COMPARISON OF GENE XPERT AND FLUORESCENT MICROSCOPY FOR DIAGNOSIS PULMONARY TUBERCULOSIS IN HUMAN IMMUNODEFICIENCY VIRUS INFECTED PATIENTS AT MNAZI MMOJA HOSPITAL, ZANZIBAR

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMETAL STUDIES (ENVIRONMENTAL HEALTH STREAM) OF THE OPEN UNIVERSITY OF TANZANIA

2016

CERTIFICATION

The undersigned certifies that he has read and hereby recommends for acceptance by The Open University of Tanzania the dissertation titled "Assessment of Two Techniques for Diagnosis Pulmonary Tuberculosis in HIV Patient at Mnazi Mmoja Hospital Zanzibar" in partial fulfillment of the requirements for the degree of Master of Science in Environmental Studies (Environmental Health Stream) of The Open University of Tanzania.

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Prof. Emmanuel S.P. Kigadye

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Date

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DECLARATION

I, **Abdalla Said Mohamed**, do hereby declare that this Dissertation is my own original work and has not been presented, and will not be presented, to any other university for a similar or any other degree award.

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Signature

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Date

DEDICATION

This piece of work is dedicated to my beloved wife Asma O. Mwinyi, my two sons Tirmidhy and Junaid as well as my daughter Rumaisar.

ACKNOWLEGEMENT

I would like to thank Prof. Emmanuel S. P. Kigadye (supervisor) for his encouragement and advice on how to develop this Dissertation whose experience, constructive criticism, generous support and encouragement gave me energy, strength and self-confidence to finish this study. Dr. Saria (course coordinator), for his recommendation for title setting of this study. Dr. Ahmed and Dr. Ali of Zanzibar Integrated HIV, TB and Leprosy Program, for their information about HIV and TB in Zanzibar. All staff of Microbiology department at Mnazi Mmoja Hospital for their support in this study. All staff of CTC and TB clinic at Mnazi Mmoja Hospital for their support in specimen collection. I would like to extend my gratitude to Ministry of Health Zanzibar and Health Research Unity Zanzibar. Also I sincerely express my gratitude to Dr. Jamala, Director of Mnazi Mmoja Hospital Zanzibar and Miss Fauzia Ameir, the Laboratory manager at Mnazi Mmoja Hospital Zanzibar. Thankful to my wife and my children for their patience and love during this study. Lastly but not least thanks to Mr. Hussein and Mr. Kibwana who worked hard in data analysis assistance.

ABSTRACT

HIV associated TB is not easy to make diagnosis and is linked with extreme disease and death. The spread of MDR-TB, together with increasing harmful of HIV infection and inadequate availability of quick examination instrument have lead to cause disappointment of global TB control. The intention of the study was to evaluate Gene Xpert MTB/RIF technique and smear auramine LED FM technique for detection of pulmonary tuberculosis (PTB) in people living with HIV at Mnazi Mmoja Hospital Zanzibar. The study was experimental type of design that involved laboratory analysis of sputum specimens for determination of *Mycobacterium tuberculosis* as well as rifampicin resistance for HIV patients. The specimens were processed in Gene Xpert MTB/RIF and smear auramine LED FM. The sum of 246 patients sputum specimens were analyzed for the existence of *Mycobacterium tuberculosis* by means of Gene Xpert MTB/RIF and LED FM techniques. The results showed that 169 (68.7%) spot samples (sample I) and 169 (68.7%) morning samples (sample II) were positive for Gene Xpert MTB/RIF technique. Then 112 (45.5%) spot samples (sample I) and 118 (48.0%) morning samples (sample II) were smear positive (LED FM). The sensitivity of LED FM in spot samples and morning samples were 66.3% and 69.8% respectively. Among 169 positive TB cases 2 patients were found with rifampicin resistance. The outcome of this study indicated that the performance of Gene Xpert MTB/RIF machine is more accuracy than LED FM in diagnosis of PTB in HIV patients suspected with TB. Gene Xpert MTB/RIF may be significant as a single quick method for PTB case finding in HIV patients suspected with active TB.

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AFB	Acid Fast Bacillus
ART	Antiretroviral Therapy
BSCs	Biological Safety Cabinets
CD4	Clusters of Differentiation
Cfu	Colon form unit
CI	Confidence Interval
CNS	Central Nervous System
CPT	Co-trimoxazole Preentive Therapy
CTC	Care and Treatment Counseling
DNA	Deoxyribonucleic Acid
DST	Drug Susceptibility Test
ELISA	Enzyme Linked Immunosorbent Assay
EQA	External Quality Assurance
EPTB	Extra Pulmonary Tuberculosis
FM	Fluorescent Microscopy
HEPA	High Efficiency Particulate Air
HIV	Human Immunodeficiency Virus
IPT	Isoniazid Preventive Therapy
LED	Light Emitting Diode
MDR-TB	Multidrug Resistant Tuberculosis
MMH	Mnazi Mmoja Hospital
MTB	Mycobacterium tuberculosis

NAAT	Nucleic-acid Amplification Test
OUT	Open University of Tanzania
PHCU	Primary health care
PTB	Pulmonary Tuberculosis
RIF	Rifampicin
rt-PCR	real time Polymerase Chain Reaction
SPO	Standard operating procedure
SPSS	Statistical Package for Social Science
SR	Sample Treatment Reagent
TB	Tuberculosis
USA	United States of America
UVGI	Ultraviolet germicidal irradiation
WHO	World Health Organization
XDR-TB	Extensively Drug-Resistant Tuberculosis
Xpert	GeneXpert
ZIHTLP	Zanzibar Integrated HIV, TB and Leprosy Program
ZN	Ziehl Neelsen

CHAPTER ONE

INTRODUCTION

1.1 Background

Tuberculosis is still a main public health problem in the world. WHO estimate that between 2000 – 2020, about one billion people will be newly infected, 200 million will become sick, as well as 35 million people will die from TB. Currently, HIV infection is known as the greatest risk factor for development of latent TB infection to active TB. Co-infection HIV and TB particularly in combination with drug resistance have caused outbreaks of increasing death rate. *Mycobacterium tuberculosis* is passed in airborne particles (droplet nuclei) generated when patients with pulmonary tuberculosis cough. These particles, 1 – 5 µm in size, are kept suspended by normal air current. Infection occurs when a susceptible person inhales the droplet nuclei. Once in the alveoli, the organisms are engulfed by alveolar macrophages. Normally, the cell mediated immunity of a host act to resist the multiplication and spread of MTB. However, some MTB can still be viable but inactive for several years after the early infection. The clinical features of PTB are cough, weight loss, night sweats, low grade fever, dyspnoea and chest pain (Murray, 2003).

Global prevalence of TB in 2013 was 159 per 100,000 populations (WHO, 2014). In the year 2013 nine million people who were infected with TB in the world, more than half (56%) were in the South-East Asia and Western Pacific Regions. One quarter was from Africa Region, which also had the maximum morbidity and mortality rates in relation to population. India and China, both accounted for 24%

and 11% of total cases respectively (WHO, 2014). Therefore, in 2013 Africa region scores the highest proportional of new infection of TB per population, which is 280 per 100,000 populations (WHO, 2014). The prevalence of TB in United Republic of Tanzania is 295 per 100,000 populations. This shows that TB is still a major burden in the country, and in Zanzibar TB prevalence is 124 per 100,000 population (MOHSW, 2013).

Today the TB epidemic facilitated by the most important factor in resource limited setting is bad economic condition, which is direct linked to poverty, malnutrition, poor settlement, lack of availability good health care services in free cost as well as community to go to tradition healers to search treatment which may facilitate spread of TB to the community (Cegielsk, 2004). In addition TB epidemic will continue to happen, particularly in Sub-Saharan Africa, due to co-infection of TB and HIV (Karp, 2007).

So far, in third world countries a laboratory which performs TB culture is challenged by inadequate infrastructure, obsolete instrument and the issue of biosafety and biosecurity measures. Also lack of competent staff and financial issues add-up to the problem in these countries. Unfortunately, there is few reference laboratories which have ability to perform correctly TB culture and drug susceptibility test (Parsons, 2011). Smear ZN stain and smear auramine stain for sputum by using light microscopy and LED FM are the standard methods to identify AFB in Zanzibar. If bacilli are present, the patient has sputum smear positive for PTB. When the MTB cannot be identified, chest X- ray is taken to assist with diagnosis (ZIHTLP, 2013).

The foundation of TB examination and case finding in large numbers of TB control programs is smear microscopy (Lawn, 2011). It is cheap and does not need sophisticated equipment and easy to perform because there are a few technical needs. In regions where burden of TB infection is high smear microscopy is suitable since it has the capacity of generating high positivity rate despite the difference range of its sensitivity of 35 to 80% (Mathew, 2002).

The reagent of carbol fuchsin used in Ziehl Neelsen (ZN) stain technique is not suitable for the environmental as well as human in health. Whereas basic fuchsin, phenol and alcohol contain toxin, that cause damage to human through breathing, assimilation, drinking or eating. Accumulation of methanol poison less than 30 milliliters in human has been reported to cause fatality when eating or drinking. This poison causes abnormality of CNS such as depression, convulsion, also causes headache, nausea, dizziness, pupils dilatation and intoxication. The outcome of this problem may be coma or mortality. In addition to that, loss of vision may happen due to methanol poison. Phenol is very toxic; when it comes into contact with human skin it causes chemical burns. In the environment alcohol reagent is decompose naturally, but basic fuchsin and phenol are not biodegradable, hence dangerous to plant and wildlife (MCC, 2014).

Gene Xpert MTB/RIF is friendly to the environment. Xpert MTB/RIF assay present very low risk to testing personnel. The Xpert MTB/RIF assay starts with addition of highly tuberculocidal sample treatment reagent (SR) to each sputum sample; it reduces the viability of MTB in sputum after 15 minutes of incubation. After incubation the sputum SR mixture is transferred to the plastic assay cartridge, the

cartridge lid is closed and pressed in Xpert instrument. The remainder of the assay is performed within the closed cartridge. The use of closed cartridge system further reduces biohazard risk by performing sample processing in an aerosol resistant enclosure (Banada, 2010).

