infection in HIV epidemic. In addition it is estimated that one third of TB cases between 1985 and 1993 would not have occurred in sub Saharan Africa if pre 1985 trends had continued. A cohort study done in Zaire showed that HIV positive women had a 26-fold increased risk of developing TB compared with sero-negative women.

Catherine et al reported that 22 countries account for 80% of all the new cases half being concentrated in countries including Bangladesh, China, India, Indonesia and Nigeria with Kenya being the twelfth country. Individuals with HIV infection are especially vulnerable to TB and may be the first infection that causes a patient with HIV to seek health care. Since TB has a long period of latent infection, those new infections are added to the pool of the existing infections, which represent a source for potential TB cases in the years and decades to come. One strategy for global TB control would be to implement an effective screening program in high-risk population that would identify individuals with latent TB infections and treat by employing TB control strategy known as Direct Observed Treatment Short course (DOTS).

Report from the Ministry of Health (MOH), 2005 showed that for the last 5 years (2000-2005) there was an average annual increase of 11% in the country with a range of 6% in coast province to 15% in North rift valley province. The report also indicated the age group with highest TB notification remained at 25-34 years in both males and females, as has been the trend over the last decade. This is the same age category with a high HIV prevalence, are both sexually and economically active in the society. The only survey done in 1994 showed that
about 40% of registered TB cases were HIV sero positive. TB is therefore one of the top priorities in Kenya having been ranked the 6th leading in patient mortality in 1996 after HIV.

The WHO has a goal of detecting 70% of all TB cases and effectively treating 85% of detected cases by the year 2015. The position in Kenya is that, detections remain under 30% on coverage far below WHO goals and consequently is now in the high priority group of disease in the new development plan for the MOH 2005-2010.

The other contributing factors leading to TB infection are worsening socio-economic conditions and increasing urbanisation with a deteriorating urban infrastructure, poverty, poor program management, (inadequate case detection), and population increase.

The Government of Kenya (GOK) launched the National Leprosy and Tuberculosis Program (NLTP) in 1980 combining existing TB control activities which had been in place since 1956, with several leprosy control projects in western Kenya, coast, eastern province initiated since early seventies into one programme. Activities of the NLTP ranged from diagnosis treatment supervision and logistic support. The program has been providing diagnostic tools like microscopes, laboratory reports for sputum analysis and training of laboratory technicians besides stationery, drugs transport and training of health workers.

In 2006 MOH/NGO and some private institutions delivered services through 1800 health units that were managed. Smear microscopy services were available at 777 of these health units.

The laboratory is an essential component of the diagnosis, treatment promotion and control of TB. Delays in the laboratory confirmation of TB can lead to delays in initiation of therapy, prolonged infectiousness, and inappropriate therapy or missed opportunities to prevent transmission. Sputum microscopy is the most efficient way of identifying sources of TB infection, diagnosis of TB in persons.
with suspected pulmonary TB and in monitoring of treatment. This method is reproducible, has a short turn around time and is one of the cornerstones of WHO. Mycobacterium culture is useful in definitive confirmation of tuberculosis, hence more definition of cure, failure and relapse.

In developed and developing countries, the presumptive diagnosis of the infectious cases of TB is based on the identification of acid fast bacilli in sputum specimen from suspected cases and the sensitivity this of this technique ranged from 25 to 65%. The major limitation of direct smear microscopy are its low sensitivity and at best it can only detect 20 to 70% of the TB cases.

To achieve the Millennium Development Goal (MDG), a recommendation that was done by WHO recognized the use of centrifugation for concentration of bacilli. A mean of 18% increase in sensitivity was noted in 14 studies. These studies have evidence that concentrate microscopy technique is worth being adopted in the TB control program because of its increased sensitivity. Many cases that were not captured in direct smear microscopy can be diagnosed using concentrate microscopy and treated promptly.

The gold standard for TB diagnosis is the demonstration of MTB on culture. Culture technique on solid media e.g. Loweinstein Jensen (LJ) is more sensitive than smear microscopy. Pacelaceous et al recovered 52.5% from smear positive and 66.4% in LJ medium, Elizabeth et al obtained 34.7% from culture and 27.1% in microscopy among other studies.

Other methods of isolation include the enriched liquid culture such as BACTEC 460 method and Mycobacterium Growth Indicator Tube (MGIT) that are now widely used to provide more rapid results. In addition the use of nucleic acid amplification is most promising.

Garg et al reported that, the diagnosis has continued to pose serious problems, mainly because of difficulties in differentiation of patients with active TB and those with healed lesions; BCG vaccinated and unvaccinated manteux positives.
Laboratories still use conventional methods such as Ziel Neelsen (Z-N) staining, fluorochrome stain, sputum culture and conventional identification techniques. Although tuberculin test has aided in the diagnosis for more than 85 years, its interpretation is difficult because sensitization with non-tuberculous Mycobacterium leads to false positive test. Furthermore, it has been difficult to identify a suitable antigen because MTB shares a large number of antigens with other saprophytic mycobacteria. With the advent of molecular biology technology, there have been significant advances in nucleic acid-based amplification and hybridization, which are used to rectify existing flaws in diagnosis of TB.

Mbagathi District Hospital has been in existence since 1956 and was formally known as Infectious Disease Hospital (IDH). The hospital that has now been converted to general, eye wards, HIV support center and paediatric services, used to serve as a TB treatment center and patients used to be transferred from all over the country. Besides all this changes, the hospital has maintained its TB clinic and patients suspected to be suffering from TB are sent to the laboratory for diagnosis. Direct microscopy of unconcentrated sputum is employed in the laboratory. Positive cases are given treatment and followed up. HIV counselling and screening of patients is also offered.
CHAPTER 2

2.0 LITERATURE REVIEW

2.1 History of *Mycobacterium tuberculosis*.

The organism that causes TB is known as *Mycobacterium tuberculosis* and was first discovered by Dr Robert Koch in 24th March 1882. The disease was earlier known as phthisis and consumption, a term that meant to "waste away". During the 17th and 18th centuries, phthisis was reported to cause 20-30% of all deaths in London. The propensity of tuberculosis was known to take away the strength of the patient.

John Runyon's work highlighted by Conner et al, described TB as the captain of all men of death in 17th C. Tuberculosis had a tremendous impact on human societies throughout the ages. More than a century ago tuberculosis was the most common killer in western world claiming those of all ranks.

Collins described humans as the only natural reservoir of the bacterium *Mycobacterium tuberculosis* causing pulmonary tuberculosis, which is contagious and extra pulmonary tuberculosis. Mycobacterium TB complex include *M. tuberculosis*, *M. Bovis* and *M. africanum* which display greater than 95% DNA-DNA homology. However MTB has specific regions in their genome that are deleted in other members of the complex hence contributing to infection. Other human pathogens belonging to the mycobacterium genus of atypical nature include *M. Avium*, which causes a TB like illness that is especially prevalent in AIDS patients. In addition some species cause chronic pulmonary disease examples being, *M. aciatica*, *M. kansasii*, *M. simiae* *M. gordonae* and *M. xenopi* among other species.
2.2 General features and characteristics of *Mycobacterium tuberculosis*

Morphologically Murray et al described the organism as being large, non-motile, non-sporing, slightly curved rod measuring 1-10 μm long and 0.2 – 0.6 μm wide. They occur in singly or in pairs often terming obtuse angles or small bundles of parallel bacilli 26.

The high lipid material in their cell walls in which on staining with hot undiluted carbol fuschin (Ziel Neelsen stain) allows impregnation by the dye which is retained despite attempts to remove it with acid alcohol. The retaining of arylmethane dyes on treatment with mineral acids is a property termed as acid fastness 2.

Auramine –Rhodamine fluorescent stain makes mycobacteria to appear yellow orange against a dark background obtained by counterstaining with potassium permanganate.

On the nutritional requirements and growth, Duguid J.P et al described the organism as a slow grower with a generation time of 18-20 hours on commonly used agar based and egg based media. The organisms usually require media enriched with whole egg and incubated aerobically at 37°C for 10-14 days or as late as 6-8 weeks. A source of carbon from glycerol is added to the medium and in presence of mineral salts to encourage growth of human strains (eugonics). Malachite green is an inhibitory dye added to egg media to prevent growth of contaminants. Discrete colonies are raised, irregular shaped with wrinkled or mamilated surface or have rough tenacious consistency, the colour at first is creamy and later becomes buff 27.

BACTEC radiometric and BACTEC MGIT fluorescence that are automated systems contain growth supplements such as, Middlebrook 7H10 essential for
growth of mycobacteria and a mixture of antimicrobial agents used to suppress the growth of contaminating bacteria.

According to Cook et al, the organism is a strict aerobe, and thrives aerobically at 37°C and grows best at partial oxygen (PO₂) of 140mm Hg, explaining their tendency to cause disease in the sub-apical portion of the lung. Organisms become scarce in necrotic tissue lacking blood borne oxygen and growth is usually inhibited by pH < 6.5 and by long fatty acids.

MTB could survive for several weeks to months in inanimate objects if protected from light and is usually is resistant to acids and alkalis like malachite green, quaternary ammonium compound and hexachlorophene, which have bacteriostatic activity. The bacterium can also survive in 15% sulphuric acid (H₂SO₄) and 3% nitric acid. This is due to its hydrophobic nature of the cell wall surface and their clumped growth in sputum survives for 20-30 hours and 8-10 days in droplets. Culture can be stored for 2 years in a deep freezer at -20°C. However MTB are susceptible to heat at 60°C for 15-20 minutes, ordinary daylight, chemicals such as tincture of iodine in 5 minutes, 80% ethanol in 2-10 minutes, ethylene oxide and formaldehyde vapour.

2.3 Mechanisms of pathogenicity

MTB normally does not have virulence factors such as those described for other bacterial pathogens e.g. toxins, adherence molecules and invasion molecules. However, the structural components and products of the cell wall complex are responsible for the organism's virulence and the ability to survive in harsh environmental conditions.

This cell wall is usually surrounded by atypical gram-positive peptidoglycan, which is attached via disaccharide bridges to a layer of arabinogalactan that in turn is linked to the mycolic acids and mycoserosic acids. The mycolic acids are
of particular importance because they are believed to play a crucial factor in virulence.

The cytoplasmic membrane containing phosphatidylinositol that serves as an anchor for the mannophosphoinositolides and lipoarabinomannan also contains proteins. This phalanx of macromolecules makes the MTB wall complex impermeable.

The Cord factor (trehalose 6,6 dimycolate) an important component of MTB which causes the organism to appear as a rop-like lateral aggregates in microscopy. This factor inhibits fusion of macrophage lysosomes with phagosome hence ability of MTB to survive within macrophages intracellularly. Studies have shown that MTB lacking cord factor are unable to cause disease. The sulfatides abundant in MTB, plays a role in blocking or reversing up the priming of monocytes by lipopolysaccharide or interferon gamma. This causes the monocytes to produce little superoxide radical which may prevent intracellular killing of MTB.

The arabinogalactan and lipoarabinomannan work in concert with cord factor to produce tuberculous granulomas and tissue necrosis and both elicit a strong but ineffective antibody response. The MTB organisms bind directly to mannose receptors of macrophages via the cell wall associated mannosylated glycolipid or indirectly via certain receptors like the complement receptors or Fragment Crystallisable receptor (FCr). They enter the cell and grow intracellularly inhibiting phagosome-lysosome fusion MTB interferes with toxic effects of reactive oxygen intermediates (ROI) by presence of glycolipids, sulfatides and down regulates the oxidative cytotoxic mechanisms.

Macrophage (MΦ) uptake via complement receptors (CIR) may bypass the activation of a respiratory burst. The antigen 85 complex proteins secreted by
MTB are known to bind fibronectin and aid in walling off the bacteria from the immune system and may facilitate dissemination 30.

2.4 Pathogenesis

The infected droplet nuclei are inhaled and taken up by the alveolar macrophages. At this point the infection begins between 7-21 days and is called primary tuberculosis. The large droplets are washed on the upper airways and are cleared by mucociliary blanket. The bacilli in the un-activated alveolar macrophages multiply unrestricted until the macrophages burst and infect other macrophages that extravagate and phagocytose the bacilli. As this cycle is repeated, some organisms migrate to the draining lymphatic vessels, where they multiply intracelularly and cause lymph nodes to swell. The hilar and mediastinal lymph nodes are most commonly affected and lymphadenopathy is common in children. The combination of the tuberculosis primary focus with lymphangitis and lymphadenitis is termed as the primary complex 24.

A simple lesion (ghon focus) is usually found either bilateral or multiple foci are very infrequent and 1.0 – 1.5cm area as consolidation circumscribed from surrounding parenchyma cells2 Patients with cavitary pulmonary or laryngeal TB are most likely to spread MTB if they have a chronic cough, have acid fast bacilli in their sputum, fail to cover the face when they cough, patients shedding many bacteria infect about 29% of their close contact and about 15% of semi-close contacts. Clinical manifestation usually occur late during the disease and include inexplicable weight loss, constant fatigue, temperature may reach 40°C night sweats, shortness of breath and chest pain. Cavitations lead to development of productive cough, bloodstained and frankly purulent sputum 24.
2.5 Epidemiology and transmission

2.5a Epidemiology

Globally, reports indicate that, TB has killed more people among other diseases in history. The number of yearly deaths continues to increase over 2 million people die of TB every year and one death occurs every 15 seconds. There are almost 9 million new cases of TB annually and the third of the world's population is infected with TB Bacillus. TB death burden is not evenly spread, 98% deaths in developing world (USAID), 22 countries account for 80% of all the TB deaths 31.

The HIV pandemic has drastically worsened the TB situation in countries with a high prevalence of TB/HIV co-infection rates varying widely with regions. At the point in time, this is particularly the case in Sub-Saharan African countries. HIV may alter the course of TB directly by leading to reactivation of latent infection or by direct progression from new infection to disease depending upon which comes first.

Swaminathan et al estimated the rate of development of active TB in a cohort of HIV positive patients as 6.9/100 persons-years (p-y) providing information regarding the high risk of developing active TB and its associated mortality in HIV infected 32.

In Kenya the number of reported TB by MOH (2005), cases has increased nine fold from 11,625 cases in 1990 to 108,401 cases in 2005. The average annual increase over the past years is approximately 14% for all forms of TB. However, the annual increase of notified TB cases is slowing down, and the average annual increase over the past 5-year period is 11%. Case notification rates (CNR) increased from 53/100,000 population for all forms of TB and 32/100,000 population for sputum positive TB cases in 1990 to 324/100,000 population and 121/100,000 population respectively in 2005.
The TB case finding as at 2004 showed that 41,167 (39%) were smear positive. The age group with the highest TB notification in 2004 remained 25-34 years in both males and females, as has been the trend over the last decade. The major reason for the increasing burden of TB in Kenya is the concurrent HIV epidemic. The HIV is known to be most potent risk factor for reactivation of latent infection and it also increases the risk of progression to disease of newly acquired TB infection. HIV infected TB patients are also at increased risk of recurrent TB.

The only near country wide survey of HIV prevalence in TB patients was done in 1994 in 17 districts. It showed an HIV sero-prevalence among TB patients of between 11.8% and 79.6%. The average HIV sero prevalence in TB cases obtained from this study was 40.7% for the 17 districts involved. The present status is estimated that over 60% of TB cases are HIV sero positive.

Tuberculosis case finding in Kenya in 2005 report showed that 41,167 (39%) being new smear positive. In Nairobi province, new smear positive were 7586 (39%) and new smear negative were 5617 (34%). In Lang'ata constituency new smear positive were 1495 (28%) and new smear negative 1978 (38%).

The sex distribution of new smear positive PTB in Kenya showed that the females were 17,197 and males were 23,192. In Nairobi province alone the females were 3,097 and males were 7,587.

2.5b Transmission

Redier quoted that; the risk of becoming infected with tubercle bacilli is largely exogenous in nature. Koch suspected that primarily an airborne route transmits TB in his seminal line of reasoning for the proof of tubercle bacilli as the cause of TB. Because he recognised that the majority of cases began in the respiratory tract, he deduced that ".... bacilli are usually inspired with the air..."
Droplets produced by a patient with tuberculosis may contain tubercle bacilli. Tubercle bacilli may still be contained in droplets below the critical size of setting before evaporation. Thus, such a droplet nuclei containing one or more tubercle bacilli that remain buoyant in the ambient air for a long period of time, is the principal source of transmission of MTB. It is known that, talking, coughing, sneezing or singing produces droplets. Loudon and Roberts demonstrated that one cough was equivalent to about 5 minutes of loud talking in terms of the resulting number of droplet nuclei, about half of which were still suspended in air 30 minutes after coughing.

Infectious droplet nuclei, is usually small enough to reach an alveolus in the periphery of the lung while the large particles generally drop more rapidly to the ground or if inhaled, are trapped in the mucociliary system of the tracheobronchial tree, swept up and rendered harmless.

A study of a tuberculosis outbreak on a United States navy ship provides information on the importance of inhalation, air circulation and ventilation. On this ship, 138 of 308 persons previously known to be tuberculosis skin test negative ultimately became infected from a single source. Men sharing the same compartment with the cases had the highest frequency of skin test conversion. Nevertheless a very high proportion of those living in other compartments and of those who did not work or significantly socialize with the index case, but whose compartments were connected through a close circuit ventilation system, also become infected. Conversion continued for a prolonged period of time after removal of the infectious source case, suggesting that air in closed circuits may contain viable infectious droplet nuclei for a prolonged period of time.

