DIAGNOSTIC VALUE OF A LATERAL-FLOW URINE LIPOARABINOMANNAN ASSAY IN ADULTS WITH ACTIVE PULMONARY TUBERCULOSIS.

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A dissertation submitted in part fulfillment of the requirement for the Degree of Master of Medicine in Internal Medicine.

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DECLARATION

This dissertation is my original work and has not been presented before any institution for the purpose of obtaining a degree award.

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DEDICATION

I would like to dedicate this dissertation to the memory of my late grandparents Mr. Raphael Odenyo, Mr. Lawrence Ochieng and Mrs. Mary Awuor Ochieng.

My paternal grandmother Regina Aoko; my parents, Dr. Fredrick A. Okoth and Dr.(Mrs.) Ursulla A. Okoth; my brothers Vincent Okoth, Nicholas Okoth and Victor Omondi; my sisters Angela, Winnie, Janet and Valerie Okoth; my sister-in-law Hellen and lastly my nephews Jason and Jude.

Thank you for all your support and prayers through the years. Be greatly blessed in all your endeavours.

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ABBREVIATIONS

AIDS Acquired Immunodeficiency Syndrome

ART Antiretroviral therapy

CD4+ Cluster of differentiation 4

CrI Credible Interval

CI Confidence Interval

DNA Deoxyribonucleic acid

DOTS Directly Observed Treatment,Short-Course

EDTA Ethylenediaminetetraacetic acid

ELISA Enzyme linked immunosorbent assay

ERC Ethics and Review Committee

HAART Highly active antiretroviral therapy

HIV Human Immunodeficiency Virus

KNH Kenyatta National Hospital

MDGs Millenium Development Goals

NPV Negative predictive value

PLWHA Persons living with HIV/AIDS

PPV Positive predictive value

PTB Pulmonary tuberculosis

TB Tuberculosis

UoN The University of Nairobi

WHO World Health Organisation

LF-LAM Lateral flow-lipoarabinomannan assay

1.ABSTRACT

Background Tuberculosis, as an infectious illness, remains a leading cause of morbidity and mortality globally. The burden of disease is felt mostly in the middle and low income countries. Among persons living with HIV/AIDS, tuberculosis is the leading cause of morbidity and mortality. Diagnosis of tuberculosis remains a challenge to date despite advances in the diagnostic tests. Currently, there are limited point of care tests in use for diagnosis of tuberculosis. The lateral flow-urine lipoarabinomannan assay is such a point of care test. It detects lipoarabinomannan, a component of the mycobacterial cell wall, using an imunochromatographic assay. The assay is read in about 35 minutes and therefore provides an opportunity to diagnose tuberculosis at first point of contact with the health facility.

Objective To determine the diagnostic value of the lateral flow lipoarabinomannan (LF-LAM) assay among adult patients with active pulmonary tuberculosis

Methodology This was a cross sectional study conducted at the out-patient TB clinics, chest clinics and the in-patient facilities at Mbagathi County Hospital and Kenyatta National Hospital in Nairobi. The study subjects were adults presenting with features suggestive of pulmonary TB. Urine and sputum samples were obtained from the patients. Tests conducted were urine LAM, sputum ZN microscopy, sputum geneXpert® and sputum TB culture BACTEC MGIT 960. HIV testing was also offered. HIV positive patients had their CD4+ counts determined. The sensitivity, specificity, positive and negative predictive values of the TB diagnostic tests were determined; the liquid culture was used as the gold standard.

Results The sensitivity of the urine LAM assay was low when it was applied to a heterogenous population,28.6%(95% CI, 20.6-38.3%). This was lower in the HIV negative population at 12.7% (95%CI 6.4-23.5%). This was in contrast to a sensitivity of 58.8% (95%CI 42.2-73.6%) when it was applied to a HIV positive population. When it was combined with sputum ZN microscopy, the sensitivity of the combined tests increased to 70.6%. The sensitivity increased to 96.2% when the LAM assay was combined with the sputum geneXpert®. The specificity of the urine LAM assay was lower when it was applied to the HIV positive population, than in the HIV negative population, 85.1%(95%CI 74.4-91.8%) versus 93.6% (95% CI, 86.4-97.3%) respectively. Among the HIV positive, it was also noted that the sensitivity of the LAM assay increased as the CD4+ count decreased whereas the specificity decreased as the CD4+ count decreased.

Conclusion The lateral flow urine LAM assay was able to diagnose pulmonary TB among HIV positive patients especially those with low CD4+ counts of 200 and below. The sensitivity of the assay was low in an unselected population and among the HIV negative**.** The LAM assay improved on diagnosis of pulmonary tuberculosis among the HIV positive when used in combination with ZN microscopy or the sputum geneXpert® assay.

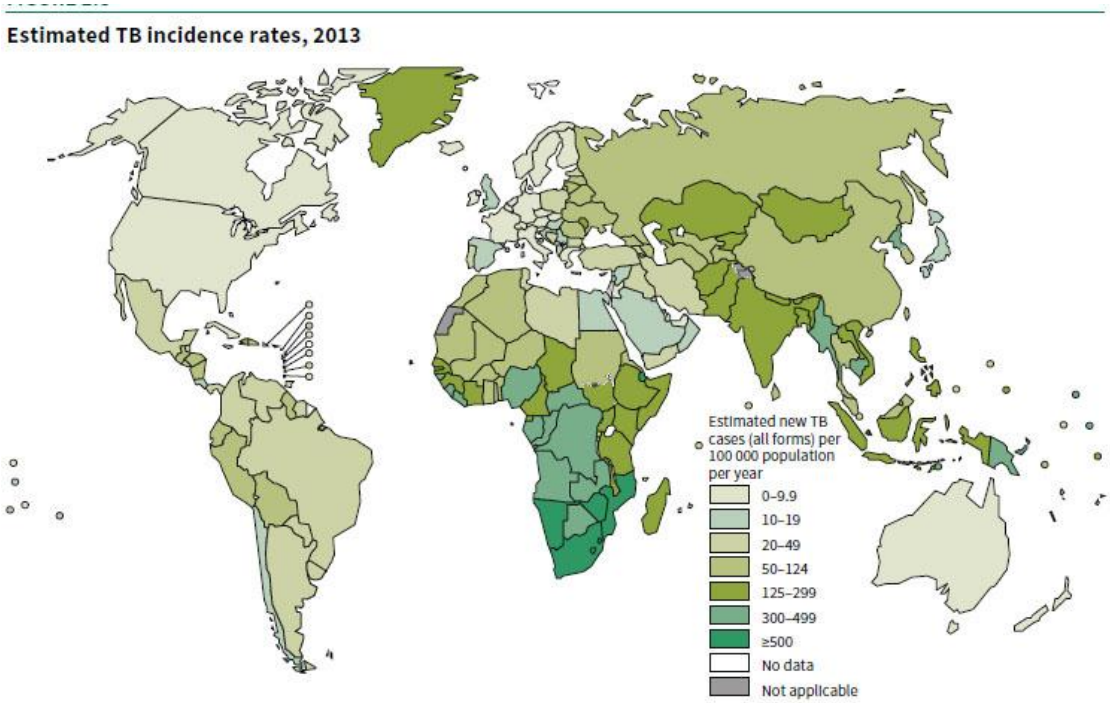
2.INTRODUCTION

EPIDEMIOLOGY

Tuberculosis remains a significant cause of morbidity and mortality globally. In 2013, the global incidence for active TB was 9 million, with a prevalence of 11 million. There were 1.1 million cases (about 13%) among persons living with HIV/AIDS. Africa, which hosts 9 out of the 22 high burden TB countries in the world, had an incidence of 2.6 million with a prevalence of 2.8 million. *[1]*

The statistics for Kenya reported an incidence of 120,000 and a prevalence of 130,000. Of the 9 million cases of TB globally, 3 million did not receive the required treatment. Deaths from TB alone were 1.14 million globally, 390,000 in Africa and 9,100 in Kenya. The mortality attributed to HIV/TB co infection for that year was 360,000 globally, 300,000 in Africa and 9,500 in Kenya. In 2013, as many people died of TB alone as from HIV and infections related to it. TB/HIV coinfection is highest in countries in the African region, about 34% (in some countries up to 50%) of patients with TB were estimated to have HIV coinfection (about 78% of TB cases among PLWHA globally). *[1]*

Figure 1 Estimated global TB incidence,2013 (The World Health Organisation)



As shown in the global map above, the burden of disease is mostly felt in low and middle income nations with high morbidity and mortality rates in Sub-Saharan Africa and South East Asia. In 2013, there were 22 high burden countries that accounted for about 80% of the global TB burden, 9 of these were from Africa. Africa had approximately 25% of the global

cases and had the highest rates of cases and deaths relative to population (280 incident cases per 100,000 on average, global average was 126 incident cases per 100,000). The South East Asia accounted for about 40% of the global TB cases in 2013.India had 24% and China 11% of the world total which were the largest number of cases in 2013. *[1]* Kenya was at that time and is still listed among the top countries with a high TB burden. In addition, it is also listed among the countries with a high TB/HIV and high MDR-TB burden.

Factors contributing to the re-emergence and persistence of tuberculosis include poverty, the HIV pandemic, emergence of drug resistant forms of tuberculosis and poor health policies and infrastructure.

Multidrug resistant-TB(MDR-TB)/Extremely Drug Resistant-TB (XDR-TB).

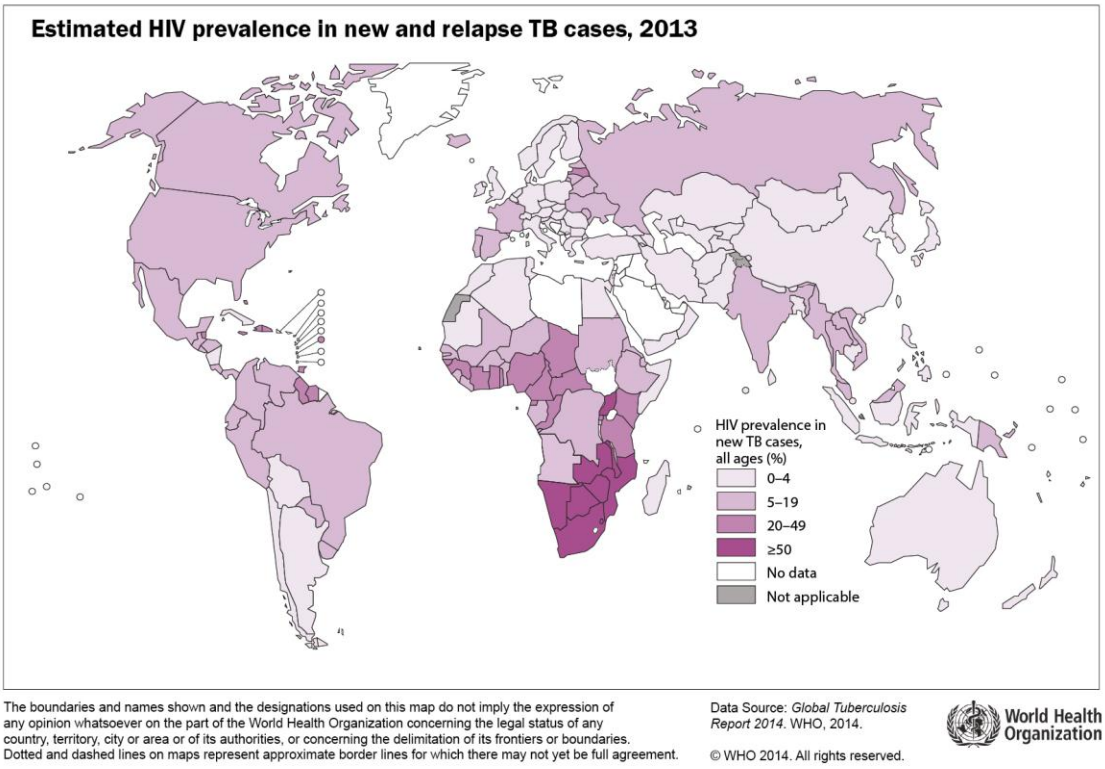
Multidrug resistant TB refers to a form of tuberculosis that is resistant to isoniazid and rifampicin. Extremely drug resistant-TB refers to the form that is resistant to isoniazid, rifampicin, any of the fluoroquinolones and to at least one of the three parenteral drugs, amikacin, capreomycin or kanamycin).