Health care sites which located in peripheral for the time being have a challenge concerning availability of a quick method for diagnosis of active TB infection. Therefore, patients with co-infection TB and HIV or active TB cases in endemic areas miss laboratory diagnosis and are treated clinically with improper drugs. Hence forth, facilitate TB infection to spread in society (Horries, 2010).

Success of accurate diagnosis of TB infection in people living with HIV is not very easy compared to immunocompetent people (Sterling, 2010). An HIV patient infected with TB infection tends to have very small number of TB organisms, that's why the accuracy of sputum smear microscopy is limited (Sterling, 2010; Getahun, 2010). Besides patients suffering with advanced immunocompromise are highly susceptible to infection with TB. Rapid and accurate diagnosis of TB is very important in HIV patients in order to initiate early treatment and thus decrease death related to TB (Carriquiry, 2012).

The verification of TB and MDR-TB in the laboratory is useful in ensuring that people who develop sign and symptoms are properly examined and immediately provided with effective treatment. In 2013, out of 4.6 million cases (new and relapse) PTB reported in the world, 2.8 million were identified positive to MTB by all methods recommended by WHO (WHO, 2014). Identification of TB without

performing DST examination is not proper as it can cause unsuccessful treatment and facilitate to disseminate drug resistance strain in the community and more money used to cure patients (WHO, 2014).

In places with inadequate resources for TB diagnosis and where the prevalence of HIV is high, urgent measures are required to implement new and rapid detection of TB. Whereas, laboratory examination of MTB from Culture is the gold standard. However, culture of MTB takes a number of days to provide results and is costly in terms of laboratory facilities, hence not a recommended method for management of severe cases. Recently, WHO authorized the implementation of Gene Xpert MTB/RIF and LED FM for national TB control programmes in third world countries (WHO, 2011).

The Xpert MTB/RIF is a new fully automated diagnostic molecular test with an analytic sensitivity of five genome copies of purified DNA and 131 cfu/ml of *M. tuberculosis* in sputum, moreover, it is able to detect more than 99.5% rifampicin resistance mutations, an indicator of MDR-TB, in less than two hours. The Gene Xpert machine does not requires sophisticated laboratory facilities. It needs minimum biosafety and training and data from many clinical validation studies indicate 92% sensitivity compared to culture utilizing a single specimen (WHO, 2011).

However, these data come from clinical trials, and information about the performance of LED fluorescent microscopy and Xpert MTB/RIF in real-life situations is desirable before worldwide implementation. The aim of this study was

assess the two techniques (i.e., Gene Xpert and LED FM technique) for detection of PTB in HIV patients.

1.2 Statement of Research Problem

Environmental factors which facilitate the possibility of the threat for MTB dissemination to increase are contact to TB in small closed area, insufficient ventilation which provides infectious TB droplets, unable to follow safety procedure effectively during samples handling, improper decontamination of medical instruments and recirculation of infectious TB droplets in the air (CDC, 2005).

Repeated visit for collection of spot and morning sputum samples causes the problem of patients dropout in health care facilities. More patients faced a challenge to get fare transport for submission of samples to heath care facilities and compilation of result. In fact the sensitivity of smear microscopy depend on the quality of samples which has been collected, quality of stain reagents and slide smear prepared, that is why there are variation range of 20% to 85% concerning sensitivity (Steingart, 2007). In addition, sputum smear microscopy loss its sensitivity for diagnosis of HIV patients infected with TB and for children due to small number of MTB in the patients (Fox, 1999).

The HIV infection leads to decreased number of cell mediated immunity, this situation not only lead to boost the TB disease but also change the way of clinical diagnosis of TB infection. When HIV infection continues to develop the number of CD4 T- lymphocytes decrease and facilitate to stimulate the growth and replication of MTB. CD4 T- lymphocytes play the role for immune response against infection.

HIV associated TB is not easy to diagnize and is linked to extreme disease and death. The spread of MDR-TB, together with increasing harmful of HIV infection and inadequate availability of quick diagnostic instrument have lead to disappointment of global TB control. Furthermore, due to changing of clinical diagnosis of TB and interruption of treatment of TB infection, contribute to increased dissemination of TB within societies.

In general to attain successful control of TB it is important to collaborate with stakeholders for case finding to the community particularly to HIV patients and provide immediate effective treatments to those TB patients. The present study assessed two techniques GeneXpert assay and LED auramine fluorescent microscopy for diagnosis of PTB in HIV victims at MMH Zanzibar.

1.3 Objectives

1.3.1 General Objective

To compare Gene Xpert MTB/RIF technique and LED fluorescent microscopy technique for diagnosis of pulmonary TB in HIV victim.

1.3.2 Specific Objective

(i) To evaluate the accuracy of Gene Xpert machine and LED FM for diagnosis of PTB.

(ii) To determine the sensitivity and specificity of smear auramine FM for PTB diagnosis in the study area.

(iii) To investigate the occurrence of MDR-TB in the study area.

1.4 Hypotheses

(i) Gene Xpert MTB/RIF is more accuracy than LED FM in diagnosis of PTB.

(ii) Smear auramine FM is less sensitivity but has high specificity for PTB diagnosis.

(iii) The prevalence of MDR TB is less in the study area.

1.5 Significance of the Study

The issue of safe environment in diagnosis of MTB in laboratory is very vital. The use of engineering controls (For example BSC and area exposure to air) and personal protective equipment (like oxygen mask) can help to protect laboratory personnel against exposure with TB infection in linked with breathing of infectious droplets. On the other hand, the major significant concept in the testing area is to diminish the threat of generating aerosols within and outside the work environment so that to decrease the danger of exposure with infectious agents.

There is a lack of accurate and rapid diagnosis technique for TB in many health centers in Zanzibar. Definitely this study will provide useful and valuable information to policy makers about accurate and rapid diagnosis technique for TB. Determination of accurate and rapid diagnosis technique for TB could help to reduce death by providing proper treatment to those patients suffering with HIV and TB co-infection as well as to keep environment well against contamination with aerosol when processing sputum. Then the importance of this study was to find rapid and accurate technique for diagnosis of PTB in patients suffering with HIV and TB co-infection.

CHAPTER TWO

LITERATURE REVIEW

2.1 Common Knowledge Concerning Tuberculosis

Tuberculosis is a persistence infectious illness caused mostly by *Mycobacterium tuberculosis* (and rarely by *Mycobacterium bovis* or *Mycobacterium africanum*). Another name of these organism is known as acid fast bacilli (AFB) because they are decolorized by acid alcohol. The lungs are affected when organisms penetrate the body through breathing. Normally there are two kinds of tuberculosis: first PTB which damage the respiratory organs, this type of disease is common to the community. The second is extra pulmonary tuberculosis (EPT) is the infection that damage other organ than the respiratory organ such as joint, lymph nodes, spine, brain, pleural and genital urinary tract (TURT-MOH, 2006).

2.1.1 Spread of Tuberculosis

TB is a communicable disease; the route of infection is person to person contact through inhalation. The individual who is affected by PTB can transmit the infectious droplets when coughing, talking or sneezing into the recirculated air. The exposure of this facilitated by environmental factors such as small closed area and insufficient ventilation provide infectious TB droplets. These infectious droplets can be eradicated in the building by providing well ventilation. TB organisms die within minutes when exposed to direct sunlight; however they are alive in a dark area for 24 to 48 hrs. Health individual staying together with a patient confirmed PTB positive in a small room that lack good ventilation for a long time is susceptible to TB infection. The danger of dissemination of TB from individual with negative smear PTB is low.

Smoking and silicosis boost the vulnerability to disease. However, facilities which have nice ventilation dissolve the mass of infectious agents (TURT-MOH, 2006).

The threat of development infection to active TB disease depends on the condition of the immune system. Normal people with HIV negative are able to resist TB disease to about 90% even though they acquire infectious agent of TB due to the active immune response. Only tuberculin skin test may be indicating positivity of TB infection to immunocompetent individual. The condition of immunocompromise stimulates the dormant bacilli to be active and cause TB disease to an individual because the cells associated with immune response become weak and fail to fight against infectious agents (TURT-MOH, 2006).

Currently, HIV infection is the source which cause immune suppression and lead to reactivation of TB in Tanzania. Co-infection TB and HIV individuals have 29 – 30 times chance of progression to active TB disease than HIV negative individuals. Also there are other conditions such as recurrent infection of any kind, malnutrition and diabetes mellitus may cause reactivation of the TB infection (TURT-MOH, 2006).

2.2 Prevalence of *Mycobacterium tuberculosis*

In 2013, about 1.1 million (13%) of the 9.0 million people who were infected with TB disease globally were HIV positive. The African region accounted for 78% of the estimated number of HIV positive incident TB cases. Since 2004 the mortality rate of people with co-infection HIV and TB has been decreasing. Nevertheless, in 2013 the mortality of HIV related with TB was still 360,000 in the world (WHO, 2014).

Although majority of the TB morbidity and mortality arise among men, also the number of TB cases among women is high. About 3.3 million cases of TB and 51,000 TB mortality among women, and about 550,000 TB cases and mortality 80,000 among children happened in the year 2013. The high number of deaths would be preventable, if the individuals may arrive at health facilities for diagnosis and appropriate treatment, this would decrease the TB mortality rate.

In fact the first line drugs for TB available at health facilities for decades may heal TB disease about 90% (WHO, 2014). Zanzibar is estimated to have 7,200 people living with HIV, this account for the prevalence of HIV to about 0.6%. HIV infected clients attending care and treatment services are screened for TB at each encounter. These include patient on ART, Isoniazid Preventive Therapy (IPT) or stopped IPT, adult and children, male and female, with exception of those on TB therapy.

Percentage of patients screened for TB has increased from 97.1% in 2011 and 2012 to 99% in 2013. Table 2.1 shows the number of HIV patient who were screened for TB out of those who received care during the period and those started on TB treatment (ZIHTLP, 2013). Currently there are two sites providing comprehensive TB/HIV activities (Mnazi Mmoja and Chake Chake Hospitals).

TB patients who are diagnosed as HIV positive are treated at TB clinic and receive ARV drugs until they finish TB treatment when they are referred to care and treatment counseling. A total of 71 patients received ART at TB/HIV under one roof clinic in 2013 (ZIHTLP, 2013).