2.6 TB and HIV co-infection

According to Konman et al, the HIV pandemic that has not been largely controlled in many parts of the world has contributed significantly to the spread of TB. These two diseases are synergistic at every level from the molecular to the
epidemiological aspects. The presence of HIV is known to promote the intracellular replication of tubercle bacilli hence promoting transmission. TB cases in adults are attributable to HIV infection. In 1997 approximately 11 million people have been thought to be co-infected and the strongest association is recorded in sub-Saharan Africa. By the end of 2003, countries in Sub Sahara were a home to 67% of all adults and children living with HIV/AIDS. 65% all new HIV and 77% of all HIV deaths occur in this countries. The range rates among adults and children are from 20-70% and there are some countries such as Malawi, Zambia where HIV sero-prevalence rates are constantly above 50%. It has been shown that in sub-Saharan a significant number of new cases of TB and recurrent cases of TB result from recent transmission attributed to HIV pandemic.

A study done in Kinshasa showed that women had a 26-fold increased risk of developing TB compared with sero negative women after a follow-up of 32 months.

In New York incidence of TB in HIV positive drug users was 24 times higher than HIV negative individuals. HIV positive patients have a high rate of sputum negative for AFB because their immunity is compromised and hence no productive cough and quality specimen is received.

Molecular DNA has shown that, in almost 2/3 of HIV infected persons; TB is due to recent infection rather than reactivation of the latent infection. Co-infection tends to accelerate the progression of the disease caused by HIV infection. If TB occurs in the early stages of HIV infection when immunity is only partially compromised, the features will be a characteristic of those that occur in first primary TB in HIV negative persons. About 40% of registered TB patients' cases were HIV sero-positive in a survey done in 1994. A recent experience from sites that have been piloting routine test of TB for HIV suggested that a natural average prevalence may be over 60%.
2.7 Diagnosis

Although tuberculin test has aided in the diagnosis of TB for more than 85 years its interpretation is difficult because sensitisation to environmental mycobacteria leads to false positive test. False negative also occur in severe TB and AIDS. Upto 10% to 20% of patients with early stages of active TB may have a negative tuberculin test \(^2\). Chest x-ray is another important diagnostic technique for PTB. It is reported that chest x-ray cannot confirm that a person has TB. A variety of illnesses may produce abnormalities whose appearance on a chest x-ray resembles TB. In patients who are infected with HIV, PTB may have an unusual appearance of chest x-ray or may appear entirely normal.

Reliability of chest x-ray showed 70% and AFB microscopy 98% hence unreliable for diagnosis and monitoring treatment of TB. On specificity X-ray was 50% and AFB microscopy 98%. Tomans showed that X-ray does not diagnose 10% to 15% of culture positive TB patients and that 40% of patients diagnosed to have TB on the basis of that alone do not have active disease \(^3\).

2.7.1 Introduction to the Laboratory Diagnosis

It has been shown that, the early diagnosis of TB is critical in the appropriate management of cases, lowering the medical cost of treatment and preventing its spread \(^3\). In developed as well as developing countries the presumptive diagnosis of infectious cases of TB based on the identification of Acid Fast Bacilli in sputum (AFB) specimen \(^9\). However the diagnosis of TB continuous to pose serous problems, mainly because of difficulties in differentiation of patients with active TB and those with healed lesions in x-ray, normal mycobacterium bovis (BCG) vaccinated individuals and mantoux skin test positive.

Laboratories still use conventional methods such as ZN staining, fluorochrome stain, sputum culture and other non-traditional methods. A number of proteinous and non-proteins have been explored from time to time. It has been difficult to develop an Enzyme Linked Immunosorbent Assay (ELISA) suitable antigen
because MTB shares a large number of antigenic proteins with other microorganisms advent of molecular biology technology has been significant advances in Nucleic Acid-based Amplification (NASBA) and hybridization, which are used to rectify existing flaws in the diagnosis of TB.

2.7.2 Laboratory methods

(a) Direct microscopy

This is used for a rapid diagnosis of TB since most patients have a high bacterial load and are more likely to spread TB. Results of direct microscopy are available within 24 hours. It is important to detect the presence of Acid Fast Bacilli (AFB) as rapidly as possible for implementation of appropriate patient care and public health measures. Moreover, direct microscopy on successive specimen can also be used to monitor the success of chemotherapy for smear positive patients.

(i) Direct microscopy from unconcentrated sputum

Those parts of the world where pulmonary tuberculosis is most prevalent, the standard diagnostic test recommended is ZN stains of three (spot,morning,spot) unconcentrated sputum to detect AFB. This method is economical and has proven a reproducible record and is one of the cornerstones of World Health Organization (WHO) TB control strategy. However this is not the case anymore because of HIV infection leading to less or unproductive cough.

The suspected part of the sputum specimen is usually selected especially the muco-purulent, purulent and blood stained. The smear (2x1 cm) of uniform thickness is prepared on a clean slide using broken ends applicator stick or wire loop. The smear is then air dried, fixed and stained with ZN stain. Upto 300 oil immersion fields are examined. If 5000 bacilli / ml are present the entire smear will have 50 bacilli. Less than 5000 bacilli per ml cannot be identified in direct microscopy. After examination the grading for the number of observed was recorded according to the International Union Against TB and Lung Disease (IUATLD) recommendation.
Direct microscopy using acid-fast stains is a relatively insensitive diagnostic procedure with reported sensitivity ranging from 25% to 65% when compared to culture that is the gold standard. Though this method has been the cornerstone for WHO for years, factors that relate to the HIV/TB pandemic including large number of individuals being screened and poor quality sputum specimens further make diagnosis by direct unconcentrated smear microscopy inadequate\textsuperscript{10}

A study done in Peru by Tomans demonstrated that, 210,905 smear examinations carried out in 1990 lead to identification of 24,023, representing only 11.4% of PTB cases. The proportion of positive smears is an indirect indicator of the impact of the NLT Program in reducing the prevalence of TB and HIV impact leading to many smear negative cases\textsuperscript{34}

Cumulative results for initial 3 specimens from 2693 patients detected 34% of MTB, while L. Apers et al demonstrated that the direct microscopy gave 67.5% positivity out of a total of 234 specimens investigated\textsuperscript{11,36}. This two studies are considered to be of low sensitivity according to W.Githui's reviews ranging from 25-65%.

A yield of 20.2 % was obtained when 5776 samples were subjected to direct smear microscopy done by Dhingra et al\textsuperscript{13}. This was far much lower than those reviewed by Githui suggesting that much more cases were missed. Another low yield value was observed by Ukwandu et al giving a 15% positive cases out of 160 samples processed\textsuperscript{12}.

More studies done by Farnia et al at a set up in Iran with a sensitivity of direct microscopy of 25-50% managed to obtain a 9.5% yield among 430 patients screened in this set up\textsuperscript{18}. This shows that in as much as the sensitivity levels in some countries have been determined, the results obtained at different times and the population set up is not generalizable. This may be partly contributed by poor quality of specimens, poorly trained personnel and HIV epidemic leading to smear negative cases.
L. Lawson et al demonstrated that in Sub Saharan Africa the number of patients presenting with scanty bacilli has increased to 98%. The major contributing factor is due to the high incidence of HIV in the region. They obtained a yield of 29.9% from a total of 3204 patients who participated in the study. Yahya et al working on patients from a rural setting in Tanzania did another striking observation. They noted that out of 61, 85 suspected TB cases analysed 18.9% had positive direct smear microscopy. It may be thought that rural areas have a low prevalence of TB in rural areas compared to urban centers having so many socio economic factors that can enhance the spread of TB.

A study done in Zambia realized 43.3% of PTB cases using the direct microscopy. Elizabeth et al observed that out of 844 samples analysed only 27.1% of the PTB cases were positively identified.

The overall observation is that direct smear microscopy has been quicker and easy to perform but has a very poor sensitivity despite the fact that it still remains the cornerstone of WHO TB control strategies for case finding and monitoring of cases that have been diagnosed and are already on short course chemotherapy through DOTS.

The direct microscopy can lead to false positive results due contaminants like food particles, precipitated stains, environmental acid-fast bacilli from one smear to another. The implication of this is that the patients are started on anti-TB drugs, delay in correct diagnosis, emotional stress and financial loss. Direct microscopy can also lead to false negative results mainly due to poor quality sputum, failure to select suitable sample sites, improper examination and storage conditions in case of delay in processing, transportation and temperature conditions. The implication to the patients is misdiagnosis and wrong treatment thus infectious agent to the community.
(ii) Microscopy from concentrated sputum

The sputum must be digested and decontaminated before preparing smears and also culture. Mucin in sputum bind Mycobacteria and the goal are to eliminate normal flora and as little harm as possible to the somewhat harder Mycobacteria. Four Sodium hydroxide (NaOH) is both mucolytic and decontaminating agent. A relative centrifugal force of 3000g for time limits of 15 minutes, sediment the tubercle bacilli.

David et al work highlighted by Tomans, determined the probability of not finding any AFB in a smear. For various concentration of bacilli he examined, 41 specimens and found that the concentration of bacilli increased from 15,000 bacilli to 300,000 bacilli.

The detection of AFB on stained smears varies widely, with sensitivities varying from 22%- 78%.

A recommendation that was done by WHO in September 2005 recognized the use of centrifugation for concentration of specimen by use of various chemical methods in 14 studies. All this studies showed that there was an 18% mean increase in sensitivity ranging from –3% to + 39%. The degree of sensitivity depends on factors such as specimen type, number of specimens examined, observer experience and number of AFB Present.

L. Apers showed that out of a total of 234 specimens (204) a yield of 87 1% was obtained in concentrated method were positive compared to the (158) 67.5% in direct microscopy of unconcentrated smear that were positive This gave an incremental yield of 16% having been missed in direct microscopy. T. Foder et al showed that centrifugation of 32 cases yielded 26 representing 81.3% cases of positive results obtained than the conventional direct method. The difference in number of the bacilli was 100-200 folds in 5 cases and 10 fold in 12 paired cases. This technique was suitable in making culture and smear from the same sediment.
Ellen et al demonstrated that smear made from concentrate smear of 2693 specimen detected MTB in 58% of the suspected PTB patients compared to 34% from direct smear microscopy. This study obtained an incremental yield of 24% meaning that any cases were captured and treated hence alleviating the public health problem. Bruchfeld et al in their study stratified results according to the HIV status of the patients and noted that among the HIV positive patients, the concentration method increased the sensitivity of sputum microscopy from 38.6% to 50% giving an incremental yield of 11.4%. This was compared to the overall sensitivity which increased from 54.2% to 63.1%.

Ukwandu et al demonstrated the same method and obtained an incremental yield of 10.6% the yield having increased from 15% to 25.5% from 160 samples analysed. Farnia et al on the other hand captured an incremental yield of 6.4%, the yield having increased from 9.5% to 16% among 430 specimens analysed. In Zambia a similar study had an incremental yield of 32.9%, the yield having increased from 43.4% to 76.3%.

As an alternative to centrifugation, Miorner et al described sedimentation that gave the same results in terms of increased sensitivity although more time consuming because of employing different times. Other methods have also been described like the use of Chitin and Hypochlorite.

The concentration method results in less debris and greater concentration of AFB. Various studies have indicated that the sensitivity is high and can be employed in TB control programs in HIV high prevalent countries because HIV infected patients have lower frequencies of positive sputum smears. This method agrees with the WHO recommendation of incremental levels. Many of the cases that were not captured in direct unconcentrated microscopy can be diagnosed, treated and monitored promptly.

However the disadvantage in this method is the slight increase in time for investigations and possible increased risk in contamination for the Laboratory.
personnel doing the centrifugation. Nevertheless safety cabinets have to be put in place to reduce this risk and personal safety has to be observed. Concentration technique can also lead to killing of the bacilli because of the harsh chemical treatment thus missing out these cases during culture.

(b) Culture
The culture allows confirmation of cases and also facilitates species identification. With this method very low numbers of Mycobacteria can be detected and susceptibility assays performed. Culture methods are not normally available within most diagnostic facilities except in Central Reference Laboratories and Research Institutions. In our set up we have one public Central Reference Laboratory serving the whole country for culture and sensitivity of specimens only from who have been on treatment. However there are also some few private institutions performing culture.

(i) Conventional Methods
The Egg based media such as Lowenstein Jensen (LJ), agar base media including Middlebrook (7H10/7H11) and liquid media such as Kirchner and Dubois broth is commonly used. Because of the slow growth of the tubercle bacilli (generation time 18-24 hours), specimens like sputum should be digested and decontaminated to kill rapidly, dividing organisms including bacteria, fungi and other environmental Mycobacteria.

L-J medium base is a relatively simple medium that requires supplementation in order to support the growth of Mycobacteria. Rapid growers have colonies within 7 days and slow growers require more than 7 days for mature colony forms. Studies done by Rivera A.B et al showed that in all known stock and reference cultures of Mycobacteria, there was an overall growth of 89.3% on LJ. Both species grew taking a tie length of 29.9 days. Study done by Ukwandu et al demonstrated an incremental yield of 13.2% having increased the yield from 25.6% in concentrate microscopy to 38.8%.
Pacelaceous et al noted that out of 5208 samples analysed a yield of 66.4% was obtained from L.J in a mean time of 16.5 days compared to 52.5% from microscopy. This gave a yield of 13.9%. Also Elizabeth et al recovered a yield of 34.7% in L.J medium from 844 samples with a mean recovery time of 34.7 days compared with 27.1% obtained from smear microscopy giving a yield of 7.8%.

A prospective study of TB patients in Lusaka by Namambo et al reported that, 109 had PTB proven by sputum culture 72 were HIV-1 positive and 37 were HIV negative. 43% of culture proven HIV positive patients had negative sputum smears compared to 24% of HIV negative cases. There was a strong trend towards lower grade or negative sputum smear in HIV positive group.

Dongsi et al showed that there was a sensitivity of 59.7% in the isolation of Mycobacteria. Other associates obtained a sensitivity of 45.5% and 46.6% respectively in comparison with other techniques Harris et al compared the culture techniques and found out that the isolation rates for L.J medium was 78% from 681 clinical samples with a mean time of 19 days and a contamination rate of 4.1%.

Garcia et al isolated a total of 93 mycobacteria in culture media. Out of this, 66 representing 70% was isolated from L.J with a mean time 22.7 days. Akos et al studied and noted that the recovery rate of L.J medium was 81.8% with a mean time of 20.1 days and a contamination rate of 1.2% compared to other automated system that had a high yield. Other studies done by V.Mirovic and co workers when they evaluated 251 isolates of mycobacteria noted that 67.3% was isolated from L.J medium with a mean time of 22.1 days and a contamination rate of 7.7%.

Despite the fact that culture by L-J medium is the "gold standard" for isolation and identification of Mycobacteria, it is evident that the growth rate is slow and
delay of results is inevitable. The harsh treatment of sputum samples can lead to killing of mycobacteria and so especially the non-TB species that causes TB like infections. Also LJ medium has been basically designed for the isolation of MTB so other Mycobacteria may be missed 45. Finally contamination rate may be high in some set-up because various treatment processes involved but a 2-5% level of contamination that should be expected in a set-up with good laboratory practice.

(c) Other methods of laboratory diagnosis

Fluorescent microscopy that uses illumination from quartz -halogen lamp. Low magnification objective is used to scan smears allowing a much larger area of the smear to be seen resulting in more rapid examination 45 Fluorescent microscopy can scan a sputum smear at 250x magnification rather than 1000x magnification done by light microscope. Tomans demonstrated that fluorescence microscopy yielded a sensitivity of 88% compared to 65% of light microscopy. High cost of investment in fluorescent dyes (Rhodamine – Auramine), maintenance and care makes fluorescent microscopy makes it unavailable 34.

New culture methods include radiometric semi-automated BATEC – 460 TB systems based on the release of radiolabeled CO₂ in liquid cultures as an indicator for cell metabolism and growth. Somoskovi demonstrated the use BACTEC Mycobacterium Growth Indicator Tube (BATEC MGIT) 960 system BD. A fluorescent compound is embedded in silicone on the bottom of each of the MGIT broth tubes is sensitive to presence of oxygen dissolved in broth initially, the large amount of dissolved oxygen quenches the emission from the compound and little fluorescence can be detected. Later, respiring microorganisms censure the oxygen and allow fluorescence to be Detected 46.

A study comparing MGIT and LJ as a gold standard done by Rivera et al showed that both increased to recovery rate from 109 (63.4%) to 122 70.9% of
172 clinical specimen. Of these isolated, the yield in MGIT (99.2%) exceeded that in LJ (89.3%). The average days for detection of MTB in MGIT were done by 14.2 days. MGIT is an excellent system for the rapid isolation of Mycobacteria. It increases the recovery rate of MTB when combined with LJ mainly helps in the identification of Mycobacteria species. MGIT is known to have a high rate of contamination because any growth of respiring organism indicates a positive test.

Study done by L. Apers showed that there was high contamination rate of the MGIT. 14.7% of all specimens gave positive signal, although microscopic examination of cultures could not confirm the presence of MTB. Harris, Akos, V.Mirovic and et als noted that MGIT 960 system had 94%, 96.4% and 93.2% recovery rates of Mycobacteria. The mean times were 10.7, 12.6 and 13.8 days. The contamination rate was 5.5%, 3.7% and 8.1% respectively.

MGIT techniques are much more expensive method and make it less recommendable option for widespread use in routine district laboratory. Also the contamination rate in higher than the LJ medium but the rapid turn around time is good.