These forms of tuberculosis arise as a result of improper treatment of tuberculosis, intermittent drug shortages, poor monitoring of treatment for tuberculosis patients as well as the genuine risk of acquiring an infection with drug resistant strains. Multidrug resistant-tuberculosis accounted for 480,000 incident cases in 2013, (only 45 % of these received a for mal diagnosis). 210,000 deaths were reported to be as a result of MDR-TB. At least one case of extensively drug-resistant TB (XDR-TB) was reported by 100 countries in 2013. It is estimated that about 9% of MDR-TB cases have XDR-TB.

TB and HIV coinfection.

Countries with a high HIV prevalence are also reported to have a high prevalence of tuberculosis. Just as with tuberculosis, the burden of HIV is highest in the middle and low income countries. The prevalence of TB/HIV coinfection in 2013 is shown in the map below, countries in sub Saharan Africa including Kenya are significantly affected.

Figure 2: World map showing estimated HIV prevalence in new and relapse TB cases,2013



The threat of active TB in HIV/AIDS comes from reactivation of latent infection or from reinfection with *Mycobacterium tuberculosis*. In 2013 it was estimated that of the 34 million HIV positive persons globally, about one third or more had latent TB and approximately 10% went on to develop active tuberculosis. The risk of developing tuberculosis among the PLWHA is about 12-20 times higher than in the HIV negative population because of the impaired immunity. *[1]*

Tuberculosis is the leading cause of mortality and morbidity among persons living with HIV and AIDS. Several reasons contribute to this. There is rapid progression of disease due to immunosuppression, atypical presentation/sputum negative cases causing delayed diagnosis of TB, delayed HIV diagnosis due to stigma/not always offered in TB clinics, delayed start/ n o access to HAART and MDR-TB cases not diagnosed early enough with resultant delayed treatment.

<u>Global Targets.</u>

Global targets have been set by the organizations that form the Stop TB partnership. These organizations are committed to achieve the MDG target and the Global Plan to Stop TB

2011-2015. The following targets were set, to stop and begin to reverse the incidence of TB (and other major diseases) by 2015, to reduce the prevalence and death rates by 50% by 2015 (compared with the 1990 levels) and to eliminate TB as a global health problem by 2050. The detailed steps included measures to diagnose TB early and treat the illness successfully through the DOTS strategy, new diagnostics, drugs and vaccines and improved and accessible laboratory services for testing and identifying MDR-TB and TB-HIV co infection. Kenya currently falls in the category of countries with a reduction of between 25% and 50% HIV/TB deaths.

3.TB DIAGNOSIS

Diagnosis of tuberculosis relies on a medical history, physical examination, laboratory and radiological studies. These components are usually used in combination to start patients on antituberculous treatment.

Clinical Diagnosis.

This is made using the medical history and the physical examination findings. Tuberculosis occurs either as pulmonary, extrapulmonary or disseminated. The clinical features usually associated with TB are chronic cough, weight loss, fever, night sweats, hemoptysis, chest pain, headache, abdominal distention among others and will depend on the site of infection.

Radiological studies.

These utilize various imaging modalities to visualize the various sites affected by tuberculosis. These include plain radiography, ultrasonography, computed tomography and magnetic resonance imaging.

Laboratory diagnosis.

The laboratory diagnostic modalities have evolved significantly since Robert Koch gave his first public lecture on *Mycobacterium tuberculosis* in 1882. Earlier methods used include tuberculin skin testing, Ziehl-Neelsen staining with light microscopy on sputum and non-sputum specimen and sputum culture on solid media. Newer laboratory diagnostic methods in use include fluorescent microscopy, use of LED microscopy, liquid culture, serological tests and molecular assays.

4. LITERATURE REVIEW

Diagnosis of TB remains a challenge to date especially in middle and low income countries where the burden of disease is highest and the prevalence of HIV is also high. This delay in diagnosis in turn increases the stay in hospital, duration of infirmity for the individual eventually increasing the cost of care and increasing the risk of mortality. Diagnosis is sometimes made at postmortem as was the case in the study by *Cohen T. et al in 2010.* They conducted a postmortem study in Kwa Zulu Natal, Republic of South Africa (240 in-patients, HIV seroprevalence of 94%). The results showed for TB patients not on treatment more than 40% had culture positive TB at time of death. *[2]*

In the low and middle income countries of which Kenya is one, laboratory diagnosis of active TB relies heavily on microscopy on sputum and other non-sputum clinical specimen. The use of the Gene Xpert/ MTB/RIF assay, a nucleic acid amplification test is also increasing after its endorsement by the World Health Organisation as a diagnostic tool in 2010. Culture, which is the gold standard for TB diagnosis, is not used as widely but is available on solid and liquid culture media. Currently, there are no point-of-care tests that are readily available and affordable especially for use in the public sector and in community-level health facilities. Efforts are being directed towards development of such tests that are accurate and do not require complicated technical expertise and facilities. Such tests provide an opportunity to reduce the time taken between contact with health and start of treatment. This will go a long way in reducing the morbidity and mortality associated with tuberculosis.

As one of these newer diagnostic methods, the lateral flow urine TB LAM assay is showing much promise as a point-of-care-test that can be easily implemented in health facilities. Lipoarabinomannan, LAM, is a glycolipid component of the mycobacterial cell wall that is filtered into urine after gaining access into serum from sites of tuberculosis infection. The lateral flow assay can be done bedside, it does not require electricity, requires no sample preparation and provides rapid turnover of results. It also has a good biosafety profile, does not generate large amounts of biohazardous waste, simple to teach and apply, un used strips do not require refrigeration and it can be used in HIV positive population. It is also of benefit in sputum negative/sputum scarce cases of tuberculosis. This assay provides an opportunity to explore other clinical specimen for diagnosis of tuberculosis. Use of the assay with other tests such as sputum microscopy and the sputum geneXpert® MTB/RIF assay improves its diagnostic utility. Detecting urine TB LAM with the lateral flow assay as a point of care test can aid in revolutionizing diagnosis of tuberculosis, with the potential of diagnosing TB during the first hospital visit. This would aid in reducing morbidity and mortality currently attributed to tuberculosis especially in the vulnerable populations like persons living with HIV/AIDS.

4.1.DIAGNOSTIC TESTS USED FOR TUBERCULOSIS

4.1.1.Sputum microscopy

This refers to examination of clinical specimen under the microscope to directly visualize the *Mycobacteria* in clinical specimen. For pulmonary tuberculosis the specimen used is sputum, cerebrospinal fluid for tuberculous meningitis, ascitic fluid for peritoneal TB, lymph node aspirate for TB adenitis. These specimens are processed and after applying special stains they are examined under the different types of microscopes using different types of lighting. Conventional light microscopes, conventional fluorescent microscopes and fluorescent microscopes with LED attachment are the microscopes currently in use. *[3,4,6,7]*

Conventional light microscopy

This is a rapid, specific method that accurately identifies infectious cases of tuberculosis. Two sputum specimen, a spot and morning specimen increase yield. A third specimen only increases sensitivity by 2-5%. This runs the risk of increasing the workload for the laboratory staff. A positive smear is detected when the specimen contains 10,000 or more organisms per milliliter of sputum.

The sensitivity varies between 20 and 80%. The lower sensitivity is because of the occurrence of smear negative /paucibacillary disease and extrapulmonary disease that occurs in HIV positive patients especially those with marked immunosuppression. In HIV positive patients, sputum negative disease can account for about 25-61% of cases. Sputum induction using hypertonic saline can be used in those unable to produce sputum spontaneously or those who are sputum negative initially. This can identify an additional 25% of cases. This procedure however poses a biosafety risk. It can be used to monitor microbiological response to therapy. *[7]*

Fluorescent microscopy

Fluorescence microscopy or LED microscopy of clinical specimen after auramine +/- rhodamine staining has a higher sensitivity than conventional microscopy by about 10%. Two samples are still required for diagnosis meaning workload is still heavy for laboratory st aff and not all facilities especially at the lower level have access to fluorescent microscopes. *[7]*

4.1.2. Molecular assays: Gene Xpert MTB/RIF

Compared with culture as the reference test, the overall sensitivity of a single Xpert is 79-84%, in HIV positive patients this is lower by about 10%. For smear negative cases, the sensitivity reduces to about 67% with the first sputum produced, but increases with the second and third specimens. This means there is a chance that a third of sputum smear-negative culture-confirmed TB can still be missed by this test. The assay is not reliable for monitoring response to therapy as it detects DNA from both living and dead organisms. For this reason, it is also not to be used on its own for those who have successfully completed treatment should symptoms reccur within 6-12 months of completing a previous treatment. Culture is recommended in these circumstances.

It has a good diagnostic performance on cerebrospinal fluid, lymph node aspirates and pus with sensitivity of 79.5% and 84.9% respectively. Specificity for these specimen is 98.6% and 92.5 respectively. WHO recommends use of this for diagnosis of tuberculous meningitis and extrapulmonary TB in place of sputum microscopy. Caution should however be exercised in TBM as the test lacks sensitivity to exclude TBM in patients who present with suggestive clinical features. Treatment should be initiated in such cases even in the presence of a negative cerebrospinal fluid Gene Xpert result. With pleural fluid the sensitivity is 43.7% and specificity of 98.1%. The poor performance with pleural fluid and blood means that the test is not recommended for these forms of TB. In HIV positive patients with low CD4+ counts and disseminated disease, the assay performs well in diagnosing TB from urine.

The GeneXpert MTB/RIF, which is a nucleic acid amplification assay, has a higher sensitivity even in immunocompromised individuals and has a quick turnaround time but is not always available especially at the community level. *[3,6,8]*

4.1.3.Culture

Tuberculosis is usually considered to be definitively diagnosed when Mycobacterium tuberculosis is recovered from in vitro culture of clinical specimens, for example for pulmonary TB this would be respiratory specimens. It requires fewer organism for growth to be detected,10-100 organisms/mL. Hence culture is the gold standard diagnostic method for tuberculosis. Culture not only provides the benefit of demonstrating viable organisms, it also facilitates species identification which distinguishes tuberculous mycobacteria from non-tuberculous mycobacteria. Culture is also used for drug sensitivity testing. Drug sensitivity testing is a recommendation because of the existence of drug resistant forms of tuberculosis, these need to be identified to facilitate appropriate drug therapy and reduce morbidity and mortality. However, the necessary biohazard precautions

and aseptic techniques should be observed when conducting this culture to avoid risk of infection to the laboratory staff. Mycobacterium tuberculosis in aerosol is classified by the World Health Organisation as a Risk Group organism requiring biosafety level 3 laboratory safety precautions.

The ideal medium for isolation of tubercle bacilli should:

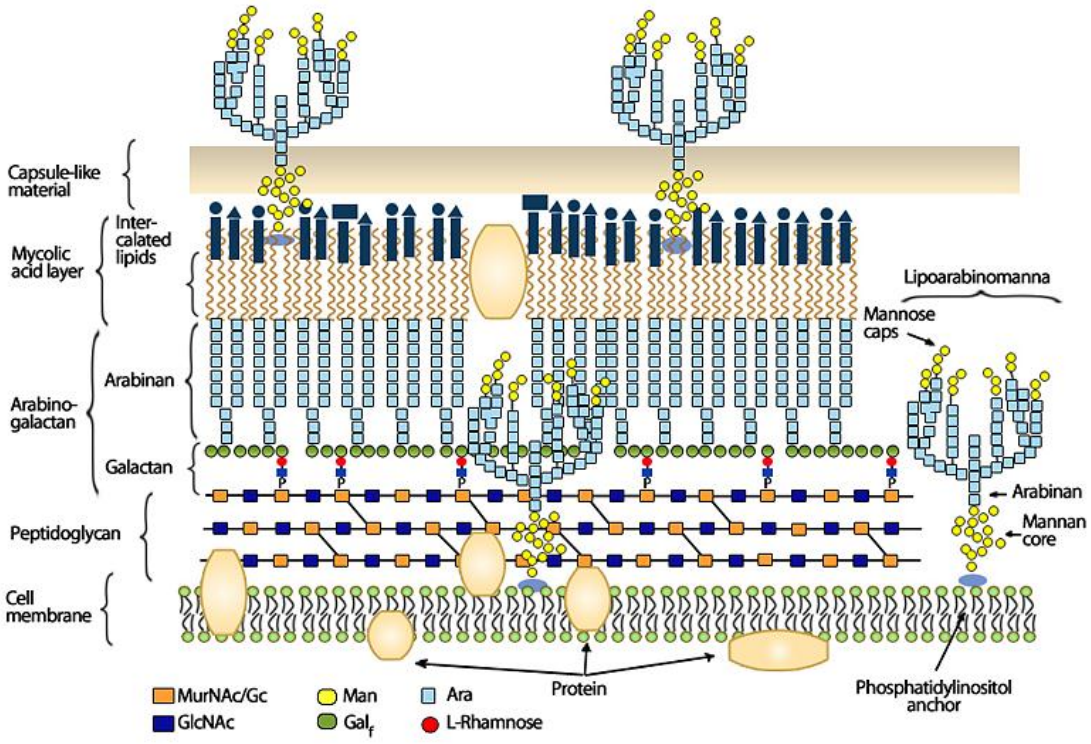
- be economical and simple to prepare from readily available ingredients.
- inhibit the growth of contaminants.
- support luxuriant growth of small numbers of bacilli
- permit preliminary differentiation of isolates on the basis of colony morphology.