Table 2.1: Number of HIV Patients Screened for TB by Facility, 2013

Care and Treatment Counseling	Total Visit	Screened for TB		Started on TB Treatment	
		< 14 Years	>14 Years	< 14 Years	>14 Years
Al Rahma	140	6	134	0	1
Bububu	377	18	359	1	8
Kivunge	224	19	205	2	4
Mnazi Mmoja	2521	216	2305	18	62
Mwembeladu	606	41	565	1	3
Chake Chake	217	23	194	1	5
Mkoani	35	2	33	0	1
Wete	157	25	132	1	10
Micheweni	63	13	50	0	2
Makunduchi	56	4	52	0	0
Total	4,396	367	4,029	24	96

Source: ZIHTLP (2013)

Among all TB patient screened in 2013 a total of 115 (17.5%) patients tested were HIV positive. This is an increase compared to 79 (16.8%) patients who were diagnosed to be TB/HIV co-infected in 2012. The number of smear positive TB cases has also increased by 5% compared to 2012. This shows that, TB infection in the community is still high. As in the previous year, most of those affected are young adults in the age group between 15 – 44 years. It shows that 77 (24, 21%) were female and 141 (44.33%) were male, as shown in the Table 2.3 (ZIHTLP, 2013).

Table 2.2: The Trend of Notification of TB for 10 Years

Year	Pulmonary tuberculosis AFB +	Extra pulmonary tuberculosis AFB +	Relapse	Failure/ Others	Returns	Total
2004	222	56	11	6	1	296
2005	211	53	14	5	5	288
2006	238	44	9	7	5	303
2007	232	48	11	3	5	299
2008	265	73	14	4	3	359
2009	235	76	25	9	3	350
2010	270	66	14	11	3	369
2011	280	73	12	14	9	388
2012	303	81	12	8	7	411
2013	318	197	18	2	5	542

Source: ZIHTLP (2013)

Table 2.3: Distribution of New Sputum Smear Positive (SSP) Cases by Age and Sex for the Year 2013

Age groups	0 – 4	5 – 14	15–24	25–34	35–44	45–54	55–64	65 +	Total
Male (SSP)	0	6	41	58	42	34	14	14	209
Female (SSP)	0	5	27	30	20	15	5	7	109
Total	0	11	68	88	62	49	19	21	318

Source: ZIHTLP (2013)

2.3 Prevalence of MDR TB

Multidrug resistant tuberculosis (MDR-TB) is defined as tuberculosis resistant to at least isoniazid and rifampicin, the most powerful anti-TB drugs. Globally, an estimated 3.5% of new cases and 20.5% of previous treated cases have MDR-TB. In 2013, there were an estimated 480,000 new cases of MDR-TB worldwide, and approximately 210,000 deaths from MDR-TB. Among patients with PTB who were notified in 2013, an estimated 300,000 had MDR-TB. More than half of these patients were in India, China and Russian federation (WHO, 2014).

Between 2009 and the end of June 2014, nearly 90,000 people with MDR-TB were detected through the expand TB project, which has established capacity to detect drug resistant TB using line probe assay, liquid culture and Xpert MTB/RIF in 27 low and middle income countries (WHO, 2014).

In Zanzibar epidemiological data for MDR-TB prevalence is low, the reasons for low prevalence of MRD-TB may be lack of accurate diagnosis for detecting MDR-TB from patients. Also community is not aware about MDR-TB. The equipment, which detects the indicator for MDR-TB, is available only at MMH, which was commissioned in 2013. The trend of MDR TB as shown in Table 2.4 (ZIHTLP, 2014).

Table 2.4: Trend of MDR TB in Zanzibar for the Past Six Years

Serial number	Year	Unguja	Pemba
1.	2009	0	1
2.	2010	2	0
3.	2011	0	1
4.	2012	0	0
5.	2013	0	1
6.	2014	2	0

Source: ZIHTLP (2014)

2.4 Current Status of TB Diagnosis

In Zanzibar a total of diagnostic centers are 51. Unguja have 32 and Pemba 19 centers. The health centers in unguja which has LED FM are 7 and 25 centers has ordinary light microscopy for the diagnosis of MTB. Also in Pemba 8 centers has LED FM and 11 has ordinary light microscopy. Only one centre (i.e Mnazi Mmoja Hospital) has Gene Xpert MTB/RIF (ZIHTLP, 2014).

Table 2.5: Zanzibar Health Centers which have LED FM

UNGUJA	PEMBA
Mnazi Mmoja Hospital	Public health laboratory
Kivunge Cottage Hospital	Bogoa PHCU
Makunduchi Cottage Hospital	Vitongoji Cottage Hospital
Rahaleo PHCU	Abdulla Mzee Hospital
Kiomba mvua PHCU	Konde PHCU
Fuoni PHCU	Chakechake Hospital
Jambiani PHCU	Wete Hospital
	Micheweni Cottage Hospital

Source: ZIHTLP (2014)

2.5 Delays in Diagnosis of TB

Diagnosis of tuberculosis (TB) is often delayed in resource-limited settings. Factors contributing to the delayed diagnosis of TB include: 1) patient delays, 2) health-

system delays and, 3) delays inherent to the conventional TB diagnostic process (Storla, 2008 and Sreeramareddy, 2009). Of these, delays related to the low sensitivity of sputum AFB smear microscopy, have been identified as the most significant contributor to the total diagnostic delay times experienced by TB patients (Millen, .2008). The consequences of delayed TB diagnosis and treatment include increased TB-related morbidity, increased mortality, and continued TB transmission (Greenaway, 2002; a Golub, 2006). In countries with a high burden of HIV, HIV infection has both reduced the sensitivity of smear microscopy contributing to further delays in TB diagnosis, while simultaneously increasing the urgency in which a rapid TB diagnosis is needed.

Two multi-centre studies: the first study using rapid molecular detection of TB and rifampicin resistance and the second study using feasibility, diagnostic accuracy and effectiveness of decentralized of Xpert MTB/RIF test for diagnosis of TB and MDR have provided strong evidence that Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), the first commercially available, automated, real-time NAAT for MTB, could reduce diagnostic delay (Boehme, 2010 and Boehme, 2011). Xpert MTB/RIF was found to be highly accurate (including 70% sensitivity in AFB smear-negative patients) and led to more rapid diagnosis and shorter time-to-TB treatment initiation compared to sputum smear microscopy (Boehme, 2010 and Boehme, 2011). Xpert MTB/RIF was also simple to perform and required minimal technician training. However, the clinical impact of more rapid TB diagnosis via Xpert MTB/RIF has not been adequately evaluated. Reducing diagnostic delays may be of particular importance in populations at high risk of early mortality.

2.6 Xpert MTB/RIF Diagnosis

In the study performed in South Africa with HIV-infected patients, the Xpert MTB/RIF increased case detection for TB by 45% compared to FM (Lawn, 2011). WHO recommended the use of Xpert MTB/RIF for diagnosis of HIV associated PTB (Golub, 2006). However, the cost of Xpert MTB/RIF can be too high for some resource limited settings. In the study which was conducted in a District Hospital in India concerning the Xpert MTB/RIF compared with LED FM the following results were obtained as shown in Table 2.6 (Alvares-Uria, 2012).

Table 2.6: Positive Results Utilizing LED FM and Xpert MTB/RIF Assay

Sample	Total	Smear positive			X-pert positive			Absolute difference		Ratio X-pert/LED	
	#	#	%	(95% CI)	#	%	(95% CI)	%	(95% CI)		(95% CI)
Sputum	166	106	63.9	(56.2 to 70.6)	124	74.7	(67.5 to 80.8)	10.8	(5.2 to 16.5)	1.17	(1.08 to 1.26)

Source: Alvares-Uria (2012)

In setting with low prevalence of MDR TB where it is possible to obtain two additional early morning sputum samples in different days, LED FM could be used with not much loss of sensitivity compared to the Xpert MTB/RIF assay for diagnosis of HIV infected patients with suspicion of PTB (Alvares-Uria, 2012).

WHO approved the Xpert MTB/RIF assay for sputum based rapid diagnosis of PTB and MDR TB. The assay can be used to accurately measure the MTB load beyond the detection limit of 131 organisms per mL in an in-vitro suspension (Helb, 2010). The Xpert MTB/RIF assay in patients with suspected TB and newly diagnosis case TB has been evaluated in several studies (Theron, 2011).

2.7 Smear Auramine FM Diagnosis

Researchers are also looking at improving the accuracy of smear microscopy, which remain the first line diagnostic test for MTB for much of the world for foreseeable future. LED FM is more sensitive than standard microscopy (Smart, 2012). Since 2012, LED FM has been introduced in about 200 medical college microscopy centers in India. Given better sensitivity (10% more) of LED FM over ZN microscopy, it may be possible that LED FM in spot specimens performed better in detecting AFB and the difference in performance between spot and early morning specimen may be lower (Cuevas, 2011).

After systematic review and meta analysis of available data, WHO reported that in comparison with direct ZN (standard) microscopy. LED FM was statistically significantly more sensitive by 6% (95% CI, 0.1 – 13%), with no appreciable loss in specificity, and LED FM was 5% (95% CI, 0 – 11%) more sensitive and 1% (95% CI, 0.7 – 3%) more specific than Conventional FM (WHO, 2011).

2.8 Importance of Accurate and Rapid Diagnosis of TB and Drug Resistant

Modelling studies have estimate that 400,000 lives could be saved each year with the introduction of an accurate, rapid and widely available TB diagnostic system with sensitivity greater than 85% for both smear positive and smear negative cases and 97% specificity (Malicotti, 2014).

The ability to rapid and accurately detect drug resistance in MTB clinical specimens is essential for appropriate treatment to be initiated in patients suffering from TB and for prevention of further spread of drug resistant strains. This is of paramount

importance for TB control of MDR-TB at national and global level (Drobniewsk, 2013).

2.9 Environmental Control in TB Diagnosis

In the diagnosis of MTB in laboratory environmental controls is very important so that to avoid the transmission and decrease the concentration of infectious aerosol in ambient air. Environmental controls include the following technologies to removal or inactivate MTB: Local exhaust ventilation, general ventilation, high efficient particulate air (HEPA) filtration, and ultraviolet germicidal irradiation (UVGI).