Considerable efforts have been invested in the use Ligase Chain Reaction (LCR) and Polymerase Chain Reaction (PCR). These techniques specifically amplify minute quantities of nucleic acid targeting either DNA or RNA. PCR amplification involves extraction of DNA from clinical specimens. Target DNA is incubated in a buffer that usually consists of magnesium chloride, primers designed to bind specifically to the target sequence of the DNA, Taq polymerase enzymes and nucleotide. Commercial kits include the gene probe Amplified Mycobacterium, TB Direct test AMTD, COBAS AMPLICOR PCR, Ligase chain reaction and LIPA Mycobacteria.

Sensitivity for PCR is 91%, culture 68% and smear 51%. In one study in Kenya reported by Jenifer et al indicate that sensitivity of PCR based diagnosis was
found to be 93% while specificity was 84%. Despite, this PCR does not have a suitable gold standard to evaluate the results. The cost and required technical skills make these technologies largely unavailable to many institutions hence a limitation for routine application of these techniques in most developing countries.

Several studies based on the detection of antibodies to a variety of Mycobacterial antigens or bacteria derived antigens have been carried out but none has proved reliable so far. Lack of sensitivity and specificity are the main problems encountered. Cytokine based assays to determine the relationship between tuberculin skin test response and cytokine profile has shown encouraging results, allowing epidemiological studies on the immunity of TB in humans. ICT test reported by Nisar Khan showed that the test is highly specific and less sensitive for diagnosis of TB. He detected 44% of smear positive and 36% of smear negative.

2.8 A typical Mycobacteria

The genus Mycobacteria contains around 100 named species usually encountered as environmental saprophytes. They occur commonly in watery environments such as marshes, ponds and lakes and rivies. They also colonize industrial and domestic water pipes. Human contacts by drinking water, inhalation of aerosols or inoculation into wound are common and regular event. Some MOTT can cause human disease especially in the immunosupressed cases. MOTT being common can contaminate containers and equipment and used to collect clinical specimens causing a Pseudo-epidemic of the disease. MOTT occurs as transient commensals on the skin, external genitalia, and the pharynx and gastro intestinal tract. Care is required in determining whether MOTT isolated from clinical specimen, especially sputum and urine is primary pathogens rather than contaminants, colonizers or commensals.

Runyon classified MOTT according to their ability to synthesise yellow or orange pigment.
**Photochromogens** – pigments are produce after exposure to light e.g. *M. Kansasii* which produce initially lemon to yellow and gradually turn orange or reddish orange owing to accumulation of beta carotene crystals. This causes chronic pulmonary disease and stains unevenly with ZN, giving a barred or beaded appearance.

**Scotochromogens** – pigments produced in the dark e.g. *M. scrofulaceum* – colonies are light yellow to deep orange and grow slowly taking 4-6 weeks producing smooth buttery colonies *M. gordonae* – yellow orange known as tap water scotochromogen.

**Non-photogromogen** – this produces no pigments e.g. *M. avium* - colonies are smooth and transparent.

**Rapid growers** – this produces any of the pigment types e.g. *M. chelonei, M. fortuitum, M. vaccae* Species producing visible growth in egg based media with 1 week on subculture is regarded as rapid growers. Paradoxically some rapid growers grow very slowly.

### 2.9 Species identification

The re-emergence of TB due to MTB and some non- MTB, species identification has now become more significant than it was before. Since the procedures used for the isolation of Mycobacteria are not usually species specific a number of techniques for identifying Mycobacteria at species level has been established.

Conventional methods include colonial morphology on solid media providing preliminary identification of different Mycobacteria species. On LJ media colonies of MTB appear rough and dry with buff colour. Growth/culture this include rate of growth preference for aerobic environment, pigment production. Temperature range where majority of Mycobacteria grow is at 37°C. Some species show optimal growth at different temperature ranges members of MTB complex best grew at 37°C, do not grow at 25°C and at 42°C, do not produce pigment. Classical identification procedures require upto 3 weeks following culture before the results are available.
Biochemical test Characteristics that include Niacin accumulation test, Nitrate reduction test, Tween 80 hydrolysis, Arylsulfatase activity and Catalase activity at 68°C among others.

Brunello et al published recovery rates of mycobacteria species to range from 53.6% to 95.9%. However the recovery of MOTT on LJ media is lower with a recovery rate of 24.6% because it is not the best media for isolation of MOTT\textsuperscript{45}. Akos et al isolated 96.5% of MTB and 3.5% of MOTT from 377 clinical specimens analysed while V.Morovic et al demonstrated a recovery of 91.2% of MTB and 8.8% of MOTT in LJ in the clinical specimens\textsuperscript{43,44}.

A retrospective analysis done by Corless et al on Mycobacterial cultures and contact tracing performed in every patient with smear positive sputum showed that, 116 patients with smear positive were identified. MTB was cultured in 57 (49%), environmental or MOTT in 37 (32%) and cultures were negative in 22 (19%) of the patients. This study done in Liverpool showed that both MTB and MOTT was a public health problem\textsuperscript{46}.

Rapid identification methods include, High performance liquid chromatography (HPLC) identifies Mycobacteria by the mycolic acid content of their walls. Gas liquid chromatography (GLC) of shorter chain fatty acid shorter and mycolic acids provide identification of MTB within a shorter time than conventional culture. Radiometric BACTEC p-nitro-α-acetamino-β-hydroxyprophenone (NAP) test evaluates specific inhibition of growth of MTB complex by NAP. However, the results obtained are considered preliminary since false positives are observed\textsuperscript{48}.

Molecular Based methods are based on specific nucleotide sequence of the hyper variable region of the MTB gene. An oligonucleotide probe allows the identification of the MTB complex. Gene targets have also been used in broad range of consensus PCR assays identification. The use of such systems
however is largely restricted to major reference centres in the industrially developed nations or at major reference centres in the tropical countries
Pulmonary TB is the most common form of TB. It is estimated that over 80% of all TB occur in the lungs, thus is a contagious infectious disease transmitted through generated aerosols either by singing, coughing, talking or sneezing. Globally TB has killed more people among other disease in history. The number of deaths per year has continued to increase and as at now over 2 million people die every 15 seconds. PTB remains a public health threat and increasing burden is due to concurrent HIV epidemic HIV is known to be the most potent risk for reactivation of latent infection and also progression to disease of newly acquired infection.

In Kenya, TB epidemic is being generally experienced affecting the economically reproductive groups (15-44 year old). The males are 1.4 times more likely to have TB than females. It is currently estimated that over 60% of TB patients are co infected. Although Kenya has a high TB burden, the cases notified might as well represent less than half of the incident cases that occur each year.

Direct smear technique is commonly employed in all the public health institution and most private institutions in order to detect AFB in a sample. It has been established that laboratory diagnosis of PTB with direct microscopy is characterized by low sensitivity (25%-65%). HIV infected patients have very scanty bacilli or no bacilli in their unconcentrated smear. This leads to missing of very many positive cases thus becoming a public health threat especially so to the vulnerable groups. There is need to compare the existing use of direct smear technique to concentrate smear technique and culture in order to evaluate the yield of MTB of each technique in finding the cases of PTB. Culture performed also will enable us identify the growth of Mycobacteria as MTB or MOTT because the mode of treatment is somewhat different. Culture is also usually taken as the "gold standard" of TB diagnosis and identification.

2.10 Statement of the problem

Pulmonary TB is the most common form of TB. It is estimated that over 80% of all TB occur in the lungs, thus is a contagious infectious disease transmitted through generated aerosols either by singing, coughing, talking or sneezing. Globally TB has killed more people among other disease in history. The number of deaths per year has continued to increase and as at now over 2 million people die every 15 seconds. PTB remains a public health threat and increasing burden is due to concurrent HIV epidemic HIV is known to be the most potent risk for reactivation of latent infection and also progression to disease of newly acquired infection.

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2.11 Objective of the study

(a) The broad objective of the study were;
To compare the yield of diagnosing PTB by use of concentrate smear microscopy and culture over the direct smear microscopy among HIV positive and negative new patients attending Mbagathi district hospital chest clinic as well as to identify the isolates.
To determine the association between the specific socio-demographic characteristics on smear and culture results.

(b) To achieve this objective, the study was guided by the following objectives

1. To determine the incremental yield of concentrated smears for the detection of AFB in HIV positive and negative patients.
2. To determine the yield of culture technique for the detection of MTB in HIV positive and negative patients.
3. To determine the percentage of smear and culture positive results according to age, gender and marital status of the study population.
4. To determine the percentage of smear and culture positive results according to size of the house, ventilation and number of occupants of the study population.
5. To determine the prevalence of MTB and MOTT in HIV positive and negative patients

2.12 Research Questions

1. Is there an incremental yield obtained by concentrating sputum for microscopy and culture over the usual direct microscopy method in the diagnosis of PTB among HIV positive and negative patients?
2. Is there a significant difference in the diagnosis of PTB to age, gender, marital status size of the house, ventilation and number of occupants of the study population?
3 What is the probability of isolating MTB compared to MOTT from culture positive specimens in the diagnosis of PTB among HIV positive and negative patients?

4 Is there a significant difference in the outcome of PTB diagnosis between direct, concentrate and culture technique in the diagnosis of PTB among HIV positive and negative patients?
2.13 Justification of the study

Pulmonary Tuberculosis is a highly prevalent infection in Sub Saharan Africa, resulting in significant morbidity and mortality occurring with the associated diseases and socio-economic factors. More infections in the population continue to explode due to missed diagnosis as a result of employing techniques with low sensitivity. This situation has lead to strain on the medical, social and economic process of the country. The financial burden of managing the disease far outweighs that of eradication and meeting the Millennium Development Goal by 2015.

Concerns to be addressed include laboratory diagnostic screening for active and latent cases for all vulnerable populations. One strategy for global TB control would be to implement an effective screening program in high-risk population that would identify individuals with latent TB infections and treat them to prevent the disease.

Ideal screening test for diagnosis of TB should have high sensitivity, which is high in all population at risk. The test specificity should be high regardless of BCG vaccination or infection with environmental Mycobacteria. Should also be reliable, stable over time and have objective criteria for a positive result. Test should be inexpensive and easy to administer.

This study is important because the main target for Millennium Development Goal (MDG) are by 2005 to detect 70% of new smear positive cases and successfully treat 85% of this cases by 2015.

To the government, the study will reduce financial costs of treating many new case that were exposed to undiagnosed cases by implementation of better diagnostic techniques.

To the community, all infectious cases will be diagnosed and treated promptly to reduce transmission to the vulnerable group especially the young, the old and the immunocompromised.
To the hospital, prompt diagnosis, reduce financial cost by treating the patient at home on short course chemotherapy and reduce transmission of infection to other patients and staff.

The study will be beneficial to the patient in that quick diagnosis will help reduce morbidity and mortality, reduce financial implication and the right kind of treatment will be administered to the patient.

To the researchers, future research on validation of the new procedures before introducing more widely in health as an intervention and promote policy making.

2.14 The scope of the study

The scope of this study will be diagnostic laboratories handling direct smear microscopy of sputum specimens from patients attending chest clinic in Nairobi. However, due to time and resources, the focus will be on specimens from patients attending chest and skin clinic in Mbagathi District hospital suspected to have pulmonary tuberculosis (PTB). Specific target will be on only new patients who have never been on any anti-TB treatment. The patients chosen will have been screened for HIV and suspected to be having tuberculosis.

2.15 Study limitations

- The study was confined to adults only who were 18 years and above seen and diagnosed in Mbagathi District Hospital, which is an urban set up. The findings need not necessarily reflect the true situation in other parts of the country.

- Some patients were not consenting to be screened for HIV thus recruiting the sample size for the target population was time consuming.

- The aspect of eliminating recall bias was difficult because some patients could not remember some socio-demographic aspects.

- The study was not able to identify the MOTT that were isolated because of lack of resources. However MOTT could be a cause of TB like illnesses or could be contaminants.
CHAPTER 3

3.0 MATERIALS AND METHODS

The study made use of descriptive and comparative desires. The researcher went to the population of interest, receiving, screening and processing samples, interviewing of patients and going through records. All this was done in the natural setting of the patients.

3.1 Study site

Patients were recruited at Mbagathi District Hospital chest clinic. This facility was a referral center for suspected PTB cases in Nairobi area especially from Kenyatta National Hospital and its catchment area. The estimated population in Nairobi province and its environs was estimated to be 3 million and TB cases estimated at 7585 new smear positive. Mbagathi District Hospital has been in existence since 1956 and was formally known as Infectious District Hospital (IDH). The hospital that as now been converted to general, eye wards, HIV support center and paediatric services, used to serve as a TB treatment centre and patients used to be transferred from all over the country. Besides all this changes, the hospital has maintained its TB clinic and patients suspected to be suffering from TB were sent to the laboratory for diagnosis. Direct microscopy of unconcentrated sputum was employed in the laboratory. Positive cases were given treatment and followed up. HIV counselling and screening of patients was also offered.

3.2 Study design

This was a cross sectional descriptive study. The study mainly targeted patients who attended the health chest clinic facility for the first time (new cases) and suspected to be suffering from PTB. In the clinic the clinician examined the patients and those who met the criteria of productive cough for more than three weeks, chest pain, breathlessness, fever and night sweats, tiredness, loss of appetite and significant loss of weight were selected. The exclusion criteria were
done on patients with cough less than three weeks, no weight loss and no night sweats among others. HIV results were obtained from the clinic register

3.3 Patients selection

The patients eligible for sputum smear microscopy were defined as those who presented with, cough of longer than two weeks in duration, blood stained sputum, night sweats, high grade fever, loss of weight and appetite and shortness of breath. The patients who accepted to participate in the study signed the consent form after understanding clearly (See Appendix I).

Therefore all patients suspected to have PTB were instructed to submit three sputum samples to the Laboratory. The first sample referred to as Spot 1(S1) was collected and submitted the tie the patient presents to the clinic, send sample referred to as Morning (M) was collected in the early morning the following day and the third sample referred to as Spot 2 (S2) was collected when the patient brought the morning specimen.

Both HIV positive and negative patients were selected. The samples were received in the laboratory by the researcher and were evaluated for processing or rejection. The following criteria were considered:

Inclusion criteria of sputum samples assessment

- Purulent sputum
- Blood stained sputum
- 5ml of sample and above

Exclusion criteria of sputum sample assessment

- Saliva
- Insufficient samples
- Nasal excretion
- Induced sample

The patients for this study were selected from an average estimate of about 1500 patients in a period of three months at MDH chest clinic. The prevalence of HIV and TB co-infection was estimated at 60% according to the MOH TB annual
Therefore a sample size of 90 was selected from HIV positive patients and 60 were selected from HIV negative patients alone. All patients who attended the clinic and send for direct smear microscopy were routinely screened for HIV using parallel testing kits i.e. Determine and Unigold techniques as already described in the text. Pre-test and post-test counseling was done in the clinic as recommended by the MOH referred to as Direct Counseling and Testing (DCT). In the event that the concentrate and or culture technique become positive for direct smear negative patients, the results were linked up to the clinician for easier tracing and treatment. The HIV result of each selected patient was retrieved from the records in the clinic. Unique identity number was used to identify all patients and the records were kept under key and lock. The information entrusted to the PI was confidential and was not being divulged to any other unauthorized person.

3.4 Sample size

A sample of 150 patients was used for the study calculated using the formula below (Fischer's et al) taking into consideration that, the prevalence of PTB in Nairobi area ranged between 10%-12%.

\[
N = \frac{z^2 pq}{D^2}
\]

\(N=\)desired sample size
\(N=\)desired sample size
\(P=\)estimated proportion, the prevalence of PTB in Nairobi
\(Q=1-p\)

\(D=\)level of statistical error at significance level of 0.05

\[
N = \frac{1.96^2 \times 0.11 \times 0.89}{0.05^2} = 150.
\]
3.5 Sampling procedure
The number of new patients (new cases, on compliant, relapse) turn over in TB chest clinic was estimated to be 1500 in three (3) months. The total sample size of 150 patients was systematically selected. Every third patient was selected for the study after meeting the rejection and inclusion criteria and also accepting to participate in the study by signing the informed consent form. Collection of samples was done for a period of three months.

3.6 Ethical consideration
Ethical approval was provided by Kenyatta National Hospital Research and Ethics Committee. An informed written consent was obtained from each participant after explaining the objectives of the study, benefits and potential risks. The information generated from the study was used in the management of the participants condition as part of the benefits. There were no risks to be incurred for participating in the study. The test did not involve any invasive procedure because only expectorated sputum is required. Pre-test and post-test counselling for HIV testing was offered to all study participants according to the national guidelines of HIV testing. Confidentiality was safeguarded between the clinician and the researcher in both TB and HIV results. There were no identifiers in the labelling of the specimens that would possibly link them directly to the participants. Participation was entirely voluntary. Refusal to participate in the study involved no penalty and services were provided without any discrimination to all individuals regardless of whether they consented to participate or declined.

3.7 Laboratory procedures
3.7a Laboratory equipments and reagents
Frosted glass slides, drying racks, sampling racks, microscopes, incubator at 35°C - 37°C, centrifuge at 3000g, vortexer disposal container, biosafety cabinets level 11, tubes 50ml, reagent bottles, refrigerator, autoclave, balance, Bunsen burner, microscope, gloves, filter paper, universal bottles, homogenizer, inssisipator, culture wash bottles, racks, masks, oil immersion, pipettes,
methanol, ZN-stain, digestant and decontaminating agent (4%NaOH), phosphate buffer, LJ media, nitrate test and niacin test.