Culture of tuberculosis which is the gold standard for diagnosis also has its challenges. The Lowenstein Jensen medium is cheap and readily available but the long turnaround time renders it unfavourable where results are required quickly. The liquid culture media such as BACTEC-MGIT 960 are much faster but are expensive, require specialized facilities and personnel. Microscopic Observation Drug Susceptibility Assay (MODS) another liquid culture medium has a high diagnostic accuracy and can also be used for drug susceptibility testing is not readily available for commercial purposes. *[4,9-16]*

4.1.4.Lipoarabinomannan assays

Lipoarabinomannan is a major lipopolysaccharide component of the cell wall of organisms of the genus *Mycobacterium* and the related *Actinomyces* (shown in figure 3 below). It is about 19,000 (+/- 8,500) daltons in size. *[17]* It can be recovered in large quantities from *Mycobacterium tuberculosis* cultures *[17]* and is detectable in serum, sputum and urine in a wide variety of tuberculosis clinical settings. *[18-22]* Due to its location on the cell surface it can readily interact with host receptors and acts as an immunomodulator. It is highly immunogenic and results in the production of anti-LAM antibodies during natural infection. *[17, 23-25]* Lipoarabinomannan and its antibodies can be found in a wide variety of body compartments including cerebrospinal fluid, sputum or bronchoalveolar lavage fluid and pleural fluid. The antigen/antibody complexes may also be found in circulation. *[26]* These antibodies were also targeted for use in the diagnosis of active TB. However, the current WHO recommendation is that antibody based tests for TB diagnosis are not reliable. Antigen based tests that detect pathogen derived antigens reflect pathogen burden better than antibody tests which rely on the immune response of the host. *[27]*

Fig.3Structure of the Mycobacterial cell wall. *(Cell wall and tissue vector-2009.igem.org/ Team:SupBiotech-Paris/Concept 1*

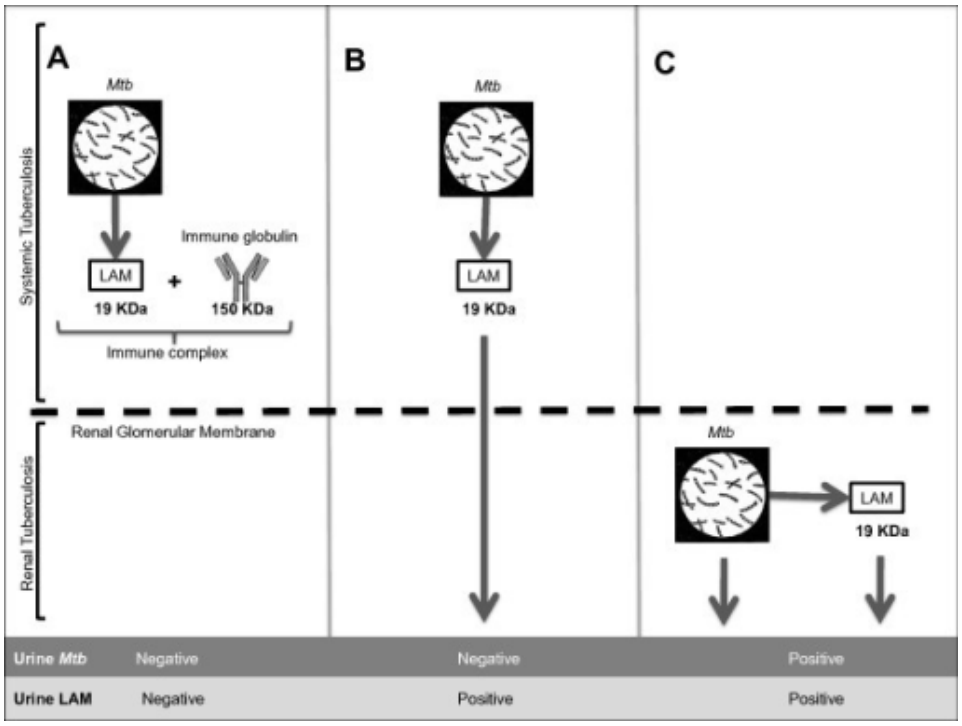


Lipoarabinomannan(LAM) was first described as a mycobacterial product shed from metabolically active or degrading cells then released into serum *[9, 52]*. It is subsequently filtered by the kidneys and thus detectable in urine as was shown in an animal study conducted by *Hamasur et al* in 2001*[20]*. It was demonstrated in mice, that when a crude cell wall extract of Mycobacterium tuberculosis cell wall was injected intraperitoneally, LAM was detected in the urine after 17 hours. LAM has a size similar to myoglobin (about 16,700 daltons) which is readily filtered through the glomerulus during muscle injury. However, being more immunogenic LAM is not readily filtered through the glomerulus when complexed with antibodies. Furthermore, LAM is found incorporated within HDL-particles, so this prevents glomerular filtration of bound LAM. *[28]* Free molecules however, can be readily filtered through the glomeruli. So in states of reduced humoral immunity, this LAM can be readily detected by assays. LAM can also be readily detected in urine when it is released from mycobacteria infecting the cells of the renal tract as this is not complexed with antibodies. *Wood et al* and *Lawn et al.* provided evidence for a direct renal source of LAM. *[29, 30]*. They demonstrated mycobacteriuria using the Xpert MTB/RIF assay in approximately half of the urine specimens found to be LAM positive in their studies conducted in 2012. The Xpert MTB/RIF assay however did not detect mycobacteriuria in those who had HIV and confirmed pulmonary tuberculosis but found to be LAM negative. In

the case of increased mycobacterial load associated with impaired immune status, LAM is likely to be filtered more readily into the urine and thus detectable by the available assays. *[31,32]* In states of immunosuppression especially marked, the infection is less likely compar tmentalized with increased risk of dissemination with renal involvement. *[33]*

Therefore, the factors that favour the appearance of LAM in urine are the reduced immune (humoral) response, increased mycobacterial load and disseminated tuberculosis, states associated with marked immunosuppression. The test was therefore favoured in areas with high TB and HIV prevalence. This is shown in figure 4 below.

Figure 4. Three proposed models of fate of LAM molecules released from systemic or urinary Mycobacterium tuberculosis organism



A.Systemically released LAM is bound to an antibody to form an immune complex within the circulation that impedes renal filtration of LAM across the intact glomerular membrane. This model gives rise to a negative LAM test in the absence of Mtb. B. Circulating LAM unattached to a specific anti-LAM antibody is freely filtered through the kidney into the urine , which gives rise to a positive LAM test in the absence of Mtb. C.Mtb within in the renal tract releases LAM directly into urine, which gives rise to a positive LAM test in the presence of Mtb [29]

LAM was then described as a diagnostic target in serum *[18,19,34]*and urine *[35, 36, 37]*. Cross reactivity of LAM molecules from non-mycobacterial sources such as *Actinomycetes*

(oral commensals) means that it is highly likely to give false positive tests when sputum is used for diagnosis. Detecting TB-LAM in urine offered the best results and in addition it offered several advantages, the most attractive being the reduced biosafety hazard.

Two formats were developed for detection of lipoarabinomannan in urine, an enzyme linked immunosorbent assay (ELISA) format *[38]* and a urine lateral flow method *[39]*. The ELISA format was developed initially and was in use prior to 2012. The challenges with the ELISA method included the need to process urine and it required more technical expertise which was not always available in resource constrained settings.

Earlier studies carried out to assess the diagnostic performance of LAM were based on the ELISA format. Several studies were conducted in Africa using this format. Patients presenting with features of tuberculosis were enrolled in the studies and the diagnostic performance of the assay established. The studies that enrolled both HIV positive and negative patients with a presumptive diagnosis of TB were conducted by *Boehme et al in Tanzania, Dheda et al in South Africa, Mutetwa et al in Zimbabwe, Daley et al in India and Reither et al in Tanzania [22,34,35,36,40]*. *Gounder et al and Lawn et al* conducted their studies on the LAM ELISA assay, recruiting only HIV positive patients who were suspected to have active tuberculosis. *[32, 41*].

Meta-analysis of seven of these studies, that assessed test accuracy in microbiologically confirmed cases only, sensitivity ranged from 13% to 93%, while specificity ranged from 87% to 99%. In five studies that assessed accuracy in clinical and confirmed TB cases, sensitivity ranged from 8% to 80%, while specificity ranged from 88% to 99%. In five studies with results stratified by HIV status, sensitivity was 3–53% higher in HIV-positive than HIV-negative subgroups; sensitivity was highest with advanced immunosuppression. *[45]*.

The search for a point-of-care test led to the development and evaluation of the lateral flow assay for rapid LAM detection. The lateral flow format was in use from the year 2012 to date. The strip currently in use is marketed by Alere, USA, it is known as the 'Determine' TB-LAM urine strip. The test is an immunochromatographic assay.

Studies conducted on suspected TB patients by *Jonathan Peter et al and Stephen Lawn et al; [42,46]* used the lateral-flow urine assay and the LAM ELISA concurrently. The final analysis done on the HIV positive patients showed that the two formats were comparable. The results concurred with the previous studies done which showed that the sensitivity of the test is variable with sensitivity increasing as the degree of immunosuppression worsened.

Thereafter, the studies on the LAM assay were conducted using the lateral flow format and based on the results from previous studies only HIV positive were recruited.

Stephen Lawn et al evaluated 6 studies that used the lateral-flow urine LAM assay between 2012 and 2013. The studies were conducted in Cape Town, Kampala, Johannesburg and Durban. The sensitivity of the assay in these studies was between 24.2 and 68.8% and the specificity ranged between 89.8 and 98.9%. The higher sensitivity just as with the ELISA for mat was seen in the HIV positive patients with highest sensitivity among those with lower CD4+ counts (CD4+ count less than 200 cells/uL). It was also demonstrated that when used with other diagnostic modalities as an add-on test the sensitivity was improved. *[39]*

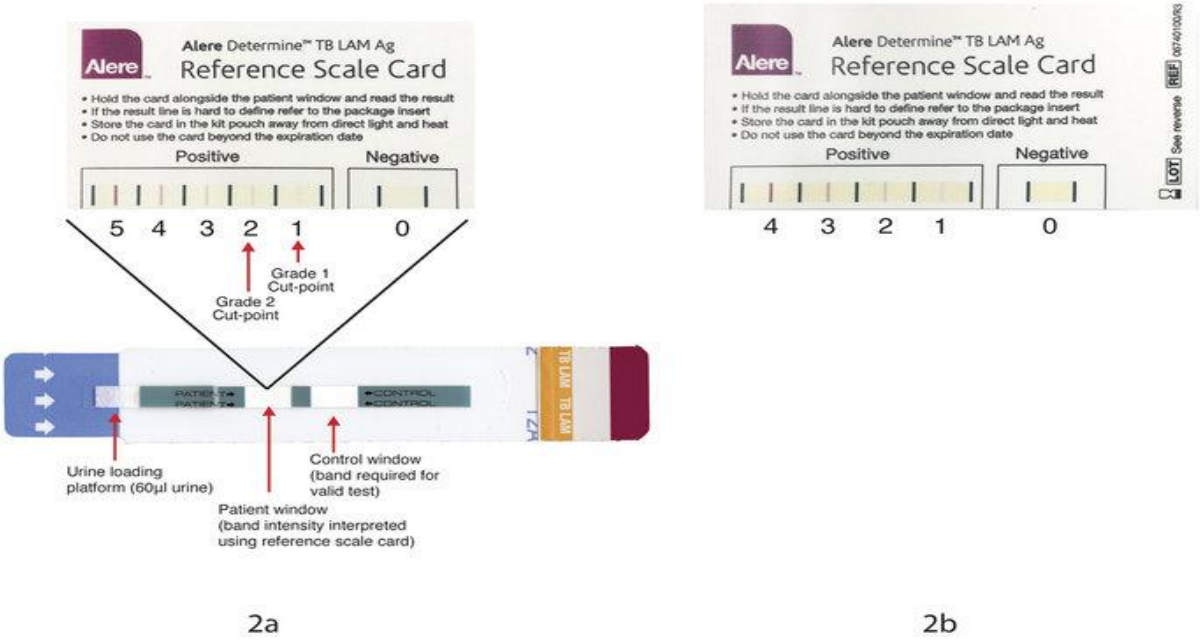
At the conference for Retroviruses and Opportunistic Infections held in Boston, Massachusetts in February, 2014 several findings were presented. These showed the importance of the lateral flow-urine LAM assay as an add-on rather than a stand-alone test. The studies showed favourable results where it was used together with other diagnostic tests such as Xpert MTB/RIF and sputum microscopy. In a study conducted in Uganda by *Shah et al.* the sensitivity of Xpert MTB/RIF and Determine LAM combined was superior to either test used alone. *[47]* For *Steve Lawn et al.* there was an increase in detection from 26.6% to 80.6% when the Determine LAM assay was added to an Xpert MTB/RIF test. When combine d, they detected 69.1% of active-confirmed cases, enabling them to find MTB infection in 85% of people with CD4 cell counts 100/ mm3 or less. *[48]* For *Taye Balcha et al.* in Ethiopia in people with HIV it was found that the Determine TB LAM assay performed best in those who had CD4 100/mm3 or less and that it could be used with sputum microscopy in this group. *[49*]. A study conducted in hospital and outpatient settings in Uganda and South A frica (by *Susan Dorman et al.)* found that in HIV positive adults with symptoms of TB who had CD4 cell count 100 cells/mm3 or less, the assay detected over half of culture positive TB samples in less than 30 minutes. *[50]* Early initiation of treatment was enabled in those with advanced HIV after diagnosis was made early using the lateral flow assay. Based on the favourable results, the quick turnaround time, use as a point-of-care test and it being cheap,it was recommend that the WHO review the evidence available on the test. In so doing it would provide guidance on large-scale application of the test in care of PLWHA especially those with marked immunosuppression. The WHO launched its policy guideline in 2015. This gives advice on how to use the lateral flow urine lipoarabinomannan assay among HIV positive persons. *[57]*