These controls help to stop the transmission and decrease the concentration of airborne infectious agents. Environmental controls are the second line of defense in the TB infection control program, and they work in harmony with administrative controls. The reduction of exposure can be facilitated through the effective use of environmental controls at the source of exposure (e.g., coughing patient or laboratory specimen) or in the general work place environment. General ventilation can be used to dilute the air and remove air contaminants and to control air flow patterns in room or in health care setting. Air cleaning technologies include HEPA filtration to reduce the concentration of MTB droplet nuclei and UVGI to kill or inactivate the microorganism so that they no longer pose a risk of infection (CDC, 2005).

Class II BSCs in Figure 2.1 provide personnel protection (containment), product protection (a virtually particle free work area to help minimize contamination of culture or other products), and environmental protection (helps prevent contamination of laboratory, the building and the community). Characteristics that

are shared by all types of class II BSCs have two HEPA filters, a supply filter and an exhaust filter. All of the air leaving the work area is HEPA filtered by either the supply filter or the exhaust filter. The exhaust filter provides environmental protection by preventing particulates from escaping via the exhaust air duct (Fleming, 2006).

Xpert MTB/RIF assay posses a smaller biohazard risk than the performance of a direct AFB smear. Any type of sputum manipulation, including AFB smear preparation, can generate potentially infectious aerosols from a sputum sample containing a sufficient number of bacteria. The combination of an effective tuberculocidal SR and the closed configuration of the Xpert MTB/RIF cartridge effectively reduce the risk of infectious aerosol formation to below that of AFB smear preparation. These features help to make the Xpert MTB/RIF assay suitable for near patient detection of TB and drug resistance in settings where biocontaiment facilities are not available (Banada, 2010).

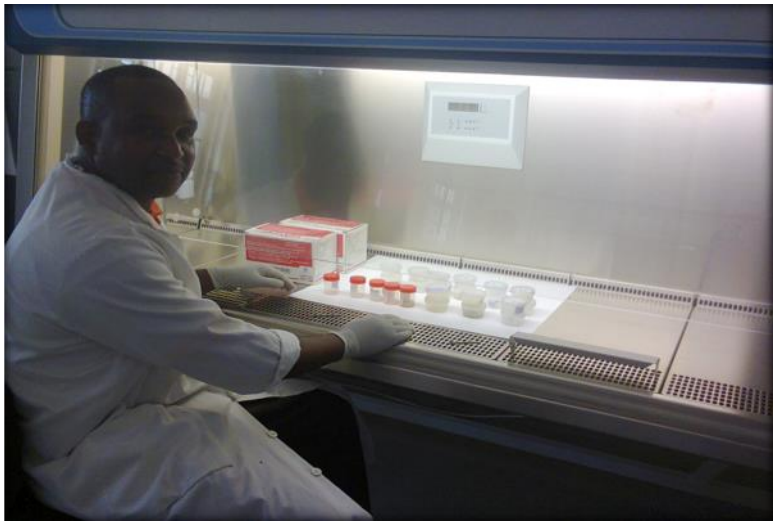

Figure 2.1: Sputum Samples Processed in Class II Biological Safety Cabinet
Source: Pathology Laboratory Mnazi Mmoja Hospital Zanzibar, (2015)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

The study was experimental type of design that involved laboratory analysis of sputum samples for determination of AFB and rifampicin resistance for the HIV patients attending CTC at Mnazi Mmoja Hospital from all districts of Unguja Island.

3.2 Study Area

The study was done at Mnazi Mmoja Referral Hospital in Zanzibar specifically at CTC where the samples were collected and then laboratory test conducted at TB section in microbiology department. The study was conducted from 5th April to 10th August 2015. Therefore, this study in Zanzibar provides a base line data to other studies.

Mnazi Mmoja is the main referral hospital in Zanzibar. The hospital itself is located at Urban west region in the Stone Town, the historic centre of Zanzibar City. It comprises of outpatient clinic, specialized clinics and several wards for the inpatient services. Although termed as referral hospital, basic outpatient services are also provided to the nearby communities.

The hospital contains 450 beds. The specialty departments include paediatrics, surgery, internal medicine, acupuncture, physiotherapy, occupational therapy, obstetrics and gynaecology, maternity services, dental and eye (ophthalomology). The hospital is fairly well-staffed with doctors, nurses, mid-

wives, nursing students and a number of foreign medical students. Regularly foreign doctors also work at the hospital, which is the product of medical exchange programmes between Zanzibar and other countries such as Cuba, China, Egypt, Russia and Sweden.

This hospital has a good laboratory with well-equipped equipment. This equipment includes; CD4 count machines, automatic tissue processor, ELISA reader, teach microscopes and genexpert machine. The laboratory has six departments which are microbiology, parasitology, clinical chemistry, haematology, histopathology and blood transfusion. The laboratory is three stars ranked and has about 55 staffs. Among these; they have one pathologist, four laboratory scientists, twenty one technologists, twenty three technicians and six laboratory assistants.

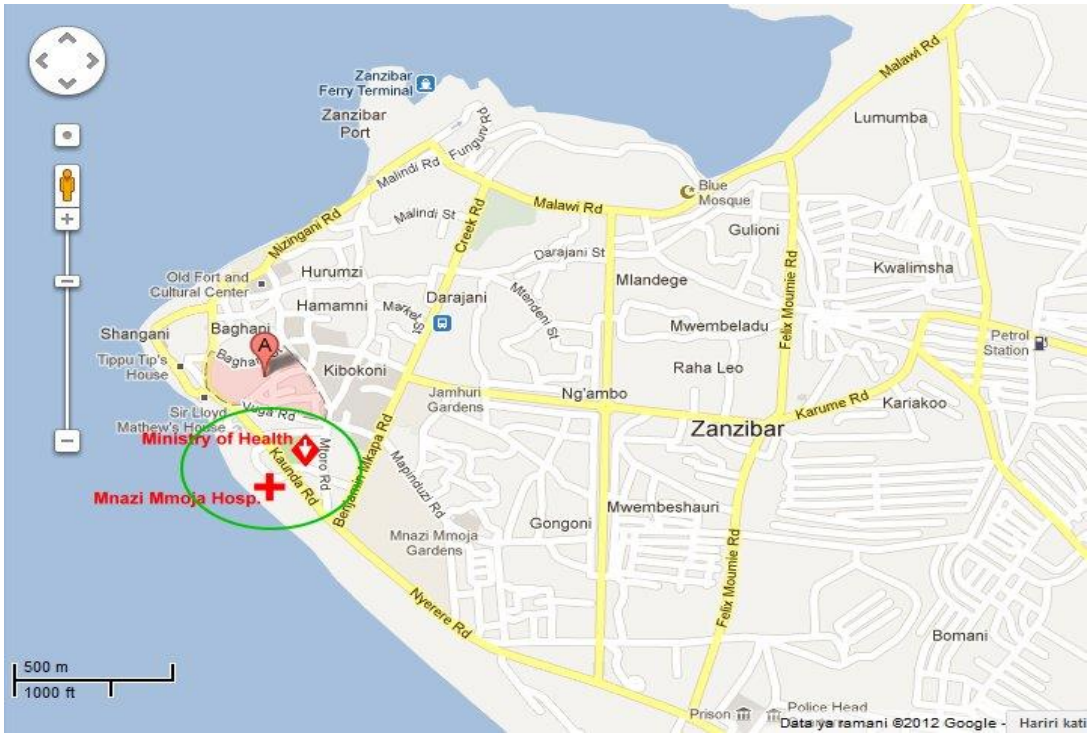

Figure 3.1: Map of Zanzibar Stone Town showing Location of Mnazi Mmoja Hospital where Sputum was Collected

Source: https:/www.google.oc.tz/maps/placemnazimmojaHospital

Zanzibar consists of two sisters Islands named Unguja (Zanzibar) and Pemba, the land area of the two Islands is approximately 2,332 square kilometers. Zanzibar is 1,464 square kilometers. The population of Zanzibar is approximately 1.303,569 based on 2012 National census.

It is located about 35km off the cost of Dar es Salaam (commercial capital city of Tanzania), between 39 degree longitudinal and 6 degree latitude south of equator. Zanzibar Island has 5 regions; Unguja Island has 3 regions named Urban west, North and South. Pemba Island has 2 regions named North and south.

Figure 3.2: Map of Unguja Island showing TB Diagnosis Centres

Source: www.faysafaris.com/Tanzania Maps.htm

3.3 Study Population

Study population included all HIV patients male and female suspected with TB attending CTC and TB clinic at Mnazi Mmoja Hospital. All agreed and signed the informed consent form for the study purpose.

(a) Inclusion criteria of patient

(i) All HIV patients suspects with TB overt 18 years

(ii)Must have capability of producing two quality sputum samples

(iii) Cough duration must be two weeks or more

(b) Exclusion criteria patient

(i) HIV patient(s) less than 18 years old were not allowed to participate in the study.

(ii)HIV patient(s) who were not able to produce quality sputum was not involved.

(iii) HIV patient(s) with less than two weeks of coughing episodes were excluded.

3.4 Sample Size

The formula for calculating sample size was available in (htpp://www.caribvet. 13th February, 2015). The prevalence was determined by regarding the number of PTB cases during a specified time period in the study area. The size of the population of PTB patients occurred in study area. The prevalence was then calculated by dividing the number of PTB incidence during specified time period by the size of population of HIV patients, the result is expressed as a percentage. The total numbers of PTB

cases reported in Zanzibar between 2009 – 2013 were 1506 and the total numbers of the population were 7200. The prevalence was calculated as shown 1506/7200 = 0.2 (20%). Therefore the prevalence of PTB was 0.2. In this study 246 HIV patients male and female attended CTC at MMH were examined. Those HIV patients suspected with PTB were used in the study to represent the population. The numbers of sputum samples collected are shown below.

$$n = \frac{Z^2 1-\alpha/2\ P(1-P)}{e^2}$$

Where n= number to sample

$Z^2 = (1.96)^2$ for 95% confidence (i.e. $\alpha = 0.05$)

P= 0.2

e= maximum tolerable error for the prevalence estimation 0.05

From formula above $n = \frac{(1.96)^2 \times 0.2(1 - 0.2)}{(0.05)^2}$

n = 246

3.5 Specimen Collection

Two sputum samples were requested from all HIV patients suspects with TB according to inclusion and exclusion criteria mentioned above. One on-the-spot sputum sample at the time of the patient's first visit, and an early morning sputum sample taken by the patient at home on the day following the initial visit. Patients were instructed on production of good quality sputum samples. Biological safety cabinet was used when specimens were assessed macroscopically to inspect the quality and quantity and a record was made.