3.7b Specimen, collection and transport.
Patients were instructed on how to expectorate the sputum sample and collect in sterile, leak proof, clear screw caped sputum mug that were provided. Container ones used was disposed and incinerated.
Three deep expectorated specimens were collected (S1, M, and S2) in Mbagathi TB Laboratory and were assessed for adequacy and quality. No induction was practiced. All samples were labelled using the code number of the patient, date received and type of sample.
Transport to the Central Reference Laboratory was done within 1-2 hours for processing. Samples were packed into a cool box with enough absorbent material incase of any leakage by the researcher and research assistant. The research tools were located separately from the specimens.

3.7c Receiving of sample
The specimens on being received from the patient were assessed for signs of any leakage whether it was adequate, quality in terms of macroscopic appearance. A laboratory number was given to the sample and the request form. Direct microscopy was done in Mbagathi TB Lab. Those patients who consented to the study and met the criteria had their samples given the research code number and transported to Central Reference lab for further diagnosis and identification.

3.7d direct microscopy
The researcher labelled the clean grease free slides using Lab numbers and suggestive parts of all sputum samples were used to prepare a standard smear of 2 x 1cm under a safety wood. Also known positive and negative sputum samples control were included.
The smears will be left to dry, heat fixed and stained by Ziel-Neelsen stain (ZN) technique. This technique uses 5% standard carbol fuchsin as the basic stain, 3% methylene blue as the counter stain and IUATLD 25% sulphuric acid for decolourization. (See Appendix iii a)

The smears were examined using x 100 oil immersion. Upto 100 fields were examined and another 100 if the smears were negative or one bacillus was observed.

The results of the direct microscopy will be entered in the request form and also to the research tool in accordance with the grading system of IUATLD. (See Appendix iii e).

3.7e Sputum preparation for concentrate smear and culture

Sputum was digested and decontaminated before inoculation and preparing concentrate smear for microscopy. Four percent Sodium hydroxide was both mucolytic and decontaminating. This done to remove mucin that binds to mycobacteria, to eliminate the normal flora and doing as little harm as possible to the somewhat harder mycobacteria.

10ml of sputum or less was transferred to 50ml plastic screw capped centrifuge tube and an equal amount of 4%NaOH Solution was added. The tube was capped tightly and mixed on a vortex-type mixer until the specimen is liquefied. The mixture was left to stand at room temperature with occasional gentle shaking to avoid over treating the specimen.

A freshly prepared phosphate buffer, Ph 6.8 or sterile distilled water was added mixed and centrifuged at 3000g for 15 minutes.

The supernatant was poured off into a splash proof vessel containing disinfectant (Sodium Hypochlorite). Small quantity of phosphate buffer PH6.8 or sterile distilled water was added to resuspend the sediment. The suspension was used to make smears like in direct microscopy and cultured on LJ medium and MGIT tube 38. (See Appendix iii b)
3.7f Culture of concentrated sputum samples

A traditional component of media, albumin in agar base Lowenstein Jensen (LJ) media was used. This was used for isolation and propagation of Mycobacterium species. MGIT tubes was also used to compare isolation rates on morning samples.

LJ media was a relatively simple formulation that required supplementation in order to support the growth of Mycobacteria. Glycerol and egg mixture were added prior to the inspissation process. The substances provided fatty acids and protein required for metabolism of Mycobacteria. The coagulation of egg albumin during sterilization provided a solid medium for inoculation purposes. L-J medium also contained malachite green as an inhibitor to micro organisms other than acid-fast bacilli.

A 1-3 drop of the concentrate sputum specimen was inoculated on LJ slant. Incubation was done at 35°C – 37°C in 5 – 10 % CO2 atmosphere, caps of tubes were loosened and incubated in a horizontal position for the first 24 hours then vertically. Growth was then being checked daily for the first 7 days for rapid growers and then once a week for upto 6 weeks for slow growers.

A suspension of positive and negative control organisms was also included i.e. HRV 37 MTB strains and M.Kansasii. In the event that there was no growth after 8 weeks, culture was recorded and discarded.

500ul was added into supplemented BBL tubes and entered into BACTEC MGIT960 to be incubated at 37°C and monitored every 60minutes for increasing fluorescence for 42 days.

3.7g Identification of MTB from MOTT

Confirmation of mycobacterial growth for all culture systems was done by ZN direct stained smears. Purity of MGIT system was checked by inoculation on nutritional Muller Hinton agar containing 5% horse blood and incubated aerobically at 370c for 48hours and then examined for growth.
Identification of growth on LJ was done by conventional methods that included determination of the rate of growth, colonial morphology, pigmentation, cord formation on microscopic direct ZN stain for AFB and emulsification on slide. Biochemical characteristics such as Niacin production and nitrate reduction tests were used.

(i) **Niacin production;** this biochemical test was done on growth of 2 weeks and above. Niacin functions as a precursor in the biosynthesis of co-enzymes. The accumulation of niacin in culture medium was due to lack of an enzyme that can convert it into another metabolite. Paper strips impregnated with potassium thiocyanate and chloramine T will release cyanogen chloride, which in turn reacts with Para aminosalicylic acid to produce a yellow colour in presence of niacin. (remel Niacin Regent Strip insert www.reml.com) (See Appendix iii c).

(ii) **Nitrate reduction;** This was also done on growth of 2 weeks and above. Four drops of sterile distilled water was added on Nitrate substrate broth. Inoculate heavily with colonies and shake by hand. Incubate at 35oC for 2 hours. Add 1 drop of HCL then 2 drops of each of reagents A (0.2% aqueous solution of sulphanilamide) and reagent B (0.1% aqueous solution of n-naphthyethylene diamine dihydrochloride), observe for colour change Controls was also be included (Appendix iii d).

### 3.8 Performance of the CRL on External Quality Control

The Central Reference Laboratory is involved in External quality control in conjunction with the Supra National Laboratory based in Brisbane, Australia. The results of the quality control are satisfactory.

### 3.9 HIV screening

All patients diagnosed with PTB were screened in the clinic for infection. This was routinely done with the introduction of Direct Testing and Counseling (DTC) in the TB clinics countrywide. The secondary data results were obtained from the
register in the clinic. The councilors administered pre-test and post-test counseling to the patients in the clinic. This test was done voluntary without patients being cohersed. All secondary data was kept confidentially between the researcher and the clinician.

All PTB negative patients were given antibiotics for 2 weeks and to return back for review, meanwhile the samples were subjected to concentrate and culture technique in order to determine the yield and identification. Cases turned out to be positive were linked to the clinician in the TB clinic. All positive PTB cases were begun on anti tuberculosis regimen therapy for adults. Patients were monitored at 2 months, 5 months and 7/8 months to ensure complete cure.

3.9.1 Screening of HIV

The Kenya National recommended algorithm for HIV screening was used as follows, parallel testing with Determine (Kit from Abbot Determine™ and Unigold (kit from Trinity Biotech Uni-Gold™) with a Tie breaker using Bioline (SD standard Diagnostic,Inc).

(a) Determine

Determine HIV 1 and 2 is an immunochromatographic test for the qualitative detection of antibodies to HIV1/2. 2 drops of whole blood is added to the test pad, wait for blood to be absorbed and add 1 drop of chase buffer. As the sample migrated through the conjugate pad, it reconstituted and mixed with the selenium colloid antigen conjugate. The mixture continued to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient’s window site. This took place for a minimum of 15 minutes and results are read.

If antibodies to HIV 1 and HIV 2 were present in the sample, the antibodies bound to the antigen – selenium colloid and to the antigen at the patient window, forming a red line at the patient window site.
If antibodies to HIV 1 and or HIV 2 were absent, the antigen selenium colloid flow past the patient window and no red line is formed at the patient window site.

(b) Unigold
Recombinant proteins representing the immunodominant regions of the envelope proteins of HIV -1 and HIV-2 glycoprotein's gp41, gp120 and glycoprotein Gp36 were immobilized at the test region of the nitrocellulose strip. During testing, 2 drops of whole blood was applied to the sample part, followed by two drops of wash buffer and allowed to react. Antibodies of any immunoglobin class, specific to the recombinant HIV 1 or HIV2 proteins, reacted with the colloidal gold linked antigens. The antibody protein colloidal gold complex migrated by chromatography along the membrane to the test and control regions of the test device.
The results were read immediately after the end of the 10 minutes incubation time.
A positive reaction is visualized by pink/red band in the test region of the device. A negative reaction occurred in the absence of human immunoglobin antibodies to HIV in the analysed specimen. Consequently no visually detectable band develops in the test region of the device. If the two tests performed test showed a discrepancy the sample is collected and confirmed by use of a tiebreaker which is Bio-line done as per the manufacturer's instructions.

(c)SD Bio Line
This test contains membrane strip, which is precoated with recombinant HIV-1 capture antigen on the test band 1 region with recombinant HIV 2 capture antigen on test band 2 region respectively. The recombinant, conjugate and samples move along the membrane chromatographically to the test region and forms a visible line as the antigen antibody antigen gold particle complex form with a high degree of sensitivity and specificity. Positive and negative controls are inbuilt in the kit.
3.10 Data collection and analysis.

Data collection was done using the research tool and questionare (See Appendix ii) during the period of the study. Quality control panels of smear, which was negative, was used as the negative control and smear from known strains of MTB will be used as a positive control in all the procedures.

For identification a MTB, HRV37 standard organism was used as the control and M Kansasii was used as a negative control.

The data was tabulated into coded research tool and entered into the computer database.

Data was analyzed by use of SPSS, EPI INFO and Excel to achieve descriptive summary statistics that were presented as proportions and percentages in form of frequency tables, charts and graphs.

The chi-square and Fischer’s exact test was used to establish relationship among the variables and compare proportions from computer-generated contingency tables. Statistical significance for each parameter was done.
CHAPTER 4

4.0 RESULTS

4.1.1 Socio demographic factors of the study population

Socio demographic data presented in table 4.1 was for a total of 150 adults who were recruited into the study. Out of these patients, 60 (40%), where HIV- while 90 (60%) where the HIV +.

The findings indicate that the average age of the respondents was 32.4 years, the youngest 18 years and the oldest 68 years. Majority of the study population 84.6% were aged between 23-42 years. This was the most active group in the society.

The table 4.1.1 shows the ages of the study population recruited were grouped at an interval of 5 years. The lowest age group was 18-22 years and the highest >53 years. Among the age groups, there were 90 patients (60%) being HIV positive were as follows, 18-22 years 4%, 23-27 years 33%, 28-32 years 27%, 33-37 years 21.1%, 38-42 years, 38-42 years 11%, 43-47 1% and above 53 years 2.2%. The observation showed that the age group with majority of HIV + patients ranged from 23 through to 42 years (84.6%). These age groups represented the productive group in the society translating to a lot of economic and social losses due to their morbidity. The individuals infected HIV were also suspected to be infected with pulmonary tuberculosis. There were no patients who were between 48-52 years.

Among the age groups of HIV- patients there were 60 (40%) and the distribution were as follows; 18-22 years 10%, 23-27% 16.7%, 28-32 years 23.3%, 33-37 years 13.3%, 38-42 years 20%, 43-47 years 6.7%, 48-52 years 3.3% and > 53 years 6.7%.

45
However, the age group with highest percentage of HIV- patients was 28-32 years (23.3%) followed by 38-42 years (20.0%) and the least 48-52 years with 2 patients (3%).

Generally, few individuals who were HIV + or - were seen in the age groups of 43 years and above. This group was not so productive and were not vulnerable to HIV/AIDS and in most cases other underlying conditions and socio economic factors can lower their immune mechanism hence predisposing them to PTB infection.

Majority of the males who were 51 (56.7%) were HIV+ while the females who were 39 (43.3%) who were HIV+ out of a total of 90 patients.

On the HIV- group still the males contributed a higher percentage than the females, 31 of the males (51.7%) while the females 29 (48.3%). The data showed there was no statistical significance in the relationship between HIV status and gender (p=0.616).

Most patients seen were males suggesting that males had a higher morbidity perhaps because of their social habits of smoking and drinking alcohol and/or greater health seeking behaviors. (Males may not need to seek permission from their spouses to come to the hospital).

Table 4.1.1 showed that married patients contributed the majority of the study population.

54 of these patients (60%) who were married were HIV + and among the singles 36 patients (40%) were HIV+. The HIV- group had the married patients contributing to 43 (71.7%) and the single patients contributing to 17 (28.3%).

The indication here was that couples misfortunes and morbidity had far reaching implications. This is because dependents suffered in various dimensions
especially when the state of morbidity is long that may culminate to death if not managed. The relationship between gender distribution and HIV status was not statistically significant ($P=0.165$) at a level of 95% significance.

Fourty nine (54.5%) of the HIV+ individuals had no history of TB in the family while 41 (45.5%) had history of TB. 33 (55%) of the HIV- individuals had no history of TB in the family while 27 (45%) had history of TB infection. According to the chart 4.2.9 those who were HIV+ with previous or no TB infection were each 7 patients (50%). Those who were HIV- with previous TB infection were 83 (61%) and 53 (39%) did not have the previous TB infection. The relationship between HIV status and the previous TB infection was not statistically significant ($P=0.568$) ($X^2=0.422$).
### Socio demographic factors of the study population

#### Table 4.1.1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency (n)</th>
<th>Percent(%) n=150</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group (Years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-22</td>
<td>10</td>
<td>6.7</td>
</tr>
<tr>
<td>23-27</td>
<td>40</td>
<td>26.7</td>
</tr>
<tr>
<td>28-32</td>
<td>38</td>
<td>25.3</td>
</tr>
<tr>
<td>33-37</td>
<td>27</td>
<td>18</td>
</tr>
<tr>
<td>38-42</td>
<td>22</td>
<td>14.7</td>
</tr>
<tr>
<td>43-47</td>
<td>5</td>
<td>3.3</td>
</tr>
<tr>
<td>48-52</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>&gt; 53</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>82</td>
<td>54.7</td>
</tr>
<tr>
<td>Female</td>
<td>68</td>
<td>45.3</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td>97</td>
<td>64.7</td>
</tr>
<tr>
<td>Single</td>
<td>53</td>
<td>35.3</td>
</tr>
<tr>
<td><strong>Size of the house</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 rooms</td>
<td>141</td>
<td>94</td>
</tr>
<tr>
<td>3-4 rooms</td>
<td>8</td>
<td>5.4</td>
</tr>
<tr>
<td>No house</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Ventilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 windows</td>
<td>19</td>
<td>12.7</td>
</tr>
<tr>
<td>1-2 windows</td>
<td>107</td>
<td>71.3</td>
</tr>
<tr>
<td>3 window</td>
<td>21</td>
<td>14.1</td>
</tr>
<tr>
<td><strong>Number of respondents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 persons</td>
<td>45</td>
<td>30.1</td>
</tr>
<tr>
<td>3-4 person</td>
<td>71</td>
<td>47.3</td>
</tr>
<tr>
<td>5+ persons</td>
<td>31</td>
<td>22</td>
</tr>
<tr>
<td><strong>History of TB in family</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>67</td>
<td>44.7</td>
</tr>
<tr>
<td>No</td>
<td>81</td>
<td>54</td>
</tr>
<tr>
<td>Didn't know</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Previous TB infection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>9.3</td>
</tr>
<tr>
<td>No</td>
<td>136</td>
<td>90.7</td>
</tr>
</tbody>
</table>
4.1.2 HIV status of the study population according to the geographical distribution.

Chart 4.1.2

The data represented in chart 4.2.6 indicated that Langata Division had a total of 26 HIV+ patients (28.9%). This was the division with the highest percentage of patients with HIV followed by Embakasi Division with a total of 17 patients (18.9%). Embakasi and Langata Division were known to house people living below the income per capita level.

The divisions with the lowest number of HIV+ individuals were Central and Parklands/Westlands each with 1 case (1.1%). Outside Nairobi had a total of 13 HIV+ patients (14.4%).
The study population who were HIV- was distributed in Nairobi Division and outside Nairobi. Langata Division still had the highest number of cases, 13 (25%) followed by Embakasi with 8 (13.3%). Central Division was the least with the lowest number being 1 (1.7%). Outside Nairobi area had a total of 14 cases (23.3%). These cases were second after Langata Division.

4.1.3 Relationship between the size of the house, ventilation and number of occupants.

Table 4.1.3

<table>
<thead>
<tr>
<th>Ventilation (No. of windows)</th>
<th>Occupants (No. of persons)</th>
<th>Size of the house (Number of rooms)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1-2</td>
<td>11 (58%)</td>
<td>11 (58%)</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>8 (42%)</td>
<td>8 (42%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>19</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>1-2</td>
<td>1-2</td>
<td>70 (64%)</td>
<td>70 (64%)</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>39 (36%)</td>
<td>39 (36%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>109</td>
<td>109</td>
</tr>
<tr>
<td>3-4</td>
<td>1-2</td>
<td>5 (42%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>7 (58%)</td>
<td>9 (64%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>5+</td>
<td>1-2</td>
<td>1 (50%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>1 (50%)</td>
<td>4 (57%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2 (100%)</td>
<td>7 (100%)</td>
</tr>
</tbody>
</table>

The study population responding who lived in 1-2 roomed houses were 142 patients. Of these those who did not have ventilation were 19, 11 of them (58%) lived with 1 or 2 persons and 8 patients (42%) lived with 3 or more persons. Those who lived in 3 roomed houses and above all had ventilation. Those who had 1 or 2 windows as a source of ventilation were 109 cases, 70 of them (64%) lived with 1 or 2 persons and 39 patients (36%) lived with 3 persons and above. Those who had 3 to 4 windows as a source of ventilation were 12 cases, 5 (7%) lived with 1 or 2 persons and 7 (58%) lived with 3 persons and above. Those who had 5 windows and above had a source of ventilation were 1 case, each case (50%) living with 1-2 and 3 persons and above.
The study population who responded to live in 3-4 roomed houses were 6 patients. In all this cases 2 representing 100\% had 3-4 windows as a source of ventilation and lived with 3 persons and above.