In these studies, the sensitivity of the LAM assays was thought to be influenced by the characteristics of the test capture antibodies used, the variable concentration of the LAM in the urine samples, the patients selected for the studies, the humoral response of the subjects recruited and the proportion of urine LAM derived from either renal or extra-renal sources.

The specificity of the assays ranging between 87-99% compared to a 'gold standard' of sputum culture was attributed to the following reasons. The ability to obtain good respiratory specimens and the capability of the study laboratories to isolate and successfully culture *Mycobacterium tuberculosis.* Inclusion of patients with unrecognized subclinical disease most likely contributed to the apparent false positives whereas contamination of urine specimen with fungi or non-tuberculous mycobacteria could have led to cross reaction with the LAM antibodies used in the LAM assays. Specificity, unlike sensitivity, was not significantly affected by the proportion of urinary LAM derived from renal or pulmonary disease. It has been found that extrapulmonary disease in HIV infection is most frequently associated with pulmonary tuberculosis. *[33]*

Another issue that arose from the studies that were conducted using the lateral flow urine LAM assays was which between grade 1 and 2 cut-off point would yield the best results. Using grade 1 cut off the sensitivity was found to be higher in the studies by *Lawn et al* than in those that were conducted using grade 2 cut off by *Peter et al* and *Dorman et al*.*[42,46,50]* The specificity was however found to be higher in the grade 2 cut off group. The results from the study by *Peter et al* reported a specificity of 90% vs. 99%, p = 0.009; sensitivity was 60% vs. 45%, p < 0.001 using the grade 1 and grade 2 respectively. *[46]* For the study conducted by *Dorman et al* in Uganda and South Africa the results were as follows, sensitivity of grade 1 and grade 2 cut-points were 61.6% and 37.1%, respectively, and the specificities were 78.4% and 97.6%, respectively *[50]*

Figure.5 Lipoarabinomannan urine strip and reference scale card for interpretation.2a-reference card prior to January 2014, 2b reference card after January 2014.



2a 2b

To counter the controversy that had arisen regarding which between grade 1 and grade 2 on the uine strip provided optimal results, adjustments were made on the strip by the manufacturer.The previous strip had 5 bands on the reference card.The strip manufactured from January 2014 to date has 4 bands on the reference card. The grade 2 on the older version corresponds to grade 1 on the current strip. This adjustment made it easier to conduct and compare studies . The intensity of the band however does not reflect the burden of mycobacteria and any visible band is reported as positive.*[57]*

5.STUDY JUSTIFICATION

This study aimed to assess the diagnostic value of the lateral flow urine LAM assay in diagnosing pulmonary tuberculosis among adult patients who attended two Kenyan health facilities, Mbagathi County Hospital/Kenyatta National Hospital.

Pulmonary tuberculosis which is the commonest form of TB, was the form of tuberculosis chosen for evaluation of the assay in this study. This form of tuberculosis plays an important role in transmission of tuberculosis as this is through the inhalational route. Sputum which is the specimen of choice for microbiological confirmation is readily retrievable. This is in comparison to the clinical specimens for extrapulmonary forms of TB which are not always easy to retrieve. The yield of the organisms also tends to be higher from respiratory samples compared to other clinical samples. This therefore gives confidence that the LAM assay confirms a diagnosis of active TB even in the absence of microbiological confirmation in a clinical setting.

This was a benchmark study for the lateral flow urine LAM assay in Kenya, as the data previously in use was from other African countries. By determining its performance and factors that influence this, the study would provide information on how to best apply the assay in Kenyan health facilities in the management of patients with tuberculosis.

1. Point-of-care test: results obtained same day within few minutes, reduced number of visits to healthcare facilities, earlier start of treatment, reduced health care costs, reduced duration of hospital admission or possibility of averting hospital admission.
2. Reduced biosafety hazard with use of urine compared to sputum.
3. Suited for resource limited settings: reduced health care costs, use of unprocessed urine, no need for specialized and expensive equipment or laboratories or personnel, can be done bedside, no need for specialized disposal units, strips can be stored easily at room temperature.
4. Suited for regions with high TB and HIV prevalence such as Kenya.
5. Can be used in combination with diagnostic tests already in use such as sputum microscopy and Xpert MTB/RIF for improved diagnostic performance.

6.RESEARCH QUESTION

What is the diagnostic value of the lateral-flow lipoarabinomannan (LF-LAM) assay among adult patients with active pulmonary tuberculosis?

7.RESEARCH OBJECTIVES

7.1. BROAD OBJECTIVE

To determine the diagnostic value of the lateral flow lipoarabinomannan (LF-LAM) assay among adult patients with pulmonary tuberculosis.

7.2. SPECIFIC OBJECTIVES

Primary objectives

I)To determine the sensitivity and specificity of the urinary LAM assay in diagnosis of pulmonary TB.
II) To compare the sensitivity and specificity of the lateral-flow urinary TB LAM assay in HIV positive and HIV negative patients.
III) To determine the additive value of the TB-LAM assay to sputum microscopy and the sputum Xpert® MTB/RIF assay.

Secondary objective

I) To determine the influence of the CD4+ count on the performance of the urine TB-LAM assay in HIV positive patients

8. RESEARCH METHODOLOGY

8.1. Research design

Study design: The design employed for this study was a cross sectional study.

Study area: The study was conducted at Mbagathi District Hospital and Kenyatta National Hospital in Nairobi County. The targeted areas in these facilities were the out-patient TB clinics, the out-patient chest clinic and the in-patient wards.

Study population: The study subjects were adult patients who presented with features suggestive of active tuberculosis in the two health facilities.

8.2. Case definition

Selection criteria

Pulmonary tuberculosis

Persons who presented with cough for more than two weeks, pleuritic chest pain for more than two weeks, fever, night sweats for more than two weeks, weight loss, more than 3 kilograms in 4weeks and hemoptysis of any duration were interviewed for possible enrollment in to our study.

Inclusion criteria

In addition to presenting with the symptoms associated with the case definition of pulmonary tuberculosis, those we enrolled were persons eighteen years of age or older who gave informed consent to participate in the study and not on anti-tuberculous treatment at the time of enrollment. HIV positive patients on isoniazid preventive therapy, who presented with features of active pulmonary tuberculosis, were also be enrolled.

Exclusion criteria

Individuals who declined to give consent, who were unable or unwilling to provide biospecimens, those who had been on anti-tuberculous treatment for more than two days in the last two months or anyone who was on treatment using the medication used as second line therapy was not enrolled in the study.

8.3.Sample size

For a prospective cross sectional study such as this one, the sample size calculation requires the level of knowledge to be known, the desired level confidence should also be provided and

a tolerance error margin or width of the confidence interval so that the necessary sample size is then calculable for a given precision level. There are no previous studies assessing the diagnostic utility of urinary TB-LAM in diagnosing PTB among adult patients thus p of 0.5 will be used in this study. Consecutive sampling of eligible patients will be used until the sample size is reached.

The sample size will be calculated using the Cochran formula (1977)

$$n = z^2pq/d^2$$

Where,

n= sample size

z= standard normal variant corresponding to the 95% confidence interval, and is 1.96

p= Prevalence of 50%, expressed as a decimal

q= 1-p

d= the required precision of estimate (0.05)

Thus,

$$n = \frac{1.96^2 * 0.5(0.5)}{0.05^2}$$

n = 385

Considering the number of patients suspected to have TB, reviewed in these two facilities, the formula below is applied to adjust this for a finite population;

$$n = \left[\frac{n_0}{1 + \frac{n_0 - 1}{N}}\right]$$

n_0 is the sample size (385)

n = is the final sample size

N = Estimated number of patients with suspected TB (640)

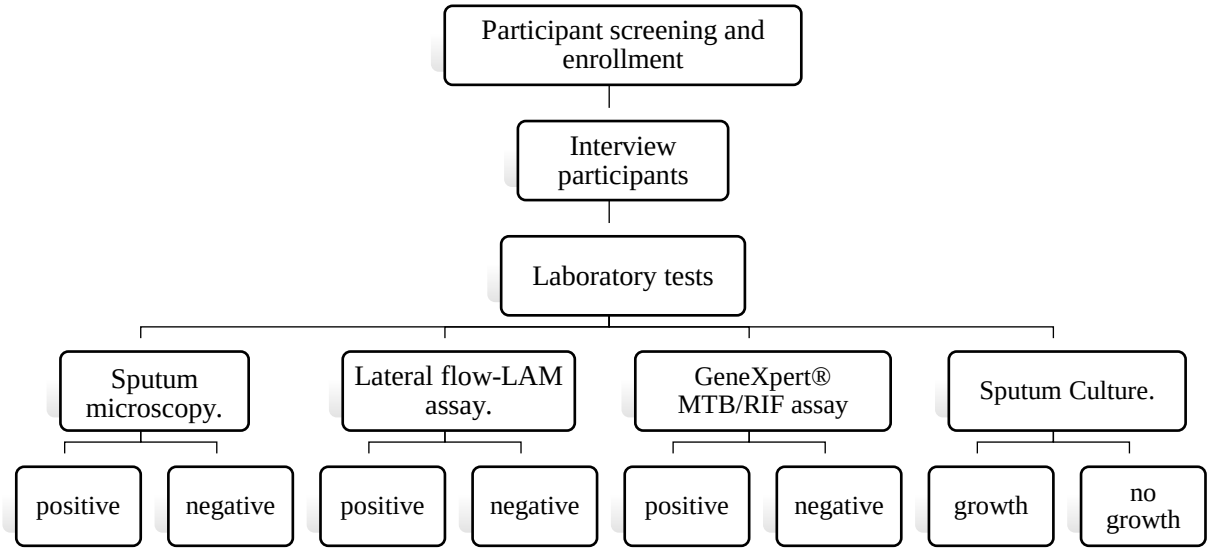
Using the above formula our final sample size is 241.

8.4.Sampling method

We used consecutive sampling, a type of non-random sampling technique. Every subject who met the criteria of inclusion was selected until the desired sample size was reached.

8.5.Patient recruitment

Figure 6. Patient enrollment, recruitment and laboratory tests.