3.6 Sample Transport

All sputum samples were transported to the laboratory in clean containers, and transported in cooler box after collection. Sample were labeled by necessary information including name and number of patient, number of sample either 1 or 2, date of collection.

3.7 Materials and Equipments

3.7.1 For Gene Xpert Technique

Reagents	Supplies	Equipment
GeneXpert Kit including: Xpert cartridges, sterile disposable transfer pipettes, Sample Reagent (SR) buffer (Pro-Gen Diagnostics) Tap Water 70% Ethanol 3.5% or 5% Sodium hypochlorite (Bleach)	Screw-capped sputum containers Pasture pipette Disposable Gloves Laboratory coat Soap Permanent marking pens Tidy wipe/paper towel Biohazard medical waste bin	The GeneXpert Dx System includes GeneXpert instrument, computer and barcode scanner, UPS, printer (Pro-Gen Diagnostics) Stop watch/timer 500ml measuring cylinder Memory stick/RW-CD's for data backup

Figure 3.3: Gene Xpert MTB/RIF Machine

Source: Pathology Laboratory Mnazi Mmoja Hospital (2014)

3.7.1.1 Diagnosis of Sputum Samples by GeneXpert MTB/RIF Technique

GeneXpert machine was easy to perform, gave results within 2 hours of sample processed and there was no operation difficult during its usage. This is automated method; both spot and morning sputum samples were processed in this machine to identify the presence of MTB in the sputum. Processed of sputum in GeneXpert MTB/RIF assay (see appendix 3)

3.7.1.2 Comparison Between GeneXpert MTB/RIF and LED FM Techniques

Comparisons of these two techniques were done after sputum samples examined. The results of sputum samples generated from GeneXpert were compared with the results yielded by LED FM technique

3.7.1.3 Investigate of MDR-TB in the Study Area

GeneXpert MTB/RIF machine is an automated machine which used to detect MTB and rifampicin resistance. The rifampicin resistance is an indicator of MDR-TB. All sputum samples collected were done in GeneXpert MTB/RIF machine, processed of sputum (see appendix 3), all results yielded were analyzed to identify if there were a results of rifampicin resistance. In fact machine itself generate this results.

3.7.2 For Auramine Staining Technique

Requirements for performing this technique were as follows:

(i) Timer

(ii) Fluorescent microscope

(iii) Bunsen burner or alcohol lamp

(iv) Slide rack to support slides over the sink

(v) Slide rack for drying stained slides

(vi) Sink with water

(vii) Forceps

(viii) Diamond pencil

(ix) Waste bin

(x) Positive and negative control slides

(xi) Paper filters 32.0 cm in size

(xii) Gloves

(xiii) Slides

(xiv) 0.1% of auramine

(xv) 0.5% of acid-alcohol

(xvi) 0.5 of potassium permanganate or 0.3 of methylene blue

Figure 3.4: Fluorescent Microscope

Source: Pathology Laboratory Mnazi Mmoja Hospital Zanzibar (2014)

3.7.2.1 Diagnosis of Sputum Samples by LED FM Technique

All spot and morning sputum samples were processed. An appropriate portion of the sputum samples (from the most purulent portion) was collected with an applicator stick or a wire loop and spread on the labelled side of the microscope slide. The slides were air dried and then heat-fixed. All smears were stained by the FM technique (See appendix 2).

3.8 Examination

All slides were examined blindly by FM at 20 - 25 X objectives to scan the smear and 40X objectives for confirming AFB. The AFB was graded according to the following scheme:

N of AFB	Report
No AFB/1 length	Negative
1-29 AFB/1 length	Record exact number
1 - 9 AFB/ fields on average	1+
10-100 AFB/field on average	2+
> 100 AFB/ field on average	3+

3.9 Permission and Ethical Consideration

Ethical clearance was sought and obtained from the concerned authorities for sample collection which is the Ethical Committee of the Ministry of Health Zanzibar. During the study, the purpose, procedures of the study, measures taken to ensure

confidentiality of the participants, the voluntary nature of the study and applicability of findings were explained to participants. Participants were assured that their participation in the study is voluntary and they are free to withdraw without any negative impact in their treatment at the clinic. Written informed consent was sought and obtained from the participants, since the study dealt with human and involved collection of sample direct from human the consensus of participants was necessary.

3.10 Quality Control

Both positive and negative slides were used for internal quality control during samples processed.

3.11 Data Analysis

All data analysis was performed using SPSS version 20 software. Comparison of performance of Gene Xpert MTB/RIF and LED FM were performed. Sensitivity, specificity and Pearson chi-square were performed to compare two diagnostic techniques. A p value (< 0.05) was considered significant.

Objective	Analytical Tool
To compare the diagnosis accuracy of Gene Xpert MTB/RIF and LED FM among TB suspect	Pearson chi-square test used to determine association between the Gene Xpert MTB/RIF and LED FM techniques using p value (< 0.05) level of significant.
To evaluate the sensitivity and specificity of smear auramine FM to TB diagnosis in the study area.	Pearson chi-square test used to determine the different of two sides of Gene Xpert and LED FM techniques to generate positive and negative results of TB using p value (< 0.05) level of significant.

To determine the prevalence of MDR-TB in the study area	Pearson chi-square test used to calculate the prevalence value of MDR-TB for those TB positive

3.12 Limitation

The limitation of this study was 10 patients failed to collect morning sample (second sample), so in this case were removed from the study because they did not adhered to the inclusion criteria. Also some of the clients refused to provide their telephone number because they thought of stigmatization.

CHAPTER FOUR

RESULTS

4.1 Characteristics of Study Participants

The age of study participants ranged between 18 – 80 years and majority of them were females 136 (55.3%) as shown in Table 4.1. A high proportional of the patients were between ages of 31 to 40 years which account 66(26.8%). Majority of study participants lived in Urban district of Unguja (40.7%) which is located in Urban West region while high number of patients were living (80.9%) as shown in Figure 4.1 and Figure 4.2.

Table 4.1: Patients Age Groups and Sex

Age group (years)	Patient gender		GeneXpert MTB/RIF technique results			
	Male N(%)	Female N(%)	Total patients	Total patients tuberculosis positive N(%)	Positive tuberculosis Male N(%)	Positive tuberculosis Female N(%)
< 20	9(3.7%)	14(5.7%)	23(9.3%)	11(6.5%)	2(1.2%)	9(5.3%)
21 - 30	25(10.2%)	33(13.4%)	58(23.6%)	44(26.0%)	23(13.6%)	21(12.4%)
31 - 40	27(11.0%)	39(15.9%)	66(26.8%)	44(26.0%)	20(11.8%)	24(14.2%)
41 - 50	26(10.6%)	32(13.0%)	58(23.6%)	41(24.3%)	21(12.4%)	20(11.8%)
51 - 60	16(6.5%)	11(4.5%)	27(11.0%)	18(10.7%)	12(7.1%)	6(3.6%)
> 61	7(2.8%)	7(2.8%)	14(5.7%)	11(6.5%)	7(4.1%)	4(2.4%)
Total	110(44.7%)	136(55.3%)	246(100%)	169(100%)	85(50.3%)	84(49.7%)

Among the male and female patients, the least proportional were in the age group above 61 years as shown in Table 4.1. Among 246 confirmed seropositive HIV/AIDS patients examined, 169 were pulmonary tuberculosis positive for Gene Xpert MTB/RIF technique. Among the HIV/AIDS patients that were positive of

pulmonary tuberculosis 85 (50.3%) were males and 84 (49.7%) were females as shown in Table 4.1.