Finally the study population who responded to live in 5 roomed houses and above was 1. This case represented 100\% and 5 windows and above as a source of ventilation and lived with 3 persons and above.

The data shows that majority of the study population lived in impoverished housing conditions i.e. Lack of or poor ventilation small size house and many occupants. This contributes to spread of airborne tuberculosis among the population. Mycobacteria organisms also thrive and survive well for a long time in a dark closed environment.
4.2.0 Laboratory investigations, diagnosis and identification of mycobacteria and relationships with socio demographic data among the HIV+ and HIV- study population.

4.2.1 Visual appearance of sputum samples according to the HIV status.

Table 4.2.1

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Visual appearance</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mucopurulent</td>
<td>Mucopurulent &amp; blood stained</td>
</tr>
<tr>
<td>+</td>
<td>62 (68.9%)</td>
<td>28 (31.1%)</td>
</tr>
<tr>
<td>-</td>
<td>51 (85%)</td>
<td>9 (15%)</td>
</tr>
<tr>
<td>Total</td>
<td>113 (75.4%)</td>
<td>37 (24.6%)</td>
</tr>
</tbody>
</table>

Table 4.2.1 indicates that all 150 specimens representing (100%) were mucopurulent. The mucopurulent alone accounted for 113 (75.3%) while the mucopurulent and blood stained accounted for 37 (24.7%). From the HIV+ patients, category of mucopurulent specimens was 62 (68.9%) while the category of mucopurulent and blood stained was 28 (31.1%).

From HIV+ patients, category of mucopurulent specimens was 51 (85%) while the category of mucopurulent and blood stained was 9 (15%). This means that most of the mucopurulent and blood stained specimens were observed in HIV+ patients than the HIV-, haemoptysis being one of the inclusion criteria of samples. However, there was no statistical significance among the study population and on the visual appearance of the sputum sample. (p=0.33).

Four hundred and fifty samples were investigated with the three techniques during the study period. All of the specimens were diagnostic specimens from clinically suspected TB patients who submitted their samples. The results from each of the 3 samples from each patient were done cumulatively so as to increase the output.
4.2.2 Results obtained with the direct microscopy.

Chart 4.2.2

Chart 4.2.2 showed that out of 150 specimens reported by direct smear microscopy 53 (35.3%) were reported as direct microscopy AFB+. 97 of these specimens (64.7%) were reported as direct smear negative meaning that the patient did not have AFB. Thereafter the yield of diagnosing clinical pulmonary tuberculosis among the study population using direct smear microscopy was 35.3%.
4.2.3 Results obtained with direct smear microscopy according to the HIV status

Chart 4.2.3

Chart 4.2.3 shows that 43 cases (47.8%) had AFB+ diagnosed by direct microscopy from the HIV + population and 10 (16.7%) were from the HIV- cases.

Forty seven (52.2%) of HIV + individuals and 50 (83.3 %) of HIV- individuals did not have AFB. The results showed that 43 (47.8%) of the individuals were dually infected. Majority of the AFB- cases came from HIV- individuals. A yield of 35.3% of AFB+ detected by direct smear microscopy among HIV+ and HIV- individuals was statistically significant ($X^2=0.0001$) ($p=0.0001$).
4.2.4 Direct positive smear microscopy according to HIV status and age groups.

Chart 4.2.4 showed that the age group that had the highest AFB+ cases by use of direct microscopy was 23-37 years adding up to 31 (58.5%) cases. The least age groups with the lowest cases were 43-53 and above years having 2 (1.9%) cases each.

According to the HIV status age groups who were HIV+ and had the majority of smear positive cases in the age groups ranging from 23-32 years accounting for 28 patients (65.1%). Age groups of 33-37 had 7 (16.3%) cases. The least age group with positive smear cases was 43-47 years with 1 (2.3%) case. In HIV- category the diagnosis of PTB by direct microscopy indicated that age groups 18-22 years, 28-32 years, 33-37 years and 38-42 accounted for 2 individuals in each group representing 20%. Age groups of 23-27 years and ≥ 53 years accounted to 1 individual each representing 10%. There were no positive smears cases in the age groups of 48-52 years.
Fourty three (81.1%) were HIV + and 10 (18.9%) of the smear positive cases were HIV -. This translated to HIV + patients were also co-infected with the TB diseases and affected mostly the productive age groups.

4.2.5 Direct positive smear microscopy results according to HIV status, gender and marital status

Table 4.2.5

<table>
<thead>
<tr>
<th>Gender</th>
<th>HIV status (n/%)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Males</td>
<td>25 (58.1%)</td>
<td>8 (80%)</td>
</tr>
<tr>
<td>Females</td>
<td>18 (41.9%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td>43 (100%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Married</td>
<td>Single</td>
</tr>
<tr>
<td>Married</td>
<td>24 (55.8%)</td>
<td>8 (80%)</td>
</tr>
<tr>
<td>Single</td>
<td>19 (44.2%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td>43 (100%)</td>
<td>100 (100%)</td>
</tr>
</tbody>
</table>

Table 4.2.5 showed that 25 (58.1%) of males who were HIV + were diagnosed to have positive smears direct smear microscopy while 18 (41.9%) of the females were diagnosed. A total of 43 HIV+ cases were diagnosed to have had positive smears in the population of HIV + patients males accounting the majority.

Eight (80%) cases of HIV- males were diagnosed by direct smear microscopy while 2 (20%) cases of HIV- females were diagnosed. A total of 10 HIV- patients had positive smears. Males contributed a majority of positive smears cases. There was no statistical relationship between the parameters ($X^2=0.199$) (P=0.286). The married individuals contributed to the majority of positive smears cases diagnosed by direct smear microscopy representing 60.4% of the HIV +
and HIV- cases. The single group contributed 31.6% of the smear positive cases in both HIV + and – subjects.

The smear positive cases were 24 (55.8%) from HIV+ among the married and 19 (44.2%) were single. Among the HIV- cases, a total of 8 married patients representing 80% and 2 singles representing 20% had positive smears.

Direct smear microscopy had been influenced by the marital status of the study population and HIV status. More married patients were infected with PTB. The relationship was not statistically significant ($X^2=0.159$) (P=0.282).

The age groups combined, gender and marital status contributed to the same number of smear positive cases diagnosed by direct smear microscopy among the HIV + and - individuals i.e. 43 (81.9%) of HIV + and 10 (18.1%) of HIV – patients.

### 4.2.6 Direct smear positive microscopy results according to HIV status and size of the house.

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Size of the house (Number of rooms)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-2</td>
<td>3-4</td>
</tr>
<tr>
<td>+</td>
<td>43 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>-</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>53 (100%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4.2.6 shows that all patients who had PTB were living in a house of 1-2 rooms. 43 representing 81.1% were co-infected and 18.9% were HIV -ve and PTB +ve. There were no PTB cases identified living in 3-roomed house and above. This meant that PTB mostly affected people living in small squeezed houses hence easy transmission from one individual to another.
4.2.7 Direct smear positive microscopy results according to HIV status and ventilation of the house.

Table 4.2.7

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Ventilation (Number of windows)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1-2</td>
</tr>
<tr>
<td>+ve</td>
<td>7 (16.3%)</td>
<td>33 (76.7%)</td>
</tr>
<tr>
<td>-ve</td>
<td>1 (10%)</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (15%)</td>
<td>40 (66%)</td>
</tr>
</tbody>
</table>

Among the PTB patients, 7 representing 16.3% who were HIV +ve and 1 representing 10% of HIV -ve patients lived in a house with no ventilation. 33 representing 76.7% of the PTB patients being HIV +ve and 7 representing 70% being HIV -ve lived in a house of 1-2 windows offering ventilation. 4.7% and 20% representing HIV positive and -ve lived in a house with 3-4 windows. Only one case representing 2.3% who was HIV +ve PTB patient lived in a house of 5+ ventilation windows. This showed that people living in houses with no ventilation and 1-2 ventilation windows were most infected by PTB because expectorated Mycobacteria organism usually thrives well in a dark environment and transmission is inevitable.

4.2.8 Direct smear positive microscopy results according to HIV status and number of occupants.

Table 4.2.8

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Occupants (Number of persons)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-3</td>
</tr>
<tr>
<td>+ve</td>
<td>33 (76.7%)</td>
</tr>
<tr>
<td>-ve</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>Total</td>
<td>47 (88.7)</td>
</tr>
</tbody>
</table>
Among the co-infected patients representing 76.7% lived in houses with 1 or 2 persons and 10 representing 23.3% lived in houses with 3 persons and above. Patients who were HIV negative but had PTB were 4 representing 40% who lived with 1 or 2 persons and 6 representing 60% lived with 3 persons and above.

### 4.2.9 Direct smear positive microscopy results according to HIV status and history of TB in the family.

**Chart 4.2.9**

<table>
<thead>
<tr>
<th>History of TB in the family</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>41.9</td>
<td>58.1</td>
</tr>
<tr>
<td>Negative</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

The co-infected patients who had history of PTB in the family were 18 representing 41.9% while those who did not have the history were 25 representing 58.1%.

Among the PTB positive patients who were HIV -ve contributed to 5 representing 50% of each case who had and who did not have history of TB in the family. It can be concluded that having history of TB in the family did not have any strong effect on contracting PTB.
4.2.10 Direct smear positive results according to HIV status and previous TB infection.

Chart 4.2.10

Table 4.2.10 shows that 3 representing 7% of PTB patients had previous TB infection and 40 representing 93% had no previous TB infection all of who were HIV +ve.

1 representing 10% of the PTB patients had previous and 9 representing 90% had no previous TB infection all of who were HIV -ve.

This showed that previous TB infection had no association with the current infections. Majority of the new PTB infections mainly is as a result of co-infection with HIV.
4.3.0 Concentrate microscopy results

4.3.1 Results obtained with concentrate smear microscopy in the study population.

Chart 4.3.1

<table>
<thead>
<tr>
<th>Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>47.3%</td>
</tr>
<tr>
<td>Positive</td>
<td>52.7%</td>
</tr>
</tbody>
</table>

Chart 4.3.1 indicated that out of 150 specimens reported by concentrate smear microscopy 79 (52.7%) were reported as AFB+ while 71 (47.3%) of these specimens were reported as AFB-.

The yield of diagnosing pulmonary tuberculosis among the study population using concentrate smear microscopy was 52.7% compared to 35.3% yielded from direct smear microscopy. This technique gave an incremental yield of 18%. These were cases that were not captured in direct microscopy.
4.3.2 Concentrate smear microscopy results according to the HIV status of the study population.

Chart 4.3.2

Sixty three (70%) smear positive cases diagnosed by concentrate microscopy were detected from HIV+ cases compared to 47.8% detected by direct microscopy. 27 (30%) of the HIV+ cases had no AFB compared to 52% who were not diagnosed in direct microscopy. Among the HIV- individuals, 16 (26.7%) had positive smears by concentrate smear microscopy compared to 16.7% in direct microscopy. 44 cases (73.3%) had negative smears by concentrate microscopy compared to 83.3% in direct microscopy. The results showed that many individuals who were HIV+ had the highest cases of PTB diagnosed by concentrate microscopy. Also few of the HIV- individuals were diagnosed as smear positive by concentrate smear microscopy. An incremental yield of AFB+ smears was seen in both status and the rate of negative smears cases was reduced in concentrate.
A yield of 53% detected by concentrate smear microscopy among the HIV- and HIV+ individuals was statistically significant ($X^2=0.000$) (P=0.000).

4.3.3 Concentrate smear positive microscopy results according to HIV status and age groups.

Chart 4.3.3

Chart 4.3.3 showed that the age group that had the highest number of smear positive cases diagnosed by concentrate smear microscopy was 23-42 years accounting for 56 (80.9%). The least age group with the lowest percentage of PTB cases was 43-47 accounting for 6 cases (7.6%). This was lower in direct microscopy.

According to the HIV status of the age groups, those who were HIV+ had the majority of smear positive cases. Ages 23-37 accounted for 51 (81.1%) compared to 75% in direct microscopy. The least age groups with the lowest smear positive cases was 43 years and above with each 1 case representing 1.6%.
In HIV- category, the diagnosis of pulmonary infections caused by mycobacteria by concentrate microscopy indicated that, the group with highest smear positives was 38-42 accounting for 4 cases (25%). The least age groups with the lowest notification of cases were ages 23-27 years and 43-47 years each accounting for 1 case (6%). There was no smear positive cases in the age groups of 48-52 years in both categories of study population just like in direct microscopy.

Sixty three (79.7%) of the smear positive cases representing were co-infected and 16 (20.1%) of the cases were not. This was compared to direct microscopy that captured only 43 cases (81.9%) and 10 (18%) respectively. This showed that majority of the individuals were co-infected with HIV and general increases in majority of smear positive cases in concentrate method.

4.3.4 Concentrate smear positive microscopy results according to HIV status, gender and marital status

<table>
<thead>
<tr>
<th>HIV status (n/%)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
</tr>
<tr>
<td>40 (63.5%)</td>
<td>13 (81.3%)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>23 (36.5%)</td>
<td>3 (18.8%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>63 (100%)</td>
<td>16 (100%)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td></td>
</tr>
<tr>
<td>32 (50.8%)</td>
<td>13 (81.3%)</td>
</tr>
<tr>
<td>Single</td>
<td></td>
</tr>
<tr>
<td>31 (49.2%)</td>
<td>3 (15.3%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>63 (100%)</td>
<td>16 (100%)</td>
</tr>
</tbody>
</table>

Table 4.3.4 shows that 40 (63.5%) of the male population who were dually infected with HIV and were diagnosed by concentrate smear microscopy compared to 25 (58.1%) diagnosed in direct microscopy. 23 of the cases
(36.5%) were from the female population compared to 18 (42%) in direct microscopy.

Thirteen (81.3%) smear positive cases were captured from HIV- males while 3 cases (18.8%) were females. A total of 16 cases of smear positive diagnosed by concentrate microscopy did not have HIV compared to 10 cases in direct microscopy. Majority of males in both HIV status contributed to the highest percentage of smear positives accounting for 53 (67.1%) while the females contributed the lowest accounting for 26 (32.9%) compared to 33(62.3%) and 20(37.7%) cases in direct microscopy respectively.

Among the HIV+ category 32 (50.8%) of the married were diagnosed to have positive smears by concentrate microscopy and 31 (49.2%) were from the single group. Among the HIV- category, 13 (81.3%) of the married were diagnosed as smear positive by concentrate microscopy and 3 (18.3%) were from the single group. More cases were captured in concentrate than direct microscopy among the marital status of the patients. Concentrates smear microscopy results had been influenced by marital status and HIV status of the study population. The relationship was statistically significant ($X^2=0.028$) ($P=0.046$).

The age groups, gender and marital status of the patients combined contributed to the same number of PTB cases diagnosed by concentrate smear microscopy among the HIV+ and HIV- individuals i.e. 63 (79.7%) of HIV+ and 16 (20.3%) of the HIV- individuals.
4.3.5 Concentrate smear positive microscopy results according to HIV status and size of the house.

Table 4.3.5

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Size of house (Number of rooms)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>1-2 (62, 98.4%)</td>
<td>63 (100%)</td>
</tr>
<tr>
<td></td>
<td>3-4 (1, 1.6%)</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>1-2 (16, 100%)</td>
<td>21 (100%)</td>
</tr>
<tr>
<td></td>
<td>3-4 (0, 0%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>78 (99%)</td>
<td>79 (100%)</td>
</tr>
</tbody>
</table>

Among the dually infected patients 62 (98.4%) lived in 1-2 rooms and 1 person (1.6%) lived in 3-4 rooms compared to 100% who only lived in 1-2 rooms in direct microscopy results. Among the HIV- individuals, 16 (100%) smear positive cases lived in 1-2 rooms and no case was found living in 3-4 rooms. Majority of the cases dually infected and not dually infected lived in 1-2 rooms. The transmission rate among these individuals was high.

4.3.6 Concentrate smear positive microscopy results according to HIV status and ventilation.

Table 4.3.6

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Ventilation (Number of windows)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (10, 12.7%)</td>
<td>63 (100%)</td>
</tr>
<tr>
<td></td>
<td>1-2 (51, 68.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-4 (3, 4.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5+ (1, 6.3%)</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>8 (12.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (12.5%)</td>
<td>16 (100%)</td>
</tr>
<tr>
<td></td>
<td>11 (68.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (12.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (6.3%)</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>2 (12.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 (68.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (12.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (6.3%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10 (12.7%)</td>
<td>79 (100%)</td>
</tr>
<tr>
<td></td>
<td>62 (78.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 (6.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (2.5%)</td>
<td></td>
</tr>
</tbody>
</table>

Most patients reported to live in houses with 1 or 2 windows as a source of ventilation. Fifty one (81%) were co-infected while 11 (68%) of were HIV- who had positive smears and were HIV -ve. Those who reported to live in houses with 5 windows and above had the lowest number of smear positive in both groups. i.e 1.6% of the HIV +ve and 6.3% of the HIV –ve respectively.
TB control is complicated by the case of transmission in poorly lighted and poorly ventilated houses.