9.LABORATORY METHODS

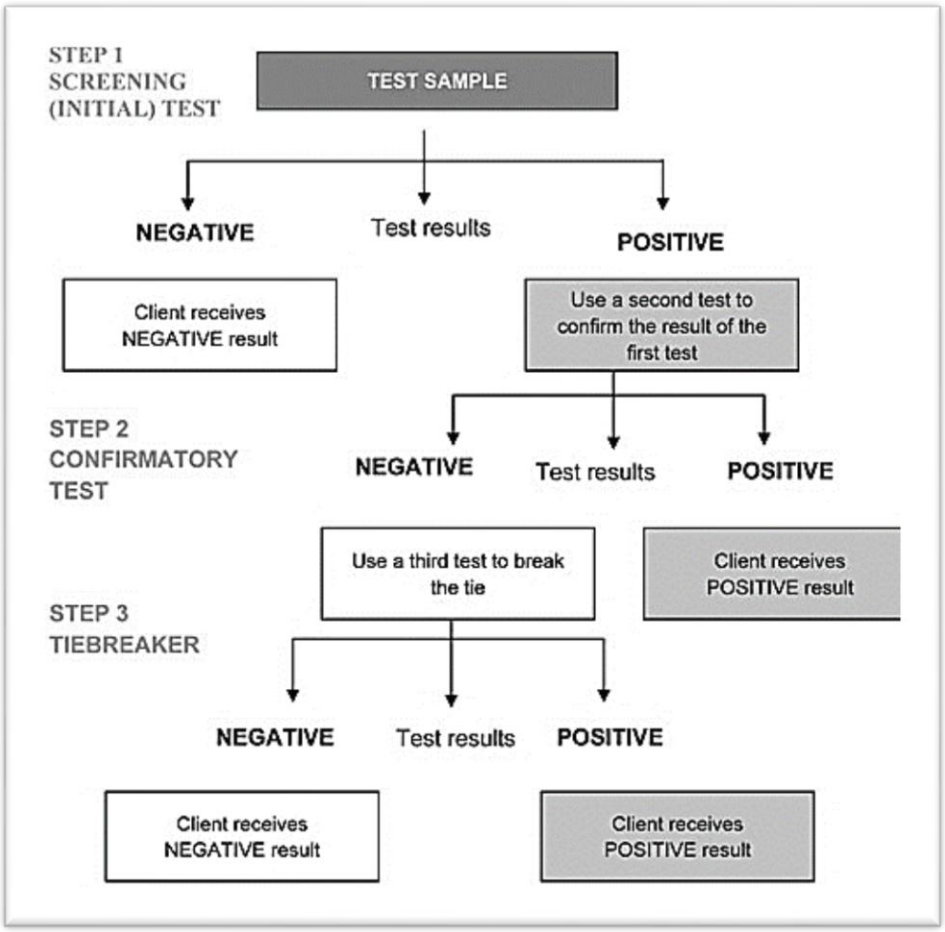
Once consent was obtained from the study participants, each participant filled in a questionnaire. This captured the patients' demographical data and symptoms at presentation. Demographical data obtained included the ages of the participants, weight, gender, residence, occupation, level of education, functional status, HIV status (if known), CD4+ count (if know n), antiretroviral therapy if used or if participant was HAART naïve, previous treatment for tuberculosis, presence of chronic lung disease and presence of other comorbid conditions.

The patients then provided two sputum samples, morning and/or spot, of about 3-5 millilitres in sterile, lockable sputum collection containers. These were appropriately stored at room temperature until the time for laboratory testing-sputum microscopy, Xpert MTB/RIF and liquid culture. Sputum microscopy was conducted in the microbiology laboratories at Lancet laboratories. The liquid culture used was the BACTEC MGIT 960 system. This also served as the gold standard to which the other TB diagnostic tests were compared. This was conducted at the microbiology laboratory at Lancet laboratories. The Xpert MTB/RIF was conducted in the microbiology laboratories that had been designated to do so at the study sites.

A mid-stream urine sample, approximately 30 millilitres was also provided by the patients in a sterile, lockable urine collection bottle. The lateral flow assay was conducted on this urine. The urine was appropriately stored until the time for analysis in the microbiology laboratory at the Kenyatta National Hospital CCC.

Blood was drawn from the patients for HIV testing using rapid tests and the ELISA method. HIV testing was done in designated areas in the out-patient and in-patient departments in the participating facilities. The rapid tests were done by personnel who had been previously trained to do so in the participating facilities. Blood was obtained by a pinprick. HIV testing using the rapid tests was conducted as per the national HIV testing algorithm (figure 6 shown below).

Figure 7. National HIV Testing Algorithm for rapid tests.



HIV ELISA was used for HIV testing in conjunction with the rapid tests in cases where there was a delay in conducting the rapid tests first. For the ELISA test, about 3-5 millilitres of whole blood was obtained by venipuncture and put into a plain vacutainer. The principal researcher, trained research assistants and clinicians from the participating facilities drew the blood for the HIV ELISA assay. The blood was tested in the immunology laboratory.

The results of the HIV test were relayed to the clients during the post-test counseling Session. For the HIV positive clients emotional and psychosocial support was provided, follow up services were discussed as well as prevention of HIV transmission. Other issues discussed included treatment initiation, nutrition, result disclosure, management of depression and associated complications such a suicide or any other psychiatric illness that may arise. The HIV negative individuals were advised on ways of maintaining their negative status and also on repeat testing in the future should the need arise.

Blood was also drawn to determine the CD4+ count for the HIV positive patients. The count was established using the protocol of the participating institutions. About 3-5 millilitres of

whole blood was drawn by venipuncture and put into an EDTA vacutainer. The blood draw was done by the principal researcher, the research assistants or the clinicians in the participating facilities. The test was conducted in the CCC laboratory or the immunology laboratories of the participating institutions by flow cytometry.

The loss of these T-helper cells, which are the primary target for HIV, results in loss of ability to mount an appropriate immune response to any pathogen and increases vulnerability to opportunistic pathogens characteristic of HIV/AIDS. The estimation of peripheral CD4 T-lymphocytes count was therefore used as the laboratory indicator of immune function in HIV positive patients. It therefore informed on the degree of immunosuppression and was used for this purpose in the study. The actual CD4+count of the participants was recorded. Where a blood sample was not available for the absolute CD4+ count, the count was estimated from the absolute lymphocyte count. *[60]*

A detailed description of the laboratory tests used in this study are described in appendix III on pages 54-61.

10.STUDY VARIABLES

The dependent variables were the HIV status and the level of immunosuppression (using the CD4+ count of the HIV positive patient).

11.QUALITY ASSURANCE MEASURES

The laboratories utilized in this study are well accredited laboratories that utilize internal and external quality assurance measures. These were the immunology, hematology, microbiology and CCC laboratories at the participating facilities. The microbiology laboratory at Lancet laboratories was also used.

All the testing kits and reagents used were checked to ensure that the expiry dates had not been passed. The kits were also used according to the manufacturer's instructions.

The sputum and urine samples were collected in sterile and lockable containers. The research assistants ensured the specimens collected were of good quality and the appropriate quantity prior to being sealed in preparation for storage. The specimens were then appropriately labelled and sealed in lockable bags. Storage of the sputum and urine samples was at room temperature. The specimens were thereafter transported to the processing laboratories between 2 and 8 hours after collection.

Sputum microscopy was conducted as per the World Health Organisation recommendations and recommendations by the National TB programs. The test was done in the microbiology laboratory at Lancet laboratories.

Xpert MTB/RIF was conducted at the CCC microbiology lab at Kenyatta National Hospital and at the TB laboratory at Mbagathi County Hospital. These were the designated areas in these facilities for this test.

Liquid culture was conducted as per the World Health Organisation recommendations and recommendations by the National TB programs. This was conducted in microbiology laboratory at Lancet laboratories.

Qualified personnel already within the participating facilities were used to conduct the rapid HIV tests as per the National HIV testing guidelines. For Kenyatta National Hospital, the HIV ELISA test was conducted in the immunology and CCC laboratories in the facility.

The CD4 count determination was done at the CCC laboratory at Kenyatta National Hospital and the CCC/hematology laboratory at Mbagathi County Hospital.

Training of the laboratory technician involved in conducting the urine LAM assays was undertaken before the start of the study. This was done by laboratory technicians previously trained on using the LAM assays at the KNH CCC laboratory.

12.ETHICAL CONSIDERATIONS

Clearance for the study was sought from the Kenyatta National Hospital/ the University of Nairobi Ethics Review Committee to ensure that the study was conducted in an ethical manner and that no harm would be caused to the participants. The study was undertaken once this clearance was provided.

Only those participants who provided informed consent were recruited into the study. A well detailed consent form showing the details of the study was provided. Those who agreed to the terms of the study ratified their consent by appending their signatures on the consent form.

Information generated from the study with regards to the health of the participants was communicated to the participants and their health care providers so that the necessary management was initiated. The health care of the participants was not compromised in any way. The care of participants who opted to withdraw from the study after recruitment was not compromised. Confidentiality of the participants' personal details, medical data and test results was maintained throughout the whole study. A unique study code was assigned to each participant and the information was entered in to a secure database to maintain this.

As per the national and international guidelines, individuals who have a presumptive diagnosis or have been diagnosed with TB also require to be tested for HIV. HIV testing was therefore offered to the participants. The five key components, the``5 Cs´´ of HIV testing and counseling services, that is consent, confidentiality, counseling (post- and pre-test), correct test results and connection/linkage to prevention, care and treatment were provided to the participants.

13. DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data from study questionnaires was stored with unique study ID so as to keep and maintain confidentiality and privacy of study participants. The data was stored in a password protected computer file. This data remained in the custody of the principal investigator. Backup was made on a compact disc (CD) and kept under lock and key with access to the investigator only.

Descriptive analysis was done to show proportions and means.

Sensitivity and specificity of the TB diagnostic tests used was calculated using the results of the liquid culture as the gold standard.

The sensitivity and specificity of the LAM assay, sputum microscopy and was determined and presented using 2 by 2 tables and bar graphs.

The sensitivity and specificity of the LAM assay in the HIV positive and negative populations was calculated. The proportion of HIV negative patients whose LAM assays were positive and culture positive was compared to the proportion of HIV positive patients whose LAM assays were positive and culture positive.

The actual CD4+ counts of the patient was recorded and the results of the TB diagnostic tests conducted, indicated alongside. The lower the CD4+ count, the worse the level of immunosuppression. This information was used to describe the effect of the degree of immunosuppression on the sensitivity of the urine LAM assay among the HIV positive participants.

The sensitivities of the tests were then compared to each other using bar graphs.

The additive value of the TB-LAM assay when added to sputum microscopy was calculated and compared to sensitivity of the LAM test used on its own. A positive result was indicated where either or both of the tests were recorded as positive.

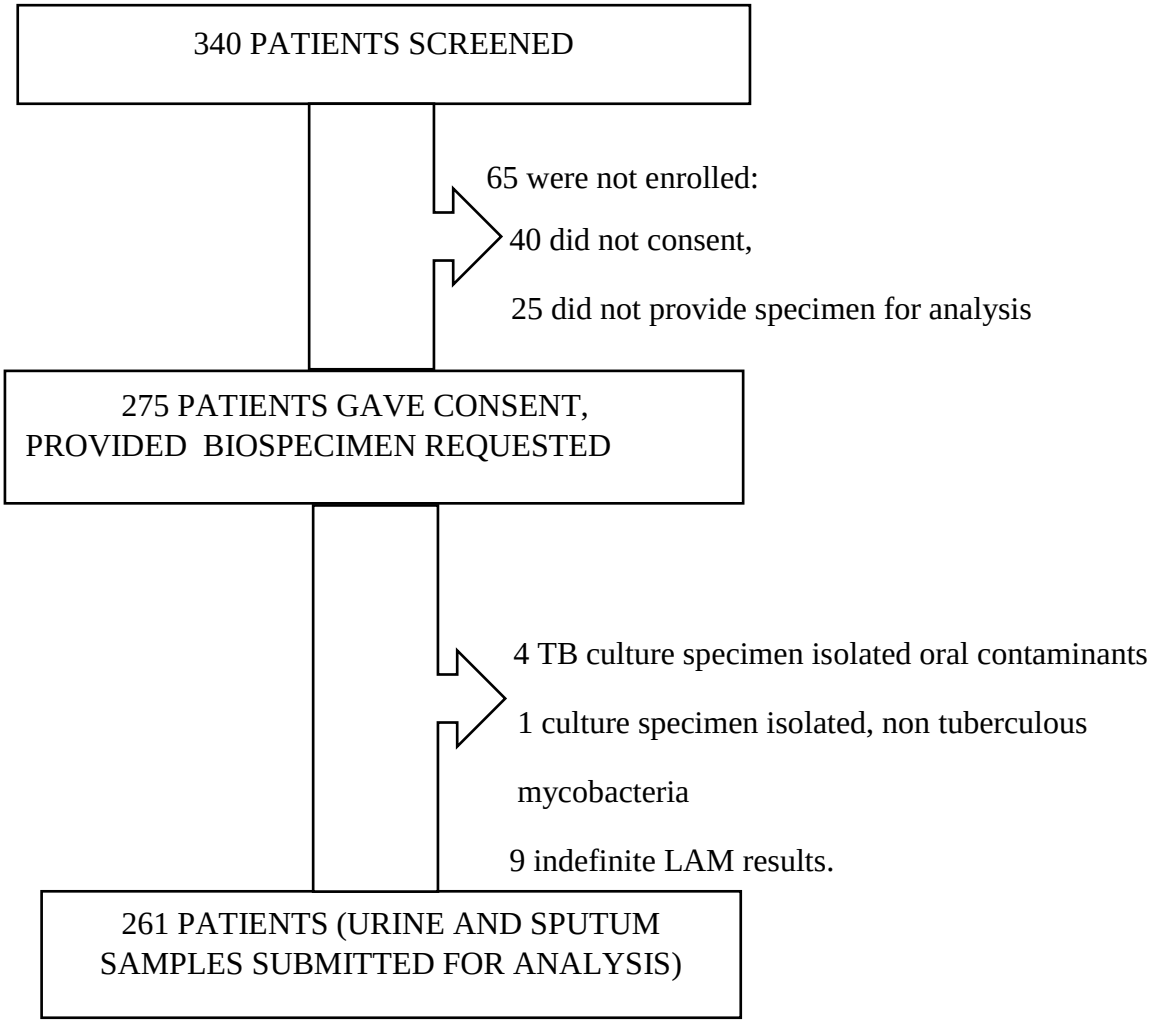
The additive value of the TB-LAM assay when added to the sputum Xpert® MTB/RIF was also calculated and compared to sensitivity of the LAM assay used on its own. A positive result was indicated when either or both of the tests were recorded as positive.