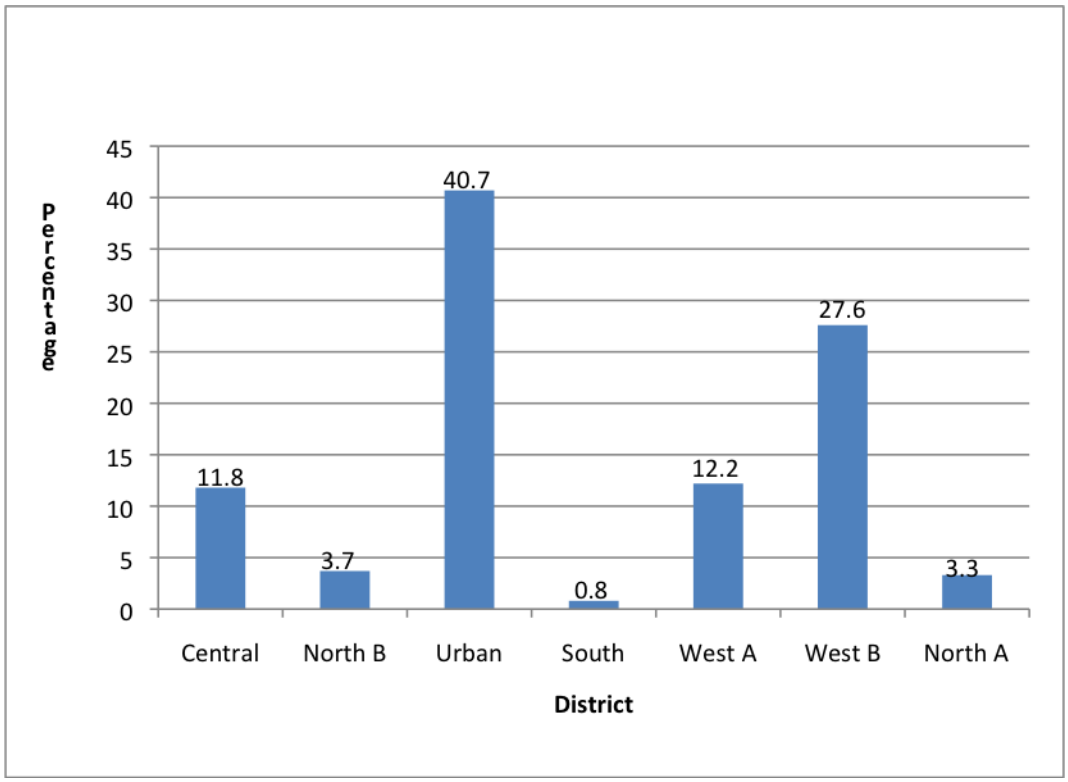

Figure 4.1: Distribution of Patients in Districts of Unguja Island

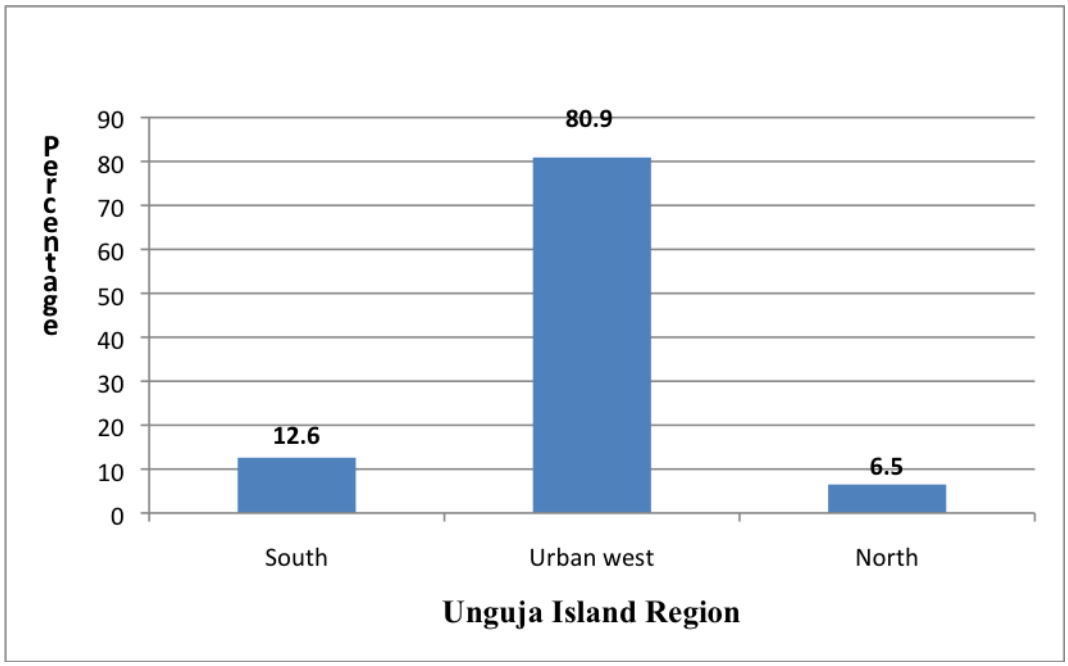

Figure 4.2: Distribution of Patients in Regions of Unguja Island

4.2 Evaluation of the Accuracy of Gene Xpert MTB/RIF and LED FM for Diagnosis of PTB

Sputum samples from HIV patients attended CTC at MMH Zanzibar who were suspected to have PTB were collected between 5th April to 10th August, 2015. A total of 246 patient's sputum samples came from seven districts of Unguja Island and were analyzed for the presence of *M.tuberculosis.* Comparison between Gene Xpert MTB/RIF and LED FM techniques was done and found that of the 246 patients examined for spot samples (sample I), 169 (68.7%) were positive for Gene Xpert technique while 112 (45.5%) were smear positive (LED FM). Also for morning samples (sample II) 169 (68.7%) were positive for Gene Xpert technique while 118 (48.0%) were smear positive (LED FM) Table 4.2. This different is statistically significant (p=0.000).

Table 4.2: Evaluation between Gene Xpert and LED FM in Positivity Results

Sample	Total number	Smear Positive LED FM		Gene Xpert Positive		Absolute Difference
		Number	%	Number	%	%
Spot sputum (sample I)	246	112	45.5	169	68.7	23.2
Morning sputum (sample II)	246	118	48.0	169	68.7	20.7

4.3 Sensitivity and Specificity of Smear Auramine FM for PTB Diagnosis in the Study Area

All 246 sputum samples collected were processed by Gene Xpert, as well as smear auramine FM in order to evaluate sensitivity and specificity of LED FM. Among 169 spot and morning samples which were positive in Gene Xpert processed by smear auramine. The sensitivity of LED FM in spot and morning samples were 66.3% and 69.8% respectively. Also the specificity for both samples was 100% as shown in Table 4.3. In Figure 4.3 and 4.4 indicate percentage of grades AFB positivity for both samples in smear examination. This difference is statistical significance of (p=0.02)

Table 4.3: Smear Auramine LED FM Results

Fluorescent microscope	Spot samples (sample I)	Morning sample (sample II)
Sensitivity	66.3%	69.8%
Specificity	100%	100%
Number of true positive	112 (66.3%)	118 (69.8%)
Number of false positive	0 (0%)	0 (0%)
Number of true negative	77 (31.3%)	77 (31.3%)
Number of false negative	57 (33.7%)	51 (30.2%)

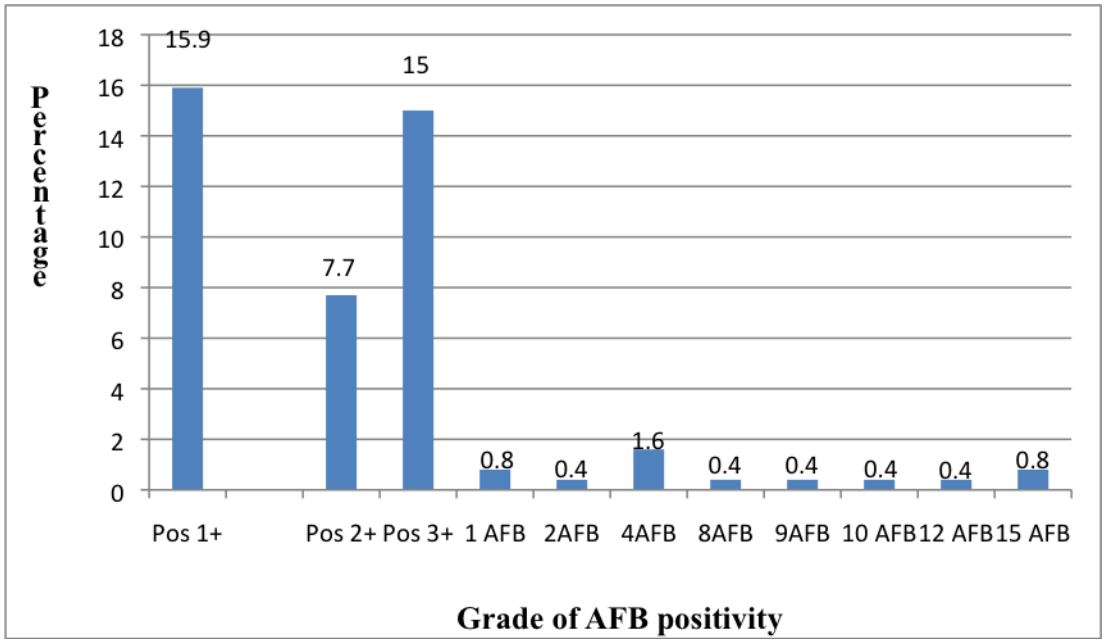

Figure 4.3: Distribution of Smear Positivity of Spot Sputum Samples

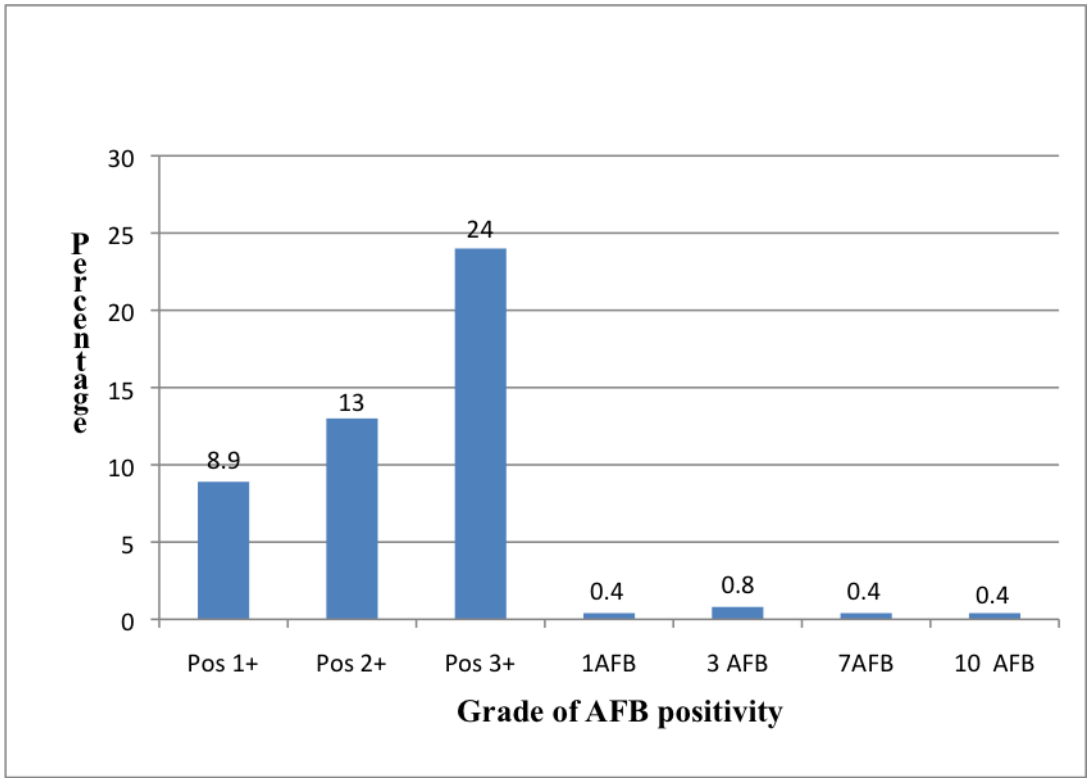

Figure 4.4: Distribution of Smear Positivity of Morning Sputum Samples

4.4 Occurrence of MDR-TB in the Study Area

Among 169 samples which were positive PTB cases two patients were found to have MDR-TB for rifampicin resistance in spot and morning samples of the same patients, so this account rifampicin resistance to about 1.2% of the positivity patients found in the study area. Therefore this indicates the prevalence of MDR-TB is 1.2%. Table 4.4 shown the result generated by Gene Xpert Machine.