4.3.7 Concentrate positive smear microscopy results according to HIV status and number occupants.

Table 4.3.7

<table>
<thead>
<tr>
<th>Occupants</th>
<th>HIV status (n/%)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1-2</td>
<td>45 (71.4%)</td>
<td>7 (43.8%)</td>
</tr>
<tr>
<td>3+</td>
<td>18 (28.6%)</td>
<td>9 (56.3%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>63(100%)</strong></td>
<td><strong>16 (100%)</strong></td>
</tr>
</tbody>
</table>

Fourty five (71%) of HIV+ individuals who had positive smears lived in houses occupied by 1-2 persons and 18 (28.6%) lived with 3 occupants and above. Among the HIV- smear positive cases 7 (43.8%) lived with 1-2 occupants and 9 (56.3%) lived with 3 persons and above.

The number of the occupants in the house has an impact on transmission of TB because of overcrowding though there is no association with the occupants HIV status and outcome of PTB (P=0.074) at a 0.05 confidence interval.

An increase was realised in concentrate compared to direct smear microscopy.
4.3.8 Concentrate smear positive microscopy results according to HIV status and history of TB in the family.

Charts 4.3.8

Thirty five (55.6%) of the HIV+ and smear positive cases had no history of PTB in the family while 28 (44.4%) had history of TB in the family. Among the HIV-, 8 (50%) each had and had no history of TB in the family but had positive smears. An increase of 20 cases was seen in HIV+ patients and 6 in HIV- patients. All cases of HIV- who had and who had no history added 3 cases each in the concentrate technique.
4.3.9 Concentrate smear positive microscopy results according to HIV status and previous TB infection.

The chart above shows 6 (9.5%) of HIV+ individuals with previous infection and 57 (91%) with no previous infection were smear positive compared to 3 and 40 cases respectively in direct microscopy. 3 (18.8%) in the HIV- patients had previous TB infection while 13 (81.3%) had no previous TB infection had smear positive sputum compared to 1 and 9 cases in direct microscopy respectively. Most of the patients who had positive smears had no previous infection in HIV+ and HIV- patients. Detection of smear positive cases had no association with the previous TB infection among the PTB cases. An increase was also realised.
4.3.10 Comparisons of direct and concentrate sputum smear microscopy results.

Table 4.3.10

<table>
<thead>
<tr>
<th>Direct microscopy</th>
<th>Concentration (n/%)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53 (67.1%)</td>
<td>0 (0%)</td>
<td>53 (35.3%)</td>
</tr>
<tr>
<td>Negative</td>
<td>26 (32.9%)</td>
<td>71 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>79 (100%)</td>
<td>71 (100%)</td>
</tr>
</tbody>
</table>

The results obtained from the direct microscopy were compared to the concentration method. All the smears that were found positive (67.1%) in direct smear microscopy were also confirmed positive with the concentrate smear microscopy. However, 26 (32.9%) smears that were considered negative with the first technique i.e. direct microscopy were rated positive after applying concentration method, meaning that 32.9% of the missed cases were captured. The agreement between the tests was 82.7% with a total number of 124 and the difference was 17.3% with a total number of 26. There was a statistical significance in the relationship between the two techniques (P=0.0001).

There was also an incremental yield of smear positive cases by 18% by use of concentration method. This meant that concentration microscopy was more superior than that direct microscopy.
4.3.11 Direct and concentrate sputum smear microscopy results according to the HIV status.

<table>
<thead>
<tr>
<th>HIV Status</th>
<th>Direct microcopy</th>
<th>Concentration (n/%)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>HIV+</td>
<td>43 (68.3%)</td>
<td>0 (0%)</td>
<td>43 (47.8%)</td>
</tr>
<tr>
<td>HIV-</td>
<td>10 (62.5%)</td>
<td>0 (0%)</td>
<td>10 (16.7%)</td>
</tr>
</tbody>
</table>

There was no false positive result. An increment yield of 22% was noted. This showed that there was a high burden of smear positive cases in the said group.

The agreement between the tests on HIV positive cases was 78% and the difference was 22% respectively.

Among the HIV- patients ten (62.5%) samples that were direct smear positive was also positive by concentration microscopy. However, employing concentration method captured six smears (37.5%) that were considered negative in direct smear microscopy. Forty-four (100%) cases were detected as negative in all the two techniques. There were no false positive cases. An incremental yield of 10% was noted among the HIV negative study population.

The agreement between the two compared tests on HIV study population was 90% and the difference was 22.2% respectively.
In both HIV+ and HIV- study population, the relationship between the HIV status and the two employed techniques i.e. direct microscopy and concentration microscopy was statistically significant (P=0.0001).

### 4.4.0 Culture results and comparisons

#### 4.4.1 Results obtained from culture (LJ) for the study population.

Chart 4.4.1

![Pie chart showing culture results](image)

Chart 4.4.1 indicated that out of 150 specimens reported by culture technique, 85 (56.7%) were reported as positive for culture. 65 (43.3%) of those specimens were reported as culture negative meaning that the patients did not have mycobacteria.

The yield of diagnosing pulmonary tuberculosis among the study population using the culture technique on LJ was 56.7%. This was compared with direct microscopy that yielded 35% and concentrate microscopy with 53%. An incremental yield of 21.4% and 4% was observed respectively.
4.4.2 Culture results according to the HIV status.

Sixty-four (75.3%) HIV+ cases were detected to be culture positive while 21 (24.7%) were culture negative.

Twenty six (40%) of the HIV- were detected to be culture positive while 39 (60%) were culture negative. The results indicated that 85 of the cases with culture positive represented 56.7% yield by use of culture method and 65 (43.3%) of the cases had negative cultures. The yield of 57% among the study population by use of culture technique was statistically significant (P=0.0001).
4.4.3 Culture positive results according to HIV status and age groups.

Chart 4.4.3

Among the HIV+ cases positive cultures was detected in all the age groups except for ages 48-52 years. The age group with the highest cases was 23-27 accounting for 21 (32.8%) followed by 28-32, 25%, 33-37 15 (23%), 38-42 6 (9.4%), 18-22 4 (6%) and 43-47 and > 53 accounting to 1 (1.6%) each.

Among the HIV- cases positive cultures was detected in all the age groups. Ages 18-22 years had 4 (19%) cases and ages 28 through to 42 had same number of cases represented by 3 (14.3%), age groups 48-52 had 1 case (4.8%), which was not diagnosed by the previous two methods. The distribution of culture positive cases was seen to range from 23 through to 37 years, once again affecting the productive age groups.
4.4.4 Culture positive results according to HIV status, gender and marital status

Table 4.4.4

<table>
<thead>
<tr>
<th>HIV status (n/%)</th>
<th></th>
<th></th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ (n=%)</td>
<td>- (n=%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Males</td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 (62.5%)</td>
<td>24 (37.5%)</td>
<td>55 (64.7%)</td>
</tr>
<tr>
<td></td>
<td>15 (71.4%)</td>
<td>6 (28.6%)</td>
<td>30 (35.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>64 (100%)</td>
<td>21 (100%)</td>
<td>85 (100%)</td>
</tr>
<tr>
<td>Marital status</td>
<td>Married</td>
<td>Single</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34 (53.1%)</td>
<td>30 (49.9%)</td>
<td>64 (100%)</td>
</tr>
<tr>
<td></td>
<td>17 (81%)</td>
<td>4 (19%)</td>
<td>34 (40%)</td>
</tr>
<tr>
<td>Total</td>
<td>51 (60%)</td>
<td>34 (40%)</td>
<td>85 (100%)</td>
</tr>
</tbody>
</table>

Table 4.4.4 indicates that 40 (63%) of the male population were HIV+ and were diagnosed to have had positive culture for mycobacteria. 24 (37.5%) of these cases were from the female population hence the total number of HIV+ individuals who were co-infected with PTB were 64. 15 (71.4%) culture positive cases were from males who were HIV- while 6 cases (29%) were HIV- females. A total number of 21 cases that had positive cultures were HIV-, hence no co-infection. Once again the majority of the groups infected with PTB were males having the highest percentage in both genders i.e. 55 representing 64.7%. The relationship was not statistically significant (P=0.601) ($X^2=0.458$).

Among the HIV+ category 34 (53.1%) cases of positive cultures were from the married group and 30 (47%) cases were from the single group who were all diagnosed by use of culture technique. A total of 64 HIV+ cases in both married and single groups were co-infected with PTB.

Among the HIV- category, 17 (81.0%) of the males were diagnosed as culture positive and 4 cases (19.0%) from the single group were captured from culture. A total of 21 HIV- cases in both married and single groups had positive cultures.
The married group had the majority of cases who had positive cultures, 51 of them representing 60%. The relationship was statistically significant ($X^2=0.024$) ($P=0.039$).

**4.4.5 Culture positive results according to HIV status and ventilation.**

**Table 4.4.5**

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Ventilation (Number of windows)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1-2</td>
</tr>
<tr>
<td>+</td>
<td>8 (12.5%)</td>
<td>51(79.7%)</td>
</tr>
<tr>
<td>-</td>
<td>2 (9.5%)</td>
<td>16(76.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>10 (11.8%)</td>
<td>67 (78.8%)</td>
</tr>
</tbody>
</table>

Most of the culture positive cases who were HIV+ lived in a house with 1 or 2 windows, 51 (79.7%) out of 64 patients. HIV- and culture positive were 16 (76.2%) out of 21 patients. Those with no ventilation followed with a representation of 12.5% and 9.5% respectively. The least number of patients occupied a house with five windows and above. Most of the PTB patients lived in a poorly ventilated environment leading to an increase in transmission of tuberculosis. This was seen mainly with an increase in HIV- patients who lived in houses with 1 or 2 windows.
4.4.6 Culture positive results according to HIV status and history of TB in family.

The chart above indicated that most PTB patients had no history of TB in the family. 37 (57.8%) were among the HIV+ and 14 (67%) were among the HIV- patients. Few cases were noted who had history of TB in the family. 42.2% who were HIV+ and 33.3% who were HIV-.

1 case was lost in culture among those who had history of TB in both HIV+ and HIV- patient and 2 cases were added in culture for the HIV- and 7 cases were added among the HIV+.
### 4.4.7 Culture positive results according to HIV status and number of occupants.

#### Table 4.4.7

<table>
<thead>
<tr>
<th>Occupants</th>
<th>HIV status</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1-2</td>
<td>44 (68.8%)</td>
<td>11 (52.4%)</td>
</tr>
<tr>
<td>3+</td>
<td>20 (31.2%)</td>
<td>10 (47.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>64 (100%)</td>
<td>21 (100%)</td>
</tr>
</tbody>
</table>

Fifty five (64.7%) of culture positive patients lived in a house with 1 or 2 occupants. Out of these 44 (68.8%) represented HIV+ patients and 11 (52.4%) represented HIV- patients. Those patients who occupied houses with three individuals and above represented 31.3% among the HIV+ and 10 (47.6%) among the HIV- patients.

There was an increase of cases in culture positive patients among the HIV- patients with occupants of both groups and 1 case reduced among the HIV+ who lived with 1 or 2 occupants and 2 cases increased with those living with 3 persons and above. Overcrowding as a factor among the cases lead to the high transmission of PTB.

### 4.4.8 Culture positive results according to HIV status and size of the house.

#### Table 4.4.8

<table>
<thead>
<tr>
<th>Size of house</th>
<th>HIV status</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1-2</td>
<td>64 (100%)</td>
<td>19 (86.4%)</td>
</tr>
<tr>
<td>3-4</td>
<td>0 (0%)</td>
<td>2 (13.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>64 (100%)</td>
<td>21 (100%)</td>
</tr>
</tbody>
</table>

Most of the PTB cases lived in 1 or 2 rooms. The highest percentage was noted among the HIV+ cases with 100% and only 19 (86.4%) was noted among the
HIV- patients. Those patients who occupied 3 or 4 rooms were only noted among the HIV- were 2 (13.6%). An increase of 2 patients was seen among the HIV+ cases and 3 patients increased among the HIV- who occupied 1 or 2 rooms. 1 case of HIV+ who occupied 3 or 4 rooms was lost during culture and 2 cases or HIV- were captured during culture that lived in 3 or 4 rooms. Poor housing is a major contributing factor of spread of TB as seen in the data above.

4.4.9 Comparison of direct/concentrate microscopy versus culture as gold standard.

Table 4.4.9

<table>
<thead>
<tr>
<th></th>
<th>Culture method (n/%)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct microscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>53 (62.4%)</td>
<td>53 (35%)</td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>85 (100%)</td>
<td>150 (100%)</td>
</tr>
<tr>
<td>Concentrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>74 (87%)</td>
<td>79 (53%)</td>
</tr>
<tr>
<td></td>
<td>5 (8%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>85 (100%)</td>
<td>150 (100%)</td>
</tr>
<tr>
<td>-</td>
<td>11 (13%)</td>
<td>71 (47%)</td>
</tr>
<tr>
<td></td>
<td>60 (92%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4.9 shows that the results for direct microscopy and concentration methods in comparison with the gold standard. All positive cases from direct smear microscopy were detected by concentrate and culture method. 74 (87%) of the concentrate smear positives were detected by the culture method out of the 79. However, culture method yielded 11 (13%) more missed in the concentrate method.

Results obtained from direct smear microscopy was compared to the culture method. All cases that were found to be the 53 (62.4%) in direct smear microscopy was also confirmed positive with the culture method. However, 32 (37.6%) of the cases that were considered negative with the direct microscopy
technique were rated as positive after applying the culture method. This meant that 37.6% more of the cases were captured in the culture method. There were no false positive results and 65 representing 100% of the cases were diagnosed as negative in both techniques.

There was also an increment yield of 32 (21%) cases by use of culture method. This meant that culture method is more superior to direct microscopy. There was a statistical significance in the relationship \(X^2=0.000\) \(P=0.0001\). For every direct microscopy positive result there was a 95% confidence that it will be the in culture method.

Results obtained from concentrate smear microscopy were compared with culture results. 74 (87.1%) cases in the concentrate smear microscopy that were found to be smear positive were also confirmed positive in the culture method. However, 5 (7.7%) cases that were reported as positive were culture negative meaning that it was false positive case or the organisms were not viable. Nevertheless, there was an incremental yield of 11(12.9%) cases from the culture positive cases meaning that more cases were captured. 60 of the cases were confirmed negative using both techniques. From the 150-sample size there was an incremental yield of 11(7%) culture positive cases by use of culture method when compared with concentration method. The comparison was statistically significant \(P=0.0001\).
4.4.10 Direct and concentrate microscopy versus culture (Gold standard) determination of sensitivity, specificity, positive and negative predictive values

Table 4.4.10

<table>
<thead>
<tr>
<th>Culture method (n)</th>
<th>Direct microscopy</th>
<th>Concentrate microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Direct Positive</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>32</td>
<td>65</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>65</td>
</tr>
</tbody>
</table>

Looking at the determinance of the test, the sensitivity of direct microscopy versus gold standard was 62.4%. The specificity was 100%, the positive predictive value was 100% and the negative predictive value was 67.0%

The sensitivity of concentrate versus gold standard was 87.1% and the specificity was 92.3%, the positive predictive value was 93.7% and the negative predictive value was 84.5%

The indication was that an incremental yield of sensitivity from directs microscopy to concentrate microscopy by 24.7%.

4.4.11 Results of the contaminated specimens from (LJ)the culture method

Table 4.4.11

<table>
<thead>
<tr>
<th>Contaminated</th>
<th>Frequency(n)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>3.3</td>
</tr>
<tr>
<td>Not contaminated</td>
<td>145</td>
<td>96.7</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>
Out of 150 specimens, 5 representing 3.3% were contaminated. Contamination may be due to under decontamination, use of a high volume of specimens or improper vortexing.

4.4.12 Results obtained from MGIT 960 system and LJ

Table 4.4.12

<table>
<thead>
<tr>
<th></th>
<th>MGIT</th>
<th></th>
<th>LJ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percent</td>
<td>Frequency</td>
</tr>
<tr>
<td>(n)</td>
<td>(%)</td>
<td></td>
<td>(n)</td>
</tr>
<tr>
<td>No AFB</td>
<td>68</td>
<td>45.3</td>
<td>81</td>
</tr>
<tr>
<td>AFB +</td>
<td>82</td>
<td>54.7</td>
<td>69</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

All morning samples were subjected in automated MGIT 960 system for a period of 42 days to rule out the negative samples. The mean time for sample to give a signal was 10 days ranging from 2.3-43.17 days/hours. The yield of mycobacteria among the samples was 82 (54.7%) compared to 69 (46%) in LJ media. The mean culture growth units in MGIT 960 system was 3,999.38 ranging from 44 to 25,149 units. MGIT system gave an incremental yield of 13 (8.7%) meaning that it was superior to LJ medium because of higher yield. Another advantage was the short turn around time.