14.RESULTS

This study was conducted between the months of April and August, 2016. The study sites were Mbagathi County Hospital and Kenyatta National Hospital. Patient recruitment was conducted at the TB clinics, accident and emergency department and the in-patient wards.

A total of 340 patients were screened, 170 from Mbagathi County Hospital and 170 from Kenyatta National Hospital.

Figure 8. Flow chart of patient recruitment.



14.1 Sociodemographic characteristics

Table 1. Sociodemographic characteristics of population recruited

		n(%)
Gender	Female	100(38.3)
	Male	161(61.7)
HIV status	HIV positive	101(38.7)
	HIV negative	157(60.2)
	Unknown	3(1.1)
Age(yrs)	Median	37.5
	Range	18-69
Highest Level of Education	None	25(9.6)
	Primary	117(44.8)
	Secondary	73(28.0)
	Tertiary/University	38(14.6)
	No data	8(3.0)
Type of Residence	Urban informal	140(53.6)
	Urban formal	41(15.7)
	Periurban	38(14.6)
	Rural	24(9.2)
	No data	18(6.9)

As shown in table 1 above, there were 261 patients enrolled in this study predominantly from an urban informal setting 140 (53.6%). There were 157(60.2%) HIV negative and 101 (38.7%) HIV positive patients in the study; 3 patients declined to be tested for HIV. The majority of patients were males, 61(61.8%). The median age for the population was 37.5 years with 117(44.8%) having attained primary level education.

14.2 Clinical characteristics

Table 2. Clinical features of the patients recruited

	n(%)
Productive cough	261(100.0)
Chronic cough (>2 weeks)	249(95.4)
Weight loss	230(88.1)
Fever	208(79.7)
Chest pain	198(75.9)
Night sweats	191(73.2)
Hemoptysis	81(31.0)
Lymphadenopathy	14(5.4)
Previous TB treatment	68(26.1)
Contact with PTB patient	29(11.1)

A shown in table 2 above,with regards to the clinical symptoms typically associated with TB, the most common clinical presentations were a productive cough that had lasted more than two weeks 249(95.4%), weight loss 230(88.1%) and fever 208(79.7%). These were also associated with chest pain 198(75.9%) and night sweats 191(73.2%). Hemoptysis was reported in 79 patients (31.0%) and was therefore not a common presenting feature in the population. Lymphadenopathy also did not feature prominently in the population. It was reported in 14 patients (5.4%). Majority had no previous treatment for TB or contact with PTB patients. However, 6 of the recruits did not know if they had interacted with a patient with pulmonary tuberculosis.

14.3.Laboratory results

All participants provided urine for LAM, sputum for both Ziehl Neelsen microscopy and TB culture (BACTEC™ MGIT™ 960 Mycobacterial Detection System).

A total of 145 sputum samples were submitted for nucleic acid amplification detection by Xpert®MTB/RIF, . One of the samples tested positive for multidrug resistant TB.

TB culture was used as the gold standard for the study.

HIV testing was offered to all study participants; 258(98.6%) were tested. The remainder opted out of HIV testing.

Mycobacteria tuberculosis was isolated from 98 (37.5%) of the sputum samples submitted for TB culutre. These 98 were the microbiologically confirmed cases and were therefore used for calculation of the sensitivity, specificity, positive and predictive values of the diagnostic tests used in this population.

Primary objectives

14.3.1 To determine the sensitivity and specificity of the urinary LAM assay in diagnosis of pulmonary tuberculosis

Analysis for sensitivity and specificity of the LAM assay was done on the 261 patients who submitted samples for the urine LAM assay.

Table 3.Overall performance of the lateral-flow LAM assay in diagnosis for pulmonary tuberculosis.

All patients		TB Culture		
		Positive n(%)	Negative n(%)	Total n(%)
Urine LAM	Positive	28(28.6)	16(9.8)	44(16.9)
	Negative	70(71.4)	147(90.2)	217(83.1)
Total		98(100.0)	163(100.0)	261(100.0)

		95%CI
Sensitivity	28.6%	20.6-38.3%
Specificity	90.2%	84.5-93.9%
PPV	63.6%	49.4-77.9%
NPV	67.7%	61.5-74.0%

Table 3 above shows the overall sensitivity of the lateral flow LAM assay which was 28.6% (95% CI, 20.6-38.3%) and the specificity was 90.2% (95% CI 84.5-93.9%). This was at 28.6 %, identifying 28 out of the 98 culture confirmed pulmonary TB cases. The assay correctly ruled out pulmonary tuberculosis in 147(90.2%) of the patients in who did not have microbiologically confirmed pulmonary TB.

14.3.2 To compare the sensitivity and specificity of the lateral-flow urine TB LAM assay in HIV negative and positive patients

The samples of 101 HIV positive and 157 HIV negative patients were analysed. The three patients whose HIV statuses were not known were excluded from this analysis.

Table 4.Performance of the lateral-flow LAM assay among the HIV positive

HIV Positive		TB sputum culture		
		Positive n(%)	Negative n(%)	Total n(%)
LAM	Positive	20(58.8)	10(14.9)	30(29.7)
	Negative	14(41.2)	57(85.1)	71(70.3)
Total		34(100)	67(100)	101(100)

		95%CI
Sensitivity	58.8%	(42.2-73.6%)
Specificity	85.1%	(74.4-91.8%)
PPV	66.7%	(49.8-83.5%)
NPV	80.3%	(71.0-89.5%)

Among the 101 HIV positive patients in the study population, there were 34 cases of culture confirmed pulmonary tuberculosis, this is shown in table 4 above. This represented 33.7% of this population. The lateral flow LAM assay accurately diagnosed 20 of these culture confirmed cases. The sensitivity was 58.8 (95%CI 42.2-73.6%). The specificity was 85.1%(95%CI 74.4-91.8%) ruling out PTB in 57 out of the 67 culture negative cases.

Table 5. Performance of the lateral-flow LAM assay among the HIV negative.

HIV Negative		TB sputum culture		
		Positive n(%)	Negative n(%)	Total n(%)
LAM	Positive	8(12.7)	6(6.4)	14(8.9)
	Negative	55(87.3	88(93.6	143(91.1)
Total		63(100)	94(100)	157(100)

		95%CI
Sensitivity	12.7%	6.4-23.5%
Specificity	93.6%	86.4-97.3%
PPV	57.1%	31.2-83.1%
NPV	61.5%	53.6-69.5%

Table 5 above shows the sensitivity and specificity of the LAM assay among the HIV negative patients. There were 157 HIV negative patients, 63(40.1%) of these patients were confirmed to have pulmonary TB. The LAM assay had a sensitivity of 12.7% (6.4-23.5%) in this population. The test diagnosed pulmonary TB in only 8 of the culture confirmed TB cases. For the 94 sputum samples that were culture negative the assay accurately identified 88 cases. Specificity was 93.6% (95% CI, 86.4-97.3%).

The sensitivity of the LAM assay in the HIV positive population was higher than that of the the HIV negative subgroup. The assay identified more true positive cases when applied to the

HIV positive. Furthermore, the probability of having pulmonary tuberculosis when the LAM assay provided a positive result was higher for those who were HIV positive.

14.3.3 To determine the additive value of the TB-LAM assay to sputum microscopy and Xpert® assay

Additive value of the urine LAM assay to sputum ZN microscopy

Table 6. Performance LAM assay among the HIV positive patients.

HIV positive		Culture		Total
		Positive n(%)	Negative n(%)	n(%)
LAM	Positive	20(58.8)	10(14.9)	30(29.7)
	Negative	14(41.2)	57(85.1)	71(70.3)
Total		34(100.0)	67(100.0)	101(100)

		95%CI
Sensitivity	58.8%	42.2-73.6%
Specificity	85.1%	74.4-91.8%
PPV	66.7%	49.8-83.5%
NPV	80.3%	71.0-89.5%

Table 7.Performance ZN microscopy among the HIV positive patients.

HIV Positive		TB sputum culture		Total
		Positive n(%)	Negative n(%)	n(%)
ZN	Positive	14(41.2)	0(0.0)	14(13.9)
	Negative	20(59.8)	67(100.0)	87(86.1)
Total		34(100.0)	67(100.0)	101(100)

		95%CI
Sensitivity	41.2%	26.4-57.8%
Specificity	100.0%	93.3-100.0%
PPV	100.0%	100.0,100.0%
NPV	77.0%	68.2-85.9%

Table 8. Performance LAM+ZN combination among HIV positive patients

HIV Positive		TB sputum culture		Total
		Positive n(%)	Negative n(%)	n(%)
LZN	Positive	24(70.6)	0(0.0)	24(23.8)
	Negative	10(29.4)	67(100.0)	77(76.2)
Total		34(100.0)	67(100.0)	101(100)

		95%CI
Sensitivity	70.6%	53.6-83.2%
Specificity	100.0%	93.3-100.0%
PPV	100.0%	100-100.0%
NPV	87.0%	79.5-94.5%

Table 9.Performance LAM assay,ZN microscopy and combination LAM and ZN microscopy.

All HIV positive (n=101)	TP/CP	Sensitivity (95% confidence interval)	TN/CN	Specificity (95% confidence interval)
LAM	20/34	58.8%(42.2-73.6%)	57/67	85.1%(74.4-91.8%)
ZN	14/34	41.2%(26.4-57.8%)	67/67	100.0%(93.3-100.0%)
LAM+ZN	24/34	70.6%(53.6-83.2%)	67/67	100.0%(93.3-100.0%)

TP=true positive CP=culture positive TN= true negative CN= culture negative

Where the two tests, the LAM assay and ZN microscopy were combined, the performance was much more improved than when each test was used alone.This is shown in table 9 above. Sensitivity for the tests combined was 70.6% (95%CI 53.6-83.2%) versus 58.8% (95%CI 42.2-73.6%) for the LAM assay alone and 41.2% for ZN microscopy alone. This combination identified 10 more cases than ZN microscopy and 6 more cases than the LAM assay. The specificity of the combined tests was 100.0%(95%CI 93.3-100.0%), for the LAM assay alone was 85.1%(95%CI 74.4-91.8%). The specificity for the tests combined was 100.0% which was higher than when the LAM was used alone but was similar to that of ZN microscopy.