Table 4.4: Gene Xpert Results for MTB/RIF Detection

Gene Xpert result	Frequency	Percent
MTB DETECTED	167	67.9
MTB NOT DETECTED	77	31.3
MTB DETECTED /RIF RES	2	0.8
Total	246	100

CHAPTER FIVE

DISCUSSION

5.1 Characteristics of Study Participants

TB and HIV/AIDS can interact and exacerbates the disease situation. TB is the most common opportunistic disease and cause of the death for those infected with HIV (Friedland, 2007). Similarly, HIV infection is one of the most important risk factors associated with an increased risk of latent TB infection progressing to active TB disease (Girardi, 2000; Meya and Mc Adam, 2007). So the WHO's policy on collaborative TB/HIV activities recommends a combination of measure to reduce the burden of TB among HIV infected individuals (WHO, 2012). These measures include intensified case finding, isoniazid preventive therapy, and infection control and antiretroviral therapy.

Among the 169 HIV/AIDS patients having TB in this study, 85 (50.3%) were male and 84 (49.7%) were female and these agree with previous reports indicating higher prevalence in male than females (Homes, 1988; Abeld, 2002). In our study, the age distribution reveals the majority of TB/HIV patients were 31 to 40 years old (26.8%), followed by 21 to 30 years old (23.6%), which represent the most sexually active age group and correlates with the study done by (Gyar, 2014).

5.2 Evaluation of the Accuracy of Gene Xpert MTB/RIF and LED FM for Diagnosis of PTB

In low income countries, TB diagnosis and treatment initiation is based on smear microscopy results. In our study, Gene Xpert MTB/RIF out performed smear microscopy for MTB detection in almost one third of the patients. The present study has several important finding: first controlled comprehensive clinical validation study of the diagnostic accuracy of LED FM in a patient suspected of having active PTB from HIV patient. Second, the study shows that LED FM has a low sensitivity and high specificity. Thirdly, the study shows that Gene Xpert MTB/RIF is more accuracy than LED FM. Fourthly, Gene Xpert assay was positive in 57 spot and 51 morning samples which were smear negative in LED FM and were classified as having PTB. Lastly, the Gene Xpert MTB/RIF was easy to perform, gave results within two hours of sample processing and there were no operational difficulties during its usage.

In the comparison between Gene Xpert MTB/RIF and smear auramine LED FM results, it was noted that Gene Xpert MTB/RIF increase the number of TB positive

results by 23.2% compared to smear auramine LED FM. The finding of this study does not concur with the study of (Alvares-Uria, 2012) who reported Gene Xpert MTB/RIF increase the number of positivity by 10.8% compared to LED FM in India. Also our finding does not concur with the study performed in South Africa with HIV infected patients that reported that Gene Xpert MTB/RIF increase case detection for TB by 45% compared to LED FM (Lawn, 2011).

The different between three studies may be explained by the difference in populations of the studies and in the way the sputum specimens were collected. In the South African study, two sputum samples were collected in a single visit to out patient clinics before the initiation of antiretroviral treatment and regardless of symptoms (Lawn, 2011). In the Indian study, they studied patient with high suspicious of active tuberculosis infection, and all patients were admitted to the Hospital (Alvares-Uria, 2012).

In this study, I examined patients with signs and symptoms of tuberculosis infection, and two sputum specimens were collected in a two visit to the CTC and TB clinics.In the present study no patient was found to have a false positive result in smear auramine LED FM. The 77 samples which were negative in Gene Xpert MTB/RIF were also processed in smear auramine LED FM, and also revealed negative results.

One important limitation of the Gene Xpert MTB/RIF assay is that it can process a maximum of four samples every two hours, so it may not be suitable for a busy laboratories receiving large number of sample in resource limited setting (Alvares-

Uria, 2012). The prompt results provided by Gene Xpert MTB/RIF would allow a timely diagnosis and prompt initiation of TB treatment. As extensively reported in the medical literature, the benefit of rapid treatment initiation of TB in HIV co-infected patients could improve individual prognosis and reduce overall TB disease transmission (Sterling, 2010).

My data suggest that smear negative TB patients could benefit from the new assay of Gene Xpert MTB/RIF assay in those areas where no culture is available. The Gene Xpert MTB/RIF assay could lead to avoid loss of patients and treatment delay in those TB suspect with a negative smear result. This could lead to the reduction of infection and improvements in TB control in the society.

5.3 Sensitivity and Specificity of Smear Auramine LED FM for PTB Diagnosis in the Study Area

The AFB staining method is the most common and supportive method in diagnosis of TB infection in resource limited settings. Although AFB staining method is a much cheaper, simple, and rapid method but also possess a low specificity and variable sensitivity (Reid, 2009). Current recommendation for the control of tuberculosis emphasizes early case detection so as to follow treatment of patients and there by limit the transmission of the bacilli. The main stay for its control is the rapid and accurate identification of the infected individuals (Prasanthi, 2005).

In this study smear LED FM have shown higher specificity of 100% so this is similar to another study carried out in South Africa which showed specificity above 95 % by

(Lawn, 2011). Also in the present study sensitivity of smear LED FM was 69.8%, this does not agree with a study conducted in Uganda by (Yoon, 2012) that showed 42% sensitivity.

Tuberculosis control, especially in TB/HIV endemic areas with poor resources, is hampered by lack of sensitivity and specificity of sputum smear microscopy which is often the only diagnosis method in place. In December 2010, WHO endorsed the Gene Xpert MTB/RIF for wide spread use. Among other indications the assay has a strong recommendation as initial diagnostic test in individual with suspected HIV associated TB (WHO, 2010). HIV infected patients are to have smear negative sputum samples. This could be confirmed by our data that showed that 33.7% of spot samples and 30.2% of morning samples of negative smears were positive when processed by Gene Xpert MTB/RIF. In the present study indicated that smear LED FM has a lower sensitivity compared to the Gene Xpert MTB/RIF assay in diagnosis of HIV patients affected by PTB.

Co-infection with TB/HIV leads to many challenges in both the diagnosis and treatment of TB. With increase in rates of drug resistance tuberculosis, including multidrug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) mortality has increased. Sputum smear microscopy in HIV infected patients are not highly sensitive therefore newer diagnostic test are urgently required that are cheaper, sensitive, specific and easy to perform and use in remote and resource constrained settings (Solomon, 2013). Treatment is very important in prevention of TB and/or HIV which can only be done with a very effective

diagnostic tool. Thus an effective diagnostic tool is very crucial in diagnosing TB in early stage and in HIV patients to reduce mortality and spread of infection.

5.4 Occurrence of MDR-TB in the Study Area

Available data on the prevalence of XDR-TB and MDR-TB in Tanzania are still low owing to improved case management. Although the levels of anti-tuberculosis drug resistance in the country are still low, the need for continuous monitoring has always been emphasized (Chonde, 2010, and Range, 2012). The most effective strategies for limiting further spread of drug resistance tuberculosis include rapid detection of drug resistance followed by prompt and effective therapy of each case. Routine surveillance linked to patient care, represents the best approach to monitor drug resistance (Hoza, 2015).

The present study has demonstrated a significant increase of positive results when comparing Gene Xpert MTB/RIF with LED FM when performing both tests in the same sputum sample. In this study two (1.2%) out of 169 samples with valid rifampicin resistant were found. This indicates that the prevalence of MDR-TB is 1.2% in Zanzibar. This results do not concur with the study of (Alvares-Uria, 2012) who reported six (4.8%) out of 124 specimens in India. Also the results do not concur with the study of (Hoza, 2015) conducted in Tanga, Tanzania Mainland that showed the prevalence of MDR-TB to rifampicin resistant was 6.3%. The reasons for the difference between the prevalence in Tanzania Mainland and Zanzibar could be that in Tanzania Mainland more study have been conducted to find the prevalence of MDR-TB compared to Zanzibar. Also population difference between Zanzibar and

Tanzania Mainland could lead to higher prevalence of MDR-TB in Mainland than Zanzibar.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The results of this study indicated that Gene Xpert MTB/RIF is more accuracy than LED FM in diagnosis of PTB in HIV patients suspected with TB. It is usefulness to detecting sputum smear negative patients needs further examination. The sensitivity of LED FM in diagnosis of PTB in HIV patient suspected with TB is lower and their specificity is higher. In our study, active case finding using the Gene Xpert MTB/RIF analysis has increased active PTB case detection among HIV infected patients.

The study has showed that spot and morning sputum samples of the same patients yielded the same results when examined in Gene Xpert MTB/RIF, therefore Gene Xpert MTB/RIF may be useful as a sole rapid test for PTB case detection in HIV patients suspected with TB, particularly in settings lacking capacity for liquid culture. Lastly the prevalence of MDR-TB rifampicin resistance is lower in Zanzibar it might be in the cause of increasing.

6.2 Recommendation

A single spot or single morning sputum sample is enough to examine PTB from HIV infected patients by using Gene Xpert MTB/RIF. This will lead to release the patients' results on the same day of the sample collection and will eliminate patients drop out as well as decrease costing of the patients to return at a health facility to submit the second sputum sample and to get the results at the following day. Policy makers should procure and disseminate the Gene Xpert MTB/RIF machine at least to the district Hospitals so that to increase case detection of PTB from the HIV infected patients in order to provide early treatment to patients who have active PTB cases and to protect environmental pollution.