Contamination of mycobacterial growth was done by ZN stained smear and inoculation on blood agar medium for purity. 5 tubes were found not to have AFBs and 10 tubes grew other bacteria after 48 hours. The contamination rate of MGIT system was found to be 10% compared to that of morning samples from LJ with only 0.7%. What was noted here was that it was important to have multiple specimens from each patient in order to capture more yield as seen in LJ where multiple specimens yielded 85. While single morning specimens compared to morning MGIT culture yielded on 69 positive cases.
4.5.0 Identification of mycobacterial growths on LJ

4.5.1 Average growth from LJ media

Table 4.5.1

<table>
<thead>
<tr>
<th>Growth length</th>
<th>HIV status (n/%)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13-19 days</td>
<td>31 (55.4%)</td>
<td>15 (51.7%)</td>
</tr>
<tr>
<td>20-29 days</td>
<td>17 (30.4%)</td>
<td>12 (41.4%)</td>
</tr>
<tr>
<td>&gt;= 30 days</td>
<td>8 (14.3%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td>56 (100%)</td>
<td>29 (100%)</td>
</tr>
</tbody>
</table>

The average time for growth of mycobacteria on LJ medium was 21 days. A total of 46 (54%) grew between 13-19 days. Among these 31 (53%) was from HIV+ group and 15 (52%) was from HIV negative group. A total of 29 (34%) grew between 20-29 days and 10 (12%) grew above 30 days. These showed that majority of the isolation was seen in the groups 13-19 days and the least seen in the groups above 30 days. HIV+ cases still accounted for higher percentages among the growth rate length.

4.5.2 Mycobacteria identified as MTB or MOTT in the study population.

Chart 4.5.2

MTB 91.8%

MOTT 8.2%
Morphological identification was done and ZN stain for the presence or absence of cord factor. Niacin and nitrate tests were performed for the identification of MTB and MOTT. For niacin and nitrate positive tests the results were 78 (91.8%) of MTB, which were identified. 7 (8.2%) were negative in all the tests or positive in either were positively identified as MOTT. Most of the specimens identified were mycobacteria tuberculosis prevalence being 52% in the study population and 91.8% among the culture positive cases.

4.5.3 Relationship between mycobacteria identified and HIV status of the patients

Table 4.5.3

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Culture identified (n/%)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTB</td>
<td>MOTT</td>
</tr>
<tr>
<td>+</td>
<td>58 (90%)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>-</td>
<td>20 (95%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Total</td>
<td>78 (92%)</td>
<td>7 (8%)</td>
</tr>
</tbody>
</table>

Fifty eight (74%) of the isolates were MTB from HIV+ patients whereas 20 (25%) were from HIV- patients. 6 (86%) of the isolates were MOTT from HIV+ patients and 1 (14%) was MOTT from HIV- patient. Prevalence of MTB among HIV+ individuals was 58 (90%) and that of MOTT was 6 (10%) prevalence of MTB among HIV- patients was 20 (92%) and that of MOTT was 1 (5%) in the PTB positive population identified in culture.
4.5.4 Relationship between identified mycobacteria, HIV status and history of previous TB infection.

Table 4.5.4

<table>
<thead>
<tr>
<th>HIV status</th>
<th>MTB (n/%)</th>
<th>MOTT (n/%)</th>
<th>Total (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5 (8.6%)</td>
<td>0 (0%)</td>
<td>5 (41%)</td>
</tr>
<tr>
<td>No</td>
<td>53 (71.4%)</td>
<td>6 (100%)</td>
<td>59 (59%)</td>
</tr>
<tr>
<td>Total</td>
<td>58 (100%)</td>
<td>6 (100%)</td>
<td>64 (100%)</td>
</tr>
<tr>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1 (5.0%)</td>
<td>1 (100%)</td>
<td>2 (9.5%)</td>
</tr>
<tr>
<td>No</td>
<td>19 (95.5%)</td>
<td>0 (0%)</td>
<td>19 (90.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>20 (100%)</td>
<td>1 (100%)</td>
<td>21 (100%)</td>
</tr>
</tbody>
</table>

Fifty-three (91%) had no previous infection, were HIV+ and MTB was isolated from them. 6 (100%) had MOTT in HIV+ population who had no previous infection. This indicated that MOTT was not isolated from previously infected individuals who got re-infected with PTB.

Nineteen (95%) had no previous infection, were HIV- and MTB was isolated from them. 1 (5.0%) had a previous TB infection of which MTB was isolated. One case representing 100% had a previous infection from HIV- patient and MOTT was isolated.
CHAPTER 5

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1.0 Socio demographic characteristics

All the patients recruited in this study were from Mbagathi District Hospital. This site was chosen because it is a public sector where free diagnostic and medical treatment is given through the NLTP program. There is also a Non Governmental Organisation (NGO) offering free management and medication of HIV infected patients.

The study group subjects were adult population with a mean age of 32.4 years ranging from 18-68 years. Among these subjects 60% formed the bulk that was HIV positive whereas 40% formed those who were HIV negative.

From the study the age groups that were most infected were from 23-37 years in both males and females though all the age groups were also infected. This was seen in both males and females but males were infected more contributing to 55%. The results also indicate that most of the PTB cases were HIV positive which obviously tells us that people living with HIV/AIDS are the major subgroups with increased incidence of tuberculosis. This result was in line with Report given by NLTP/MOH that indicate the age group experiencing a generalized TB epidemic is at 15-44 years with the highest notification in 25-34 years in both males and females that has been the trend for over the last decade. The males are 1.4 times more likely to have TB than females. This was the same age category with a high HIV socio-prevalence.  

8
A study done in Somalia indicated that the age groups who were highest infected with PTB was 35-39 years and this group had the highest HIV seroprevalence. This also affected both sexes but men were more than females. The incidence was higher in males than in females regardless of the population perhaps because of their weak immune mechanism and delayed health seeking behaviour.

Most of the infected individuals were married and contributed to the socio demographic factors that contribute to the transmission of TB. Loss of income due to long periods of illness leads to poor socio economic status coupled with poor nutrition and limited access to health services. PTB cases lived in a 1-2 roomed house and where occupants were between 1-3 and 3 and above. These factors were very important considering the poor socio economic status of the study population leading to overcrowding thus increasing burden of TB. In line with this study, studies done by Treq showed that contrary to the rural areas the number of TB patients in big cities was high in a limited space due to the population density, the high level of transmission due to overcrowding and the attractiveness of services in the big cities.

Theories indicate that following exposure to the bacillus the risk of infection with the bacillus is related to the extent of contact that is determined primarily by the proximity to and length of contact with the infectious person. A person is most likely to be infected with the tubercle bacilli if they spend long hours with an infectious person who is not on treatment. Studies done by S. Dalal et al among hospital staff of an 1800 bed hospital in Nairobi indicate that the contact per day was at an Odds Ratio (OR) of 65 being the relative risk of the staffs being infected with TB. Those infected were most likely HIV positive. This study is true considering the issue of poor housing conditions and overcrowding. The findings also indicate that most of the patients lived in the small crowded houses with little ventilation representing 88% and most lived in houses with 1 or 2 windows for ventilation representing 94% of all HIV positive cases.
6% of HIV -ve PTB cases respectively. Studies also done by S. Dalal et al on the same hospital staff concluded that ventilation should be fully addressed in the hospital because many staff become infected with TB due to lack of ventilation in the rooms besides the long contact hours.

Ventilation is the most important factor that fuels the spread of mycobacterial infections. Droplets containing bacilli generated by patients in a poorly ventilated environment remain buoyant in the ambient air for a long period of time being the principle source of transmission of MTB.

A study done in the United States navy ship provided information on the importance of air circulation and ventilation. Persons previously known to be tuberculous skin test negative ultimately became infected from a single source then sharing the same compartment with the cases that had the highest frequency of skin test conversion. Nevertheless a very high proportion of those living in other compartments and those who did not work or significantly socialise with the index case, but whose compartments were connected through a close circuit ventilation system also became infected. Conversion continued for a prolonged period of time after removal of the infectious source cases, suggesting that air in closed and poorly lighted circuits may contain viable infectious droplet nuclei for a prolonged period of time.

It is evident that the high incidence of TB in the community was in the urban slum with a high population density. Most of the PTB cases were from the divisions of Langata and Embakasi known to house the major slums. Poverty level in these areas is too high and the risk of developing the disease following infection with tubercle bacilli mainly affect those individuals who are poorly nourished, young children especially under one year of age and those with poorly immune defences especially people infected with HIV/AIDS. Current data indicates that TB cases occur mostly among slum dwellers in the large cities. Observations made by S. Kowao indicate that management of PTB is a huge challenge and a constant pre-occupation of NLTP program in urban cities. Demographic growth
in the urban areas of Africa is high and another characterization of urban areas of Africa is concentration of population in the capital cities.

The history of TB in the family and previous TB infection in the PTB cases had no strong association on finding new PTB cases. What was mainly observed was the HIV TB co-infection in a bulk of patients and socio-demographic factors including poor ventilation overcrowding and poor socio economic status that were seen to be closely related.

It is clear in the study that people living with HIV/AIDS are the major subgroups with increased incidence of tuberculosis coupled with overcrowded slums.

5.2.1 Diagnostic techniques

Proper identification of PTB cases is the pillar of TB control program and bacteriology is one of the fundamental aspects and key components of the DOTS strategy. The study population was characterised by high HIV prevalence that causes a reduction in the sensitivity of the routine laboratory direct smear microscopy test. This in turn complicates the diagnosis of tuberculosis disease, hence the search for more sensitive diagnostic tools that at the same time should be feasible in a setting with limited resources.

Ravighone et al have predicted that with the global resurgence of TB, the average number of new cases will increase to 163 per 10000 in the year 2000. While in Kenya NLTP report indicate that case notification rate rose from 51-320 per 10000 population between 1987 and 2004.

5.2.2 Yield of direct smear microscopy among HIV positive and negative patients.

The findings of the study show that out of 150 patients, 53 (35%) AFB+ cases were detected by direct smear microscopy overall. Among the HIV positive
patients a total of 43 (48%) and 10 (17%) were infected with mycobacteria. These results showed no difference among the study population and were statistically significant. Tuberculosis is the most common opportunistic infection in HIV/AIDS and accounts for a disproportionate burden of morbidity and mortality in the co-infected patients. HIV is the most significant risk factor for development of TB and HIV patients are at a risk for primary and reactivation of diseases as well as exogenous re-infections.

Thirty to forty percent of all new TB cases in many African countries occur in HIV co-infected persons, with ranges as high as 60-70% in some Eastern and South African nations.

In studies reviewed by Githui direct microscopy using acid fast stain is relatively insensitive diagnostic procedure with a reported sensitivity ranging from 25% to 65% when compared to the gold standard. This range is in line with observations made in the current study.

Also in line with the study, studies done by Ellena M. Peterson et al showed that cumulative results for initial 3 specimens detected 34% of yield of MTB. Dhengra V.K et al worked on sputum samples and got a yield of 20.2% of positive direct smear microscopy. This was far below the yield of the current study.

Ukwandu et al demonstrated a 15% yield of positivity in 24 samples out of 160 samples analysed in a West African country. This set up being an African country indicate that a lot needed to be done because of its very low yield. This translates to many cases being missed in the community. Studies done by Farnia et al showed that 41 patients representing 9.5% was direct smear positive out of 430 patients. The sensitivity of direct smear microscopy in most parts of Iran where the study was done was at 25-50%.

Studies done by L.Lawson et al on a total of 3204 direct smear microscopy yielded 29.9% were positive. In a rural setting in Tanzania Yahya A.I. et al
demonstrated that out of 61580 suspected tuberculosis cases analysed 11650 yielded 18.9% in direct smear microscopy\textsuperscript{15}.

In the current study, the 36% yield was from an urban set up compared to the 18.9% from a rural set up in Tanzania. This means that the impact of TB in urban centres is overwhelming and much more resources need to be channelled to curb the transmission of PTB by early diagnosis. It appears that sufficient numbers of Acid Fast Bacilli (AFB) are shed into deep respiratory secretion to be detected by routine microscopy in the majority of HIV infected adults with active TB.

The sensitivity of sputum direct smear microscopy in the study is 62.4% being the capacity of the test to correctly identify in a population those individuals who have the disease. The specificity of the test is 100% being the capacity to correctly identify those who are free from the disease. These two characteristics are the determinants for the accuracy of the test results. Other determinants like positive predictive value that was 100% being the probability of having the disease among the group detected by direct smear microscopy and the negative predictive value which is 67.0% being the probability of not having the disease among the group classified as negative.

Molinder et al in their study of determinance of disease observed a sensitivity of 57.4\%, specificity of 97.8\%, and positive predictive value of 96.8\%\textsuperscript{38}. A specificity and sensitivity that approaches 100\% is the best test. In the study all determinance were not approaching 100\%.

5.2.3 Yield of concentrate smear microscopy among the HIV positive and negative patients.

The findings of the study showed that out of 150 patients suspected to be infected with PTB, 79 cases were detected by concentrate smear microscopy.
representing a yield of 53%. Among the HIV positive patients a total of 63 (70%) and 27 (30%) of HIV- patients had positive smears. The yield of 53% detected by concentrate smear showed no difference among the study population meaning that the yield was statistically significant.

The results indicate that there is a substantial increase in the yield of detecting smear positive patients by use of concentrate microscopy over the direct microscopy. Generally there was an increase from 35% to 53% giving an incremental yield of 18%. The study was in agreement with a recommendation made by WHO recognised the use of centrifugation for concentration of specimens for microscopy by use of various chemical methods in 14 studies showing 18% mean increase in sensitivity ranging from -3% to +39%.

Among the HIV positive patients who had positive smears, an increase is seen from 48% by use of direct method to 70% by concentrate giving an incremental yield of these cases by 22%. Bruchfeld et al in their study stratified their results according to the HIV status of the patients and concluded that among HIV positive patients the concentration method increased the sensitivity of sputum smear from 38.5% (direct microscopy) to 50%. This was compared to the overall sensitivity that increased from 54.2% to 63.1%. The study gave a better yield of 70% compared to 63.11% of Bruchfeld.

Studies by Ellena M. Peterson et al shows that cumulative results for initial 3 specimens from 2693 patients using concentrated specimens yielded 58% compared to 34% from direct smear. An incremental yield of 22% was realised.

Ukwandu et al demonstrated the same method and gave a yield of 41 representing 25.6% from samples. An incremental yield of 10.6% was released with conventional (direct microscopy) giving 15%. Farnia et al in Iran used N-acetyl L Cystine (NAL-C) for concentration of AFB. Out of specimens analysed
16% was yielded by concentrate method. An increase was realised from 9.5% to 16% therefore an incremental yield of 6.4% \(^{18}\).

In Zambia the same method increased the sensitivity of the ZN stain from 43.4% to 76.3%. An incremental yield of 32.9% was obtained \(^{19}\). This yield was exceptionally high than the current study but was within the WHO recommendation.

It appears that the high incremental yield meant that so many PTB cases were not captured during the direct smear microscopy leading to infection to the community from those cases. Between 25% and 60% patients with PTB have smear negative results. The diagnosis of TB may be initially missed in \(\leq 50\)% of these patients \(^{51}\). The utility of concentrate diagnostic test in the early detection of smear negative TB is therefore an important clinical consideration. It is also of considerable public health interest.

The concentrate smear microscopy shows that at 95% confidence all positive smears in direct microscopy are detected.

- The sensitivity of concentrate microscopy is 87.1% being the capacity of the test to correctly identify those individuals who have the disease. Whereas the specificity of the test is 92.3% being the capacity to correctly identify those who were free from the disease. Both determinance approached 100% so the concentrate test was more accurate than direct microscopy. Positive predictive value is 93.7% and negative predictive value is 84.5% meaning that also both determinants are approaching 100% hence concentrate test is reliable in distinguishing infected from non-infected persons. Molinder et al found a sensitivity of 70.4%, specificity of 95.6% and a positive predictive value of 95.0%. All determinance were approaching 100%.
5.2.4 Yield of culture on,

(a) Solid media LJ among the HIV positive and negative patients

The findings of the study shows that (table 4.5.1) out of 150 patients suspected to be infected with PTB 85 cases representing 56.7% yielded by use of culture method being the gold standard. Among the HIV positive patients a total of 64 representing 75.3% and 26 representing 24.7% of the HIV negative patients had PTB.

The results indicate that there is a substantial increase in the yield of detecting PTB patients by use of culture method. Generally, there was an incremental yield of 32 representing 21.8% missed cases in direct microscopy. 11 cases were added representing 4% in the concentrate method. The culture method confirmed all the 53 (35.3%) cases detected from the direct smear microscopy. This means that at 95% confidence culture can be able to detect a positive direct microscopy hence referred to as the gold standard.

On the positive concentrate method, culture method isolated 74 (93.7%) positives out of 79. This indicated that the remaining 5 specimens that were positive in concentrate and negative in culture method could be false positives, contaminants or Mycobacteria that were not viable or over decontamination was done leading to death of the bacilli. However, was an addition of 11 cases that were not detected in concentrate smear.

Nevertheless, culture remains the gold standard for isolation of mycobacteria. HIV positive patients contribute to the highest percentage of PTB cases simply because TB is an opportunistic infection in HIV positive patients. Blanc L recommended that in addition to improving sputum smear microscopy there is need to introduce culture and disk sensitivity testing to improve the diagnosis and identify MTB which is very important in TB control programs. Studies done Ukwandu et al working on 162 samples demonstrated an incremental yield of 13.2% having increased from 25.6% by use of concentrate smear to 38.8%

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Dongsi et al showed that the sensitivity of LJ culture in detecting isolates was 59.7% which was in line with the current study compared to the automated systems. Also Dongsi et al highlighted Jesus et al 2001 work who observed a sensitivity of 46.6% while Lu et al 2000 observed a sensitivity of 45.5% by use of LJ medium for the isolation of MTB.

The isolation rate on LJ medium in the study was a mean time of 21 days ranging from 13-45 days. Most of the growth was seen in 13-19 days simply because of the high prevalence of TB in our country. Also recovery time for MTB in tropical countries with high HIV/AIDS epidemic and a low standard of living is 14-25 days.

The contamination rate for the LJ medium was at 3.3% being within generally acceptable range of 2%-5%. This means that the standard operating procedures were critically applied.

Harris G. compared the culture techniques using clinical samples. The isolation rates of LJ were 78% and had a mean time of 19 days. Garcia et al isolated 93 Mycobacteria, out of these 66 representing 75% was isolated from LJ and a mean time of 27 days was observed. This observation still shows that LJ medium is able to support the growth of Mycobacteria especially MTB.

A study correlating with the current study is that of Paelaceous et al where out of 5208 samples recovered 52.5% smear positive and a yield of 66.4% in LJ medium culture where LJ gave a mean growth time of 16.5 days. Akos et al studied and observed that the recovery rate of LJ medium was 81.8% compared to other automated system that had a higher yield. LJ was always used together with the automated systems because it has remained the gold standard. A mean time of 20.1 days and a contamination rate of 1.2% were observed.
Elizabeth et al observed a 27.1% positive case for AFB and in LJ culture the samples yielded 34.7%. Still making LJ the gold standard the mean recovery time on the LJ medium was 34.7 days\textsuperscript{21}. Besides LJ medium taking a long time to isolate Mycobacteria the contamination rate is very minimal compared to the new systems. This was also seen in the study.

V. Mirovic and co-workers evaluated 251 isolates of Mycobacteria isolates and 67.3% was isolated on LJ. A mean time of 22.1 days and a contamination rate of 7.7% were observed\textsuperscript{44}.

(b) On MGIT system

A total of 150 morning sample were subjected to the MGIT 960 automated system gave an 82 isolates representing 54.7% positive when compared to the LJ method that gave 69 isolates representing 46% positive. An increment yield of 9% was observed. The mean time for isolation in the MGIT system was 13 days ranging from 2.3-43.17 days meaning that MGIT system is the best because of short turn around time compared to LJ that was 21 days. A. Apers also demonstrated a turn around time of 10 days\textsuperscript{36} besides of concern was the contamination rate was 10% which was considered high keeping in mind the acceptable range of 1.8 to 8.4%. Apers demonstrated a contamination rate of 14.7% of all specimens that gave a positive signal.

Confirmation of Mycobacterial growth for all culture system was by ZN stained direct smears. Purity of the MGIT system was checked by inoculation on nutritional Muller Hinton agar containing 5% horse blood and incubated aerobically at 37°C for 48 hours and then examined for growth.

Other studies done indicate that MGIT 960 system was more sensitive than the LJ medium and the recovery rate was minimal but the contamination rate was high. Growth of 96.4% in MGIT, recovery rate of 12.6 days and a contamination
rate of 3.7% compared to LJ with 81.1% 20.1 days and 1.2% respectively. 93.2% growth in MGIT, recovery rate of 13.8 days and a contamination of 8.1% compared to LJ with 67.3%, 22.1 days and 7.7% respectively. All authors concluded that contamination with rich MGIT medium is more common than in other culture medium but short turn around time.

5.2.5 Identification of MTB compared to MOTT among the HIV positive and negative patients.

The mean growth rate time on LJ medium was 21 days that was within the acceptable range of 14-25 days in countries with high prevalence of HIV.

The prevalence of MTB in the study population was 91.8% and that of MOTT was 8.2% meaning that MTB was highly prevalent in our set-up. Among the HIV positive patients 91% had MTB and 9% had MOTT. Among the HIV negative patients 95% had MTB and 5% had MOTT. MOTT was seen in higher proportion in the HIV positive individuals than the HIV negative patients despite the fact that MOTT was of very low prevalence in the community. Presence of MOTT among the HIV positive individuals indicates that MOTT is an opportunistic infection that causes PTB like illness. Ritchter recommended that there could be a possibility of infection with MOTT and may easily be isolated as non-pathogenic. The presence of MOTT in HIV patients in high incidence countries should be considered. All isolated mycobacteria should be identified before disk sensitivity testing. Usually MOTT is known to be resistant to some or all of the regular drugs leading to false resistance.

Published recovery rates of Mycobacteria species range is from 53.6% to 95.9%. The recovery of MOTT isolates on LJ is lower with 24.6%. LJ medium is known not to be the best medium for isolation of MOTT because LJ is basically designed primarily for growth of MTB. This was in line with the present study.
Other studies done by Akos isolated 96.5% of MTB and 3.5% MOTT from 377 clinical specimens. V. Mirovic demonstrated the recovery of 91.2% MTB and 8.8% of MOTT from LJ medium. In MGIT system they made a recovery of 77.4% MTB and 22.6% MOTT. Out of 116 smear positive sputum identified 49% grew MTB and 32% grew and identified as MOTT and culture negative was in 19% of the patients. It is suggested that regional variation in the frequency of infection with environmental mycobacteria should be considered in order to keep in line with the right treatment.

Accuprobes and other molecular identification techniques are the best methods because of accuracy, precision and short turn around time. Due to its expense it is only used in research laboratories in our set up and in developed countries.
The diagnosis of PTB in our country is wanting given that only direct smear microscopy is employed and only culture technique are done in National Reference Laboratory for only patients who have been or are in treatment for DST. Direct microscopy although quick and easy to perform has been confirmed by this study and other studies of being poorly sensitive. Many authors have criticised the technique still because of perceived low sensitivity particularly so in HIV/AIDS patients.

High poverty levels among the population and poor immune defences system are seen to fuel the TB epidemic. Poverty reduction will have an important impact on tuberculosis incidence.

Concentration method added substantially to the sensitivity of direct microscopy. Although it slightly increased the time of investigation, the procedure is simple and can be applied easily in a district laboratory with basic equipment and staff training. The only disadvantage is the possible increased risk in contamination for the laboratory personnel. If safety cabinets are available and good laboratory practice done, this risk can be reduced to acceptable minimum levels. The advantage of this method for low-income countries has been described in Ethiopia and India.

Culture method gave an addition yield of PTB cases that were not identified in both techniques. Though culture was found to be the gold standard its use is important for isolation, identification and direct sensitivity testing of PTB because of the difficulty faced in diagnosis of PTB in a setting with high prevalence of HIV leading to many smear negative PTB patients. Culture method was found to be a very expensive process needing special laboratories, equipment, supplies and highly trained personnel.
Despite all the difficulties WHO second plan recommends that countries should increase their capacity for Mycobacterial culture and sensitivity tests. The identification of mycobacterial species was also very important for the purpose of sensitivity and epidemiological purposes. Many of the isolates in the study were MTB having the highest prevalence of 91.8%. The few percentage of MOTT was mainly from HIV positive individuals, indicating opportunistic infections for the immunocompromised individuals. This must be taken into consideration especially when those patients do not respond to treatment regimen.

5.2.7 RECOMMENDATIONS

The study findings can be used as the basis for a number of policy recommendations.

- The study was only done in Nairobi that is an urban set up. There is need to replicate the same in other parts of the country to come up with a more generalised view.
- Upgrading slum areas. This was seen in the study where most of the patients were living in a house with very poor conditions such small size of houses, poor ventilation and overcrowding. Since poverty and tuberculosis are closely interrelated there is need to reduce and it will have an important impact on tuberculosis incidence while TB control should be an important poverty reduction strategy.
- Concentration method was found to have a higher sensitivity therefore it is recommended that it be widely used in a setting such as Kenya, where the diagnosis of PTB still relies on bacteriological direct smear microscopy.
- It was also noted that culture techniques had a considerable advantage in that the more positives were captured though the turn around time was long. There is need to set up culture reference laboratories in all the...
provincial hospitals in Kenya so that culture can be done on all cases and sensitivity done for proper treatment results and to avoid empirical treatment especially on the smear negatives.

- It was evident from the study also that there was some prevalence of MOTT. The need to perform identification from the culture isolates is very important before performing the sensitivity because most of the MOTT are resistant to most or all anti-TB drugs used. This will lead to giving a false report on the surveillance of resistance.

- There was need to integrate closer the laboratory and clinical services for maximum management of the patients. The system should ensure that timely laboratory testing and timely flow of information among laboratories clinicians and TB controllers.

- It was also observed that cumulative results of multiple specimens yielded an increase in cases. There is need to have multiple specimens especially during culture because that is already done in the microscopy.
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APPENDICES

Appendix i

INFORMED CONSENT EXPLANATION FORM

TITLE OF THE STUDY: Diagnosis of pulmonary Tuberculosis among HIV positive and negative patients in Mbagathi District Hospital: A comparison of direct smear, concentrate and culture technique.

RESEARCH PARTICIPATION CONSENT FORM

My name is Sheilla J.Chebore. I am undertaking a postgraduate study at the University of Nairobi, Department of Medical Microbiology. I would like to request you to participate in a medical research study. The objective of this study is to determine the yield of diagnosing Pulmonary Tuberculosis by use of concentrating the sputum specimen for smear preparation and culture for isolation and identification of the causative organism. These two techniques shall be compared with the usual use of direct unconcentrated direct smear microscopy. Participants who will be diagnosed with pulmonary Tuberculosis in any of the three techniques and are either HIV positive or negative shall fulfill the objectives. The register that contains the results of HIV testing shall be confidential between the clinician who will be managing you and the researcher. The information obtained from the research shall benefit you as the participant to receive the right treatment, your family and community because transmission of the infectious disease shall be controlled and government. Before you answer any question, it's very important that you understand the following principles that apply to all the participants in the study.

1 Participation is entirely voluntary and no coercion will be done or any form of bribes given.
2 Your confidentiality will be safeguarded between the clinician and the researcher in both TB and HIV results. Names of the participants will not appear in any final report or publication resulting from the study.

3 Refusal to participate will involve no penalty. Services will be provided despite refusal to participate in the study.

4 There will be no risks to be incurred for participating in the study. The test does not involve any invasive procedure because only expectorated sputum is required.

5 If there is any part of this consent explanation sheet that you do not understand, ask the investigator before signing below.

In case of any problem or concern, you may contact my supervisors or me through the following number: 0723-679610 or the KNH Research and Ethics Committee P.O Box 20273 NAIROBI.

FOR PARTICIPATION

I-----------------------------------have fully understood the objectives of the research and hereby sign as a show of willingness to participate in the study

Signature--------------------------date-------------------------------------------

WITNESS BY

Signature--------------------------date-------------------------------------------
Appendix ii
Research tool/Questioners

CODE-------------------

1. **BIODATA**
   1. Name of health institution __________________________ Date __________________
   2. Age __________________
   3. Sex M □ □ F
   4. Marital status: Single □ Married □
   5. Residence __________________
   6. Size of the house (number of rooms): 1 2 3 4 5+
   7. Ventilation (number of windows): 0 1 2 3 4 5+
   8. Occupants (number of persons living in house): 1 2 3 4 5 6
   9. History of TB in the family: Yes □ No □
   10. History of previous TB infection: Yes □ No □
   11. HIV status: + □ □

11. **SAMPLE ANALYSIS (MACRO AND MICROSCOPIC)**

1. Visual appearance of sputum
   Mucopurulent, blood stained
   Specimen S1 □ □
   Specimen M □ □
2. Direct Microscopy results

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<tr>
<td>SI</td>
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<tr>
<td>M</td>
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<td>S2</td>
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3. Concentrate microscopy results

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<tr>
<td>M</td>
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<tr>
<td>S2</td>
<td></td>
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**111. CULTURE**

Culture method

(a) Culture results

**S1**

- No growth
- Contaminated

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### Confluent

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<td>□</td>
</tr>
<tr>
<td></td>
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<td>□</td>
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<tr>
<td></td>
<td>1-19 colonies</td>
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<td>□</td>
</tr>
<tr>
<td>S2</td>
<td>No growth</td>
<td>□</td>
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<tr>
<td></td>
<td>Contaminated</td>
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<tr>
<td></td>
<td>1-19 colonies</td>
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<td>&gt;100 colonies</td>
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<tr>
<td></td>
<td>Confluent</td>
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### (b) Identification of growth

**SI**

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<tr>
<td>morphology</td>
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<tr>
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<td>positive</td>
<td>□</td>
</tr>
<tr>
<td>Nitrate production</td>
<td>positive</td>
<td>□</td>
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</tbody>
</table>
Others positive □ negative □

Culture identified as *Mycobacterium tuberculosis* □

MOTT □

Growth rate __________ colony
morphology_________________________
Ziel Neelsen stain___________________

Niacin production positive □ negative □

Nitrate production positive □ negative □

Others positive □ negative □

Culture identified as *Mycobacterium tuberculosis* □

MOTT □

S2
Growth rate __________ colony
morphology_________________________
Ziel Neelsen stain___________________

Niacin production positive □ negative □

Nitrate production positive □ negative □

Others positive □ negative □

Culture identified as *Mycobacterium tuberculosis* □
### IV CULTURE

**Culture method**

(a) culture results

**M**

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<td>Days/hours for growth</td>
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<tr>
<td>ZN Stain</td>
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</table>

Subculture on SBA at 24 HRS

48HRS

Gram Stain on growths

113
Appendix iii

LABORATORY METHODS

(a) Ziehl Neelsen stain

Acid fast organism retains the basic stain after being treated with decolourizer and the background takes the counter stain.

Method
1. Place air dried heat fixed slide on a straining rack
2. Flood the entire slide with 5% Ziehl Neelsen carbol and heat the back of the slide until steam comes and leave for 5 minutes.
3. Rinse each slide in a gentle stream of running water.
4. Flood the slides with 25% sulphuric acid and leave for a maximum of three minutes.
5. Rinse the slide thoroughly with water and drain off excess
6. Flood the slides with methylene blue and leave for 2 minutes.
7. Rinse the slide wipe the back side and air dry

Expected results – red rods AFB.

(b) Digestion and decontamination of sputum species and culture.

This is done to reduce undesirable bacterial growth and to liquefy mucus to release bacilli.

Method
1. To 50ml of plastic centrifuge tube, add 3-5ml of the sputum and add equal volume of 4% sodium hydroxide.
2. Vertex for 15-20 seconds or until well mixed and let it stand for 15 minutes at room temperature with occasional shaking.
3. Add phosphate buffer 6.8 or sterile distilled water upto 50ml mark
4. Concentrate the specimen by centrifugation at 3000g for 15 minutes.
5. Decant all the supernatant into a container with phenol and add 2ml of phosphate buffer pH 6.8 or sterile distilled water.
6. Resuspend the deposit and transfer 2 drops of the concentrate to LJ medium and 500ul to MGIT tubes.
7. With s wire loop transfer a loopful of the concentrate into a glass slide.
8. Incubate all the medium at 37°C and MGIT tubes to the MGIT machine. Air dry the slides fix and stain with ZN.

(c) Niacin test
Niacin plays a vital role in oxidation reduction reactions, MTB accumulates the largest amount of nicotinic acid its detection is useful in diagnosis
1. Add 1ml of sterile water to the surface of LJ on when the test organism is growing and lay the tube horizontally.
2. Puncture the surface of the slant several times to allow the niacin in the medium to dissolve in the water.
3. Keep the tube tilted in room temperature for 30 minutes.
4. Transfer the suspension to a sterile screw cap test tube.
5. Insert the Niacin reagent strip, cap tightly incubate at room temperature for 15-20 minutes occasionally shaking.
6. Observe the colour of the liquid.
   Interpretation: Positive test – Yellow liquid
   Negative test – Clear liquid

(d) Nitrate reduction test
MTB is one of the strongest reducers of nitrate. In combination with niacin test a definitive diagnosis can be made.
Method
1. Add 0.2ml of sterile distilled water into screw cap tube
2. Pick colonies of suspected organism and emulsify to make a suspension
3. Add 2ml of sodium nitrate substrate and incubate at 37°C for 2 hours.
4. Add a drop of 50% hydrochloric acid and shake
5. Add 2 drops of reagent A (sulfanilamide)
6. Add 2 drops of reagent B N-Napthtylethylene – diamine and observe for a pink to purple colour.

Interpretation: Pink purple colour – positive

No pink colour and change to a red colour after addition of zinc powder – negative

(e) Interpretation of microscopy results

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Appendix iv

3.1 LABORATORY SAFETY

Studies have shown that the risk of the infection is 3–5 times higher in laboratory workers compared to administrative staff or the general community. The potential consequences of becoming infected with multi-drug resistance strains of MTB and the increasing proportion of persons infected with HIV add a new dimension to the problem and emphasize the importance of strict adherence to safety precautions in the laboratory.

A carefully developed and implemented infection control policy and an appreciation of the concept of universal precaution are fundamental for the protection of all lab personnel. Workers should wear protective garb, perform all procedures in a properly functioning biologic safety cabinet under negative pressure to the lab. A centrifuge container that is sealed to prevent leakage, guards against generation of aerosols that contain infective particles. Rigorous attention to decontamination of work area, general order of the lab and detailed understanding of Mycobacteriology lab design and safety.

Nosocomial transmission of MTB from specimens is of major concern to health care workers. Control of aerosols and other forms of Mycobacterial contamination are achieved in the laboratory by the use of properly functioning biological safety cabinet (BSC), centrifuges with safety carriers and meticulous processing techniques.

All working involving specimens or cultures, such as making smears, inoculating media, adding reagents to biochemical test mixtures, opening centrifuge cups must be performed in a BSC. The handling of all specimens suspected of containing Mycobacteria with the exception of centrifugation for concentration purposes, must be done within the BSC.