14.3.4 Additive value of the urine LAM assay to the sputum geneXpert® assay among 71 HIV positive patients

Table 10. Performance LAM assay among HIV positive patients

HIV Positive		Culture		Total
		Positive n(%)	Negative n(%)	n(%)
LAM	Positive	18(69.2)	7(15.6)	25(35.2)
	Negative	8(30.8)	38(84.4)	46(64.8)
Total		26(100.0)	45(100.0)	71(100.0)

		95%CI
Sensitivity	69.2%	49.8-83.5%
Specificity	84.4%	70.8-92.5%
PPV	72.0%	54.4-89.6%
NPV	82.6%	71.7-93.6%

Table 11. Performance geneXpert® assay among HIV positive patients

HIV Positive		Culture		Total
		Positive n(%)	Negative n(%)	n(%)
Xpert	Positive	22(84.6)	2(4.4)	24(33.8)
	Negative	4(15.4)	43(95.6)	47(66.2)
Total		26(100.0)	45(100.0)	71(100.0)

		95%CI
Sensitivity	84.6%	65.7-94.3%
Specificity	95.6%	84.2-99.5%
PPV	91.7%	80.-100.0%
NPV	91.5%	83.5-99.5%

Table 12. Performance combination LAM and geneXpert® assay

HIV Positive		Culture		Total
		Positive n(%)	Negative n(%)	n(%)
LAM+ Xpert	Positive	25(96.2)	1(2.2)	26(36.6)
	Negative	1(3.8)	44(97.8)	45(63.4)
Total		26(100.0)	45(100.0)	71(100.0)

		95%CI
Sensitivity	96.2%	79.3-100.0%
Specificity	97.8%	87.2-100.0%
PPV	96.2%	88.8-100.0%
NPV	97.8%	93.5-100.0%

Table13. Performance LAM assay, geneXpert® assay and LAM-Xpert combination

HIV Positive (n=71)	TP/CP	Sensitivity (95% confidence interval)	TN/CN	Specificity (95% confidence interval)
Urine LAM	18/26	69.2%(49.8-83.5%)	38/45	84.4%(70.8-92.5%)
GeneXpert	22/26	84.6%(65.7-94.3%)	43/45	95.6%(84.2-99.5%)
Urine LAM+Xpert	25/26	96.2%(79.3-100.0%)	44/45	97.8%(87.2-100.0%)

TP=true positive CP=culture positive TN= true negative CN= culture negative

As shown in table 13 above, the sensitivity of the LAM assay when it was used alone was 69.2% (95%CI 49.8-83.5%) and specificity was 84.4%(95% CI 70.8-92.5%). The sensitivity of the geneXpert® assay used alone was 84.6%(95%CI 65.7-94.3%) and specificity was 95.6%(95%CI 79.3-100.0%). The sputum geneXpert® assay had a higher sensitivity and specificity than the urine LAM assay among the 71 HIV positive patients who provided samples for both tests. When the two tests were combined the sensitivity increased to 96.2% (95%CI 87.2-100.0%). The specificity increased to 97.8% (95%CI 87.2-100.0%).

Secondary objective

14.3.5 To determine the influence of the CD4+ count on the performance of the LAM assay in HIV positive patients

The CD4+ count was obtained from 73 of the HIV positive patients recruited.The absolute CD4+ count was obtained from 69 of the patients. The estimated CD4+ count was obtained from the absolute lymphocyte count for 4 of the patients as they did not provide blood samples for CD4+count establishment. Three of these patients had an estimated count of less than 200cells per microliter and one had an estimated count above 200. The CD+ count ranged from three to 1040 cells per cubic millimetre with an estimated mean CD4+ count of 174 cells and median CD4+ count of 82 cells per microliter.

Table 14. Average CD4+ count for patients who tested LAM positive and LAM negative.

LAM		N	Mean	Std. Deviation	Std. Error Mean	p-value
CD4 count	Positive	24	61.83	107.641	21.972	<0.001
	Negative	45	230.64	236.074	35.192	

Table 14 shows the mean CD4+ count for those who tested LAM positive which was 61.83 while the mean count for those who tested LAM negative was about 230. Those patients whose urine LAM assay result was positive were noted to have a lower CD4+ count than those whose LAM results were negative.

The LAM assay was then evaluated with the CD4+ cut off points at 50,100 and 200 cells per microliter to assess the performance at these CD4 points. The results are shown below.

Table 15-16. The sensitivity of the urine LAM assay CD4+ threshold 50 cells per microlitre

CD4+≤50		Culture		Total
		Positive n(%)	Negative n(%)	n(%)
LAM	Positive	13(76.5)	5(45.5)	18(66.7)
	Negative	4(23.5)	6(54.5)	10(33.3)
Total		17(100.0)	11(100.0)	28(100.0)

		95%CI
Sensitivity	76.5%	52.1-90.8%
Specificity	54.5%	28.1-78.6%
PPV	72.2%	51.5-92.9%
NPV	60.0%	29.6-90.4%

CD4+>50		Culture		Total
		Positive n(%)	Negative n(%)	n(%)
LAM	Positive	3(30.0)	4(12.5)	7(16.7)
	Negative	7(70.0)	28(87.5)	35(85.3)
Total		10(100.0)	32(100.0)	42(100.0)

		95%CI
Sensitivity	30.0%	10.6-60.8%
Specificity	87.5%	71.2-95.5%
PPV	42.9%	6.2-79.5%
NPV	80.0%	66.7-93.3%

Table 17-18 Performance LAM assay CD4+ threshold 100 cells per microliter.

CD4+≤100		Culture		Total
		Positive n(%)	Negative n(%)	n(%)
LAM	Positive	13(65.0)	6(31.6)	19(48.7)
	Negative	7(35.0)	13(68.4)	20(51.3)
Total		20(100.0)	19(100.0)	39(100.0)

		95%CI
Sensitivity	65.0%	43.1-81.9%
Specificity	68.4%	45.8-84.7%
PPV	68.5%	47.5-89.3%
NPV	65.0%	44.1-85.9%

CD4+>100		Culture		Total
		Positive n(%)	Negative n(%)	n(%)
LAM	Positive	3(42.9)	3(12.5)	6(19.4)
	Negative	4(57.1)	21(87.5)	25(80.6)
Total		7(100.0)	24(100.0)	31(100.0)

		95%CI
Sensitivity	42.9%	16.0-74.9%
Specificity	87.5%	68.0-96.3%
PPV	50.0%	10.0-90.0%
NPV	84.0%	69.6-98.4%

Table 19-20. Performance LAM assay CD4+ threshold 200 cells per microliter

CD4+≤200		Culture		Total
		Positive n(%)	Negative n(%)	n(%)
LAM	Positive	17(65.4)	7(28.0)	24(47.1)
	Negative	9(34.6)	18(72.0)	27(52.9)
Total		26(100.0)	25(100.0)	51(100.0)

		95%CI
Sensitivity	65.4%	46.1-80.6%
Specificity	72.0%	52.1-85.8%
PPV	70.8%	52.6-89.0%
NPV	66.7%	48.9-84.4%

CD4+>200		Culture		Total
		Positive n(%)	Negative n(%)	n(%)
LAM	Positive	1(33.3)	2(10.5)	3(13.6)
	Negative	2(66.7)	17(89.5)	19(86.4)
Total		3(100.0)	19(100.0)	22(100.0)

		95%CI
Sensitivity	33.3%	6.2-79.5%
Specificity	89.5%	67.1-98.1%
PPV	33.3%	0.0-86.7%
NPV	89.5%	75.7-100.0%

Table 21. Performance LAM assay at varying CD4+ thresholds

CD4+count	TP/CP	Sensitivity (95% confidence interval)	TN/CN	Specificity (95% confidence interval)
≤50,n=28	13/17	76.5%(52.1-90.8%)	6/11	54.5%(28.1-78.6%)
≤100,n=39	13/20	65.0%(43.1-81.9%)	13/19	68.4% (45.8-84.7%)
≤200,n=51	17/26	65.4%(46.1-80.6%)	18/25	72.0% (52.1-85.8%)
>200,n=22	1/3	33.3%(6.2-79.5%)	17/19	89.5%(67.1-98.1%)

Using the CD4+ count as a marker of degree of immunosuppression, the HIV positive population was stratified by CD4+ count. The CD4+ thresholds used were ≤ 50, ≤100 and ≤200 and >200. The sensitivity and specificity of the LAM assay was calculated for this population at these CD4+ thresholds as shown in table 21 above. The sensitivity of the urine LAM assay was noted to increase and specificity was noted to decrease as the CD4+ count reduced. The sensitivity of the LAM assay at these thresholds was 76.5%(95%CI 52.1-90.8%), 65.0% (95%CI 43.1-81.9%), 65.4%(95% CI 46.1-80.6%) and 33.3% (95%CI 6.2-79.5%) for CD4+≤ 50, ≤ 100, ≤ 200 and > 200 respectively. The specificity was 54.5% (95%CI 28.1-78.6%),68.4% (95%CI 45.8-84.7%) ,72.0% (95% CI 52.1-85.8%) and 89.5% (95% CI for 67.1-98.1%) CD4+≤ 50, ≤ 100 and ≤ 200 and >200 respectively.

15.DISCUSSION

Our study sought to find out if the lateral-flow urine LAM assay could be used to diagnose pulmonary tuberculosis (PTB). The assay did diagnose pulmonary tuberculosis. However, when it was applied to a heterogenous population it performed poorly with a low sensitivity.It was noted on the other hand, to have an improved performance when the population was stratified by HIV status. The sensitivity of the assay was higher in the HIV positive subgroup. When the HIV positive population was further stratified by CD4+ count, it was noted that the degree of immunosuppression affected the sensitivity and specificty of the urine LAM assay. The higher sensitivity was found at CD4+ counts of 200 or less with the highest sensitivity noted at a CD4+ count of 50 and below. The specificity in contrast was noted to decrease as the degree of immunosuppression worsened. There was additive value when the urine LAM assay when was used in combination with ZN microscopy and the sputum geneXpert assay among the HIV positive.

There were 98 cases of culture confirmed pulmonary tuberculosis in this study population. The sensitivity of the lateral-flow LAM assay when all the study participants were included was 28.6%. The population in the study was composed of 101 HIV positive and 157 HIV negative patients, the majority being the latter. The HIV status of three patients was unknown. As already described LAM is readily detectable in those with a compromised immune system compared to those with a competent immune system. A higher sensitivity is therefore expected in the background of immunosuppression than in one with a background of a competent immune system.Where immunocompetent persons are tested alongside HIV positive persons, the sensitivity is expected to be influenced by the proportion of the two subgroups in that population.The low sensitivity in this study is likely a reflection of the predominance of HIV negative persons in the population.

The assay was noted to have varying sensitivity when applied to a heterogenous/mixed population in other studies with populations with a high TB prevalence. Minion et al reviewed 7 studies in 2011, with reference to TB culture. Sensitivity range of these studies was 13 to 93%. The specificity range was 87 to 99%. *[45*] A study by Mutetwa et al. in Zimbabwe in 2008 reported a sensitivity of 44% (95% CI 36-52%) and specificity of 89%(95%CI 81-94%) for culture confirmed TB cases. The overall HIV prevalence in their study was 77% with TB prevalence approximated at 50%. *[35]* The study by Daley et al in India in 2009 reported LAM sensitivity of 17.8 (95%CI 8.5-32.6%) and specificity of 87.7% (95%CI 81.3-92.3%). Though a high TB burden was reported in this population, 8.5% of their population was HIV positive. *[36]* In our study, the prevalence of HIV was 38.7% and TB prevalence was 37.5%. The sensitivity of the LAM assay was 28.6%. This figure was between that found by Mutetwa et al (higher HIV prevalence of 77%) and Daley et al (lower HIV prevalence of 8.5%). The proportion of HIV positive and negative patients in the study population likely influenced the sensitivity of the LAM assay.

When we stratified our patients according to their HIV status, the performance of the LAM assay was noted to be better in the HIV positive subgroup. (For this analysis we excluded the three patients who declined HIV testing). In the HIV positive subgroup the sensitivity was 58.8% compared to 12.7% in the HIV negative subgroup. There were 63 culture confirmed

pulmonary tuberculosis cases among the 157 HIV negative patients, this was 40.1% of this population. There were 34 cases of culture confirmed PTB among the 101 HIV positive patients which was 33.7% of this population. The LAM assay was able to identify a higher proportion of patients with PTB among the HIV positive. The specificity was however higher in the HIV negative population than in the HIV positive population at 93.6% compared to 85.1% respectively.

There is increased incidence of tuberculosis in the setting of HIV due to impairment of the immune status especially in advanced disease. This includes pulmonary TB, extrapulmonary TB and mycobacteremia/disseminated TB with likely renal involvement. It is in this setting also that LAM is readily detected in urine. The higher sensitivity in the HIV positive subgroup was therefore an expected finding and was also noted in other studies.

Minion et al reported sensitivity ranges of 3 to 53% higher in the HIV positive subgroups when 5 studies that stratified their population based on HIV status were reviewed. *[45]* In the population studied by Dheda et al., 2010, a sensitivity of 21% in the HIV positive subgroup versus 6% in the HIV negative group was reported. There was statistical significance in this difference (p value < 0.001). For Mutetwa et al. 2012, the sensitivity was also significantly higher in the HIV positive subgroup than the HIV negative subgroup. The sensitivity in the HIV positive subgroup was 52% (95% CI 43-62%) versus 21%(95% CI 9-37%) in the HIV negative subgroup, p-value was statistically significant (p<0.001). *[35]*

The sensitivity of the LAM assay in the HIV positive subgroup was 58.8%. The sensitivity for ZN microscopy was 41.2%. When the two tests were combined, the sensitivity increased to 70.6% higher than that of either tests used alone. Specificity of the combined tests was 100.0%. This was higher than that of the LAM assay used alone. This was similar to findings reported in other studies.

In the study by Paul Drain et.al.(2015), the sensitivity for the LAM assay was 42.1% (95%CI 29.1-55.9%) and that for sputum microscopy was 21.1% (95% CI 11.4-33.9%). The sensitivity of the two tests combined was 52.6%(95% CI 39.0-66.0%). *[55]* A metanalysis by Maunank Shah et al in 2015, reviewed 4 studies (Lawn 2014, Nakiyingi 2014; Peter 2012; Peter 2015). There were1876 participants and 708 (38%) had TB. They classified any patient who could not produce sputum as sputum negative. The pooled sensitivity for the ZN microscopy was 40% (27-54%) and for the LAM assay was 38% (34-42%). The pooled specificity was 98%(95%CrI 93-100%) for ZN microscopy and 95%(95%CrI 94-97%) for the LAM assay. When the two tests were combined, the sensitivity was 59%(47-70%) representing an increase of 19%(4-36%). Specificity was 92%(73-97%) representing a 6% (1-24%) decrease from sputum microscopy used alone. *[56]*

ZN microscopy has a low sensitivity when used for diagnosing PTB in HIV positive patients especially those with advanced disease. This is due to increased incidence of paucibacillary disease, patients being unable to provide good quality sputum and some patients being unable to produce sputum for analysis. The sensitivity can be lower than 50% in this population. This makes it a challenge to use ZN microscopy for diagnosis of pulmonary TB in HIV. The LAM assay on the other hand has been shown to perform better in the setting of HIV. It diagnosed pulmonary TB where patients would have been classified as sputum negative if

ZN microscopy was used alone. *[55]* It therefore had an additive value to microscopy which would have missed these cases of TB. The risk would have been delayed or missed treatment with worsening morbidity or even mortality of patients.

Among the patients who provided urine samples for the LAM assay and also provided sputum for the geneXpert® assay, the sensitivity of the LAM assay when it was used alone was 69.2%. The specificity was 84.4%. The sensitivity of the geneXpert® assay used alone was 84.6% and specificity was 95.6%. When the LAM assay and geneXpert® were combined the sensitivity of the test was 96.2% and the specificity of the was 97.8%. This combination improved on the sensitivity and specificity of the geneXpert assay and the LAM assay when they were used alone.

The review by *Maunank Shah et al*. reported a similar increase when the LAM assay was combined with the geneXpert assay. The pooled sensitivity for the combined tests was 75% (61% to 87%) while the pooled specificity was 93% (81% to 97%). The pooled sensitivity of the LAM assay used alone was 36% (31% to 42%) and specificity was 96% (94% to 98%). The pooled sensitivity of Xpert®MTB/ RIF used alone was 61% (39% to 77%) and specificity was 97% (94% to 99%). *[56]*

The geneXpert® assay being a nucleic acid amplification test is often superior to non-molecular tests even among the HIV positive. The use of urine for the LAM assay however offers advantages over use of sputum for TB diagnosis. The quality of sputum may be poor among the HIV positive and the sensitivity of the geneXpert assay can also be reduced in cases of paucibacillary pulmonary TB.

There were 34 microbiologically confirmed cases of pulmonary TB in our HIV positive population. This represented 33.7% of this population. Of the 101 HIV positive patients, CD4+ counts were available for 73 patients. With a median CD4+ count of 82, majority of the patients were in an advanced stage of HIV. Out of the 34 cases of confirmed PTB, 26 had a CD4+ count of 200 or less.

When the population was stratified by CD4+ count, the subgroup with CD4+ count ≤ 50 had 17 cases. The subgroup with ≤ 100 had 20 cases and that with CD4+ count ≤ 200 had 26 cases. The sensitivity of the LAM assay at CD4 threshold of ≤ 50 was 76.5% and specificity was 54.5%. At CD4+ threshold of ≤ 100 sensitivity was 65.0% and specificity was 68.4%. At CD4+ threshold of ≤ 200 sensitivity was 65.4% and specificity was 72.0%. The sensitivity of the LAM assay at these CD4+ thresholds was higher than the sensitivity of 58.8% reported among all the HIV positive patients without CD4+ stratification. The specificity of the LAM assay among all the HIV positive was 85.1% which was higher than that in the stratified HIV population. The sensitivity of the urine LAM assay was noted to increase and specificity was noted to decrease as the CD4+ count reduced.

There is an influence on the performance of the LAM assay by CD4+ count. This has been reported in several studies. This was first noted in earlier studies that used the ELISA format of the assay and the trend continues to be reported in studies that have evaluated the lateral flow LAM assay. Maunank Shah et al. reported this finding in 5 studies reviewed in 2016. Where CD4+ cut off was at ≤ 50, the sensitivity ranged from 52% to 73% and specificity

ranged from 67% to 98%. In studies that only considered in-patients (4 of the 5 studies), pooled sensitivity was 63%(range 49-76%), pooled specificity was 86%(range 71-94%). Sensitivity was 56% (range of 41-70%) and the specificity was 90% (range of 81-95%) in those with a CD4+ count of ≤ 100 cells per microliter or less. For those with a CD4+ count of >100, the sensitivity was 26% (range of 16-46%) and specificity was 92% (range 78-97%). For patients with a CD4+count of ≤ 200 or less, sensitivity was 49% (range of 34-66%) and specificity was 90%(range of 78-95%). Where the CD4+ cut-off was above 200, the sensitivity was 15%(range of 8-27%) and specificity was 96%(range of 89-99%). *[56]*

Below CD4+ count of 200, patient with HIV are diagnosed with the acquired immunodeficiency syndrome (AIDS). This is associated with severe immune suppression with increased risk of acquiring opportunistic infections. Tuberculosis is the most significant opportunistic infection among persons with HIV/AIDS. Majority of the cases of pulmonary tuberculosis in the population were found in patients with CD4+ counts of 200 and below. This reflects the increased incidence of TB among persons with HIV especially those with marked immunosuppression. It is at these low CD4+ counts where there is an increased incidence of paucibacillary pulmonary TB. An increase is also seen in the incidence of disseminated TB/mycobacteremia and extrapulmonary TB. The LAM assay is expected to have a higher sensitivity at these low CD4+ counts and this was shown in our study.

With regards to the specificity reported in our study, it was lowest when then CD4+ threshold was ≤50 and highest at CD4+ threshold ≤ 200. This low specificity is an expected finding in a HIV positive population especially with advanced disease. *[53, 54]* This is likely due to extrapulmonary tuberculosis/disseminated TB which was not accounted for in our study. With extrapulmonary/disseminated TB, sputum which was the clinical specimen of choice for culture in our study, is not necessarily the ideal clinical specimen of choice. Obtaining the appropriate clinical specimen for testing can be challenging. The LAM assay which is not restricted by site of TB would be positive but the sputum culture would be negative, meaning the patient would be classified as culture negative (false positive case). These false positive cases also affect the sensitivity of the LAM assay and contribute to the modest sensitivity of the assay.

The influence of the CD4+ count on the performance of the LAM assay was expected as was seen in other studies. However, the number of patients with CD4+ counts in this study was small. Therefore, these findings should be interpreted with caution.

The WHO in the 2015 policy guideline however does recommend use of the lateral-flow LAM assay in HIV positive patients with low CD4+ counts and should be used for reference.

16.CONCLUSION

The lateral-flow urine LAM assay was able to diagnose pulmonary TB. However, the sensitivity of the assay was higher in the HIV positive population especially in those with low CD4 counts of 200 and below. This was in contrast to the low sensitivity found in an unselected/mixed population and a HIV negative population. Those with these low CD4+ counts are also likely present with paucibacillary PTB, disseminated TB, extrapulmonary TB or atypical presentation of TB. They may also be too unwell to produce sputum. This makes it more difficult to diagnose TB, putting these patients at risk of worsening morbidity and even death. The LAM assay therefore offers an opportunity to diagnose TB early using urine. Treatment can therefore be started early under conditions that usually make TB diagnosis challenging using the routine tests available like ZN microscopy. The urine LAM assay also provided additive value to sputum microscopy and gene Xpert in diagnosis of pulmonary TB than when either of the tests was used alone. This allowed for accurate diagnosis of PTB in more HIV positive patients who present with symptom and signs of active pulmonary tuberculosis.

17.LIMITATIONS

It was difficult to interpret the meaning of an indefinite LAM test as 5 of the culture results did not yield growth of MTB while 3 isolated *Mycobacteria tuberculosis.*

There were few patients with CD4+ counts of 100 and below and 50 and below. It was therefore difficult to fully assess the influence of these CD4+ counts on performance of the LAM assay at these CD4+counts.

18.RECOMMENDATIONS

The lateral-flow LAM assay should be used for pulmonary TB diagnosis in the HIV positive population especially those with a low CD4+ count. Larger studies should be conducted among HIV positive patients with low CD4+ count especially ≤ 100 and ≤50 to allow for better evaluation of the influence of the these CD4+ counts on the perfotmance of the LAM assay.

Studies that use multiple clinical specimens for TB culture should be conducted to better advice on its use in cases of extrapulmonary or disseminated TB especially in the setting of advanced HIV disease. Such specimen would include pleural fluid,cerebrospinal fluid, urine, ascitic fluid, lymph node aspirates among others depending on the suspcted site of TB.

Studies should be conducted in multiple sites in the country to give a better evaluation of the lateral-flow LAM assay. This can better advice on its implementation in national TB/HIV care programs.

Studies should be conducted to evaluate the urine LAM assay in diagnosis of TB where immunosuppression is not caused by HIV.

GeneXpert® or drug sensitivity testing should be recommended early especially where prevalence of multidrug resistant TB is high and where it is suspected that an individual may have acquired a drug resistant form of PTB. The LAM assay can not distinguish drug resistant from drug sensitive forms of PTB.

Sensitive diagnostic methods for non tuberculous mycobacteria should be implemented in facilities that care for HIV positive patients.

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APPENDIX I

Patient questionnaire

Interviewee's Code: _____

Interview Date (DD/ Month/ Year): _____

Point of recruitment

Section A: Background Information

1. Year of birth of the respondent ☐
2. Gender of the respondent Male ☐ Female ☐
3. Weight in Kgs_____
4. Highest level of education of the respondent
   None ☐

   Primary ☐

   Secondary ☐

   ☐
   Tertiary/University

5. Occupation of the respondent
   a. Unemployed
   b. Employed
   c. Businessman/woman
   d. Farmer/Fisherman

6.Residence:

Section B: Symptom checklist

Symptoms	**Yes**	**No**
1. Persistent cough > 1 or 2 weeks?		
2. Blood stained sputum?		
3. Night sweats > 2 weeks?		
4. Fever?		
5. Weight loss?		
6. Chest pain?		
7. History of previous TB treatment?		
8. History of close contact with a person confirmed to have TB?		
9. Swellings in the neck or armpit or elsewhere?		
10. Diarrhea for more than two weeks?		

Section C: Patient clinical information

1. HIV status of the client: Negative ☐ Positive ☐
2. Known CD4+ count _____(For those who are HIV +)
3. Known viral load_____(For those who are HIV +)
4. ART status: Naïve ☐ Experienced ☐
5. ART regimen: 1st line ☐ 2nd line ☐
6. Cotrimoxazole use: Yes ☐ No ☐
7. Diuretic use: Yes ☐ No ☐
8. Pre existing lung disease

Pre-existing lung Disease	**Yes**	**No**
1. COPD		
2. Asthma		
3. Bronchitis		
4. Others		

9. Cigarette smoking Yes ☐ No ☐

10. Co morbidities

Co morbidities	**Yes**	**No**
1.Hypertension		
2. Diabetes		
3. Viral hepatitis		
4. Kidney disease (Acute or Chronic)		

11.Functional status_____(refer to the Karnofsky score below)

Karnofsky score.

Definition	Score	Criteria
Able to carry on normal activity and to work,no special care needed	100	Normal,no complaints,no evidence of disease.
	90	Able to carry on normal activity,minor signs or symptoms of disease.
	80	Normal activity with effort;some signs or symptoms of disease.
Unable to work, able to live at home and care for most personal needs; varying amount of assistance needed	70	Cares for self;unable to carry on normal activity or to do active work.
	60	Requires occasional assistance,but is able to care for most personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; require equivalent of institutional or hospital care, disease may