Also the study recommends expansion of MDR-TB surveillance to cover all patients with history of previous treatment for TB, all patients with infectious TB (sputum smear positive TB patients), contacts of known MDR-TB cases and all HIV positive TB patients. HIV positive patients should be screen for TB. Lastly the use of faster MDR-TB diagnostic methods such as Gene Xpert MTB/RIF to detect drug resistance

within two hours should be considered for screening among HIV positive TB and among sputum smear positive TB cases that are likely to transmit MDR-TB.

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APPENDICES

Appendix 1: Consent Form

STUDY TITLE: ASSESSMENT OF TWO TECHNIQUES FOR DIAGNOSIS PULMONARY TUBERCULOSIS IN HIV PATIENT AT MNAZI MMOJA HOSPITAL ZANZIBAR: GENE XPERT AND FLUORESCENT MICROSCOPY

You are being asked for your consent to participate in a study that involves tuberculosis suspect in HIV patients in this hospital. We are asking for your consent to participate in a medical research study .The study is done by Abdalla Said Mohamed , the Master's student of environment Health at Open University of Tanzania. I will explain the study to you and will ask if you are willing to participate. Take your time to make your decision about participating. You may discuss your decision with your family and friends and with your health care team. If you have any questions, you may ask the researcher.

Purpose of the study

To compare Gene Xpert MTB/RIF and LED fluorescent microscopy for diagnosis of pulmonary TB in HIV patients. This study is not funded by any institution. None of the researchers have potential financial gain from the study.

Study procedure

If you take part in this research study, we will ask you to give us the specimen of sputum. This is an activity that is part of the services provided by the tuberculosis clinic.

Procedure for specimen donation

If you agree to participate in this study, the following will happen:

- Your sputum will be tested and its result will be coded to safeguard their privacy per protocol.
- There will be no direct benefit to you
- There will be no human genetic tests performed to your samples.

Withdraw from the study

- You can decide to withdraw from the study at any time. You will tell the researcher if you are thinking about stopping or decide to stop. Notify the researcher at MMH (Abdalla S. Mohamed) in writing at Mnazi Mmoja Hospital, P.O.Box 236, Zanzibar, Tanzania or by Mobile: +255 (0)777 435441, and he will destroy any your identifiable. He will tell you how to stop your participation safely.

Risk and Benefits for participating in this study

Testing the specimens will not cause any risk to you.

There will be no direct benefit to you from participating in this study. You may choose not to participate. You will receive the same care whether you participate in the study or not.

Private and confidentiality

We will do our best to make sure that the results kept private.

Costs

You will not be charged for any of the study activities.

Payments for your participation

You will not be paid for taking part in this study.

Participant's right

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to

you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from the health facilities in Zanzibar as well as the whole Tanzania.

Consent

You have been given copies of this consent form.

Participation in research is voluntary

You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled.

If you wish to participate in this study, you should sign below.

_____	_____	_____
Date	Participant's name	Participant's Signature for Consent

_____	_____	_____
Date	Person Obtaining Consent	Signature

Appendix 2: SOP for Auramine Staining Fluorescent Microscopy

1. Scope

This procedure applies to all laboratory personnel who perform auramine staining to diagnose microscopically tuberculosis.

2. Principle

Sputum smears are stained with auramine which stains *Mycobacterium tuberculosis* in greenish yellow against a dark background.

3. Specimen type

Sputum

4. Material and equipment

- Timer
- Fluorescent microscope
- Bunsen burner or alcohol lamp
- Slide rack to support slides over the sink
- Slide rack for drying stained slides
- Sink with water
- Forceps
- Diamond pencil
- Waste bin
- Positive and negative control slides
- Paper filters 32.0 cm in size
- Gloves
- 0.1% of auramine
- 0.5% of acid-alcohol
- 0.5 of patassium permanganate or 0.3 of methylene blue

5. Safety precaution

- Specimen may contain live potentially infectious agents that hazardous to your health Observe Standard Universal Precautions.

- Ensure that the work surface is kept clean, free from clutter and other infectious substances to avoid contamination.
- Spills should be immediately disinfected with appropriate disinfectant solution.

6. Quality control

- Positive and negative control slides must be used
- Ensure that reagents are not expired

7. Calibration

Ensure that the fluorescent microscope is regularly maintained

8. Activity description

- Wear appropriate PPE
- Gather necessary materials and reagents
- Filter auramine solution prior to use
- Put the slides on the staining rack, smear facing upward
- Cover all the slides with the filtered auramine
- Leave the stain on slides for minimum of 20 minutes
- Gently rinse each slide with water
- Till each slide off excess water
- Cover the slides with acid-alcohol and leave on for 1-2 minutes
- Gently rinse each slide with water and do not splash other slides
- Till each slide off excess water
- Cover each slide with potassium permanganate or methylene blue for 1 minute
- Gently rinse each slide with water and do not splash other slides
- Till each slide off excess water
- Air dry away from direct sunlight. Do not examine slides until they have dried

9. Calculation

NA

10. Reading smears and reporting of results

- Place the slide on the microscope and ensure that the smear is facing upward
- Use 20-25X objective to scan the smear and 40X objective for confirming suspicious objects

1.1.1

N of AFB	Report
No AFB/1 length	Negative
1-29 AFB/1 length	Record exact number
1 - 9 AFB/ fields on average	1+
10-100 AFB/ field on average	2+
> 100 AFB/ field on average	3+

- **Reference range**

NA

- **References**

- Jane Carter &Orgenes Lema: Practical laboratory manual for health centers in Eastern Africa.
- Laboratory Diagnosis of tuberculosis by Sputum Microscopy. The Handbook Tanzania 2009.

Appendix 3: SOP for Sample Processing for Diagnosis and Rifampicin Resistance in Gene Xpert MTB/ Rif

1.0.Title

Standard Operating Procedures for TB diagnosis and Rif resistance using the Xpert MTB/Rif on sputum

2.0 Objective and Scope

This SOP describes how to use the Gene Xpert instrument and how to perform the Xpert MTB/Rif assay. To ensure that all trained health care providers are capable of performing the Xpert MTB/RIF assay for diagnosing TB in sputum specimens directly in a primary health care facility, according to the manufacturer's instructions. Standard operating procedure for sample processing for Gene Xpert used in detection of mycobacterium and Rifampicin testing.

2.1 Principle

The GeneXpert (Cepheid) is a closed, self-contained platform for the extraction, amplification and detection of Mycobacterium tuberculosis (Mtb) complex from unprocessed samples. The GeneXpert system is able to generate a result within 2 hours.

The Xpert MTB/RIF assay allows for the rapid detection of Mtb and Rifampicin (Rif) resistance by combining automated extraction, amplification and detection on a single system. Rif is one of the first line anti-TB drugs and is also a surrogate marker for multi-drug resistant TB (MDR-TB). The assay amplifies a portion of the "rifampicin resistance determining region" of the rpoB gene, the most common site for Rif mutations, in real-time, using two sets of primers. Fluorescent probes are then

used to differentiate between wild-type and mutant strains so that if one or more probes do not bind, this indicates the presence of a mutation and therefore Rif resistance.

A sample processing control (SPC) consisting of spores from Bacillus globigii, is included in the assay as an internal control to ensure adequate processing of the sample as well as to monitor the presence of PCR inhibitors. A probe check control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability.

3.0 Safety and environment

Refer to Health Laboratory Safety and Waste Management Manual for safety considerations

- The GeneXpert system should be installed on a solid, even base
- Always wear a lab coat and disposable gloves
- All sample processing should take place under a BioSafety Cabinet Class II hood OR an open, well-ventilated area.
- Treat all samples as potentially infectious
- If liquid containing potentially infectious agent is spilled, clean affected area with 3.5% hypochloride
- Dispose of all waste in a biohazard medical waste bin

4.0 Procedure

Materials

Reagents	Supplies	Equipment
GeneXpert Kit including: Xpert cartridges, sterile disposable transfer pipettes, Sample Reagent (SR) buffer (Pro-Gen Diagnostics) Tap Water 70% Ethanol 3.5% or 5% Sodium hypochlorite (Bleach)	Screw-capped sputum containers Pasture pipette Disposable Gloves Laboratory coat Soap Permanent marking pens Tidy wipe/paper towel Biohazard medical waste bin	The GeneXpert Dx System includes GeneXpert instrument, computer and barcode scanner, UPS, printer (Pro-Gen Diagnostics) Stop watch/timer 500ml measuring cylinder Memory stick/RW-CD's for data backup

NOTE: Always wear gloves and laboratory coat when processing sputum specimens and Sample preparation should take place in a Biosafety Cabinet Class II if available OR a designated open, well ventilated work area in the laboratory!

5.0 Collection of sputum specimen

- Explain the specimen collection procedure to the patient
- Provide patient with the screw-capped container.
- Instruct patient to cough deeply and spit up sputum into the screw-capped sputum container.
- Instruct patient to seal the specimen container tightly.
- Ensure the sputum in the container is sent to the laboratory.
- Label each specimen immediately with identifying information – patient name, date and time of collection
- Log the sputum specimen in laboratory log book/computer system

6.0 Sample preparation

1. For each of the sample: unscrew the lid of sputum collection container
2. Add directly in the collection container 2 volumes of the sample reagents to 1 volume of sample.
3. Replace the lid, and shake vigorously 10 – 20 times.(Note: one back and forth movement is a single shake)
4. Incubate at room temperature: After 10 minutes of incubation, shake (or vortex) the specimen vigorously 10 – 20 times.
5. After 5 minutes of incubation. Sample should be perfectly fluid before being processed, with no visible clumps of sputum. If still viscous, wait 5 – 10 further minutes before processing it in the cartridge.

NOTE: The unprocessed sputum specimen can be stored at 4^{o}C for 4-10days

7.0 Cartridge preparation

1. Label the cartridge with the sample ID by writing on the left or right side of the cartridge or affix ID label.
2. Open the cartridge ,pipette at least 2 ml with the plastic transfer pipette from the collection container to the cartridge, then close the cartridge lid.

Note: Do not put the label on the lid of the cartridge or obstruct the existing 2d barcode on the cartridge,

8.0 References

Operators Manual for the GeneXpert system (Cepheid).

Or contact: Pro-Gen Diagnostics on 011 467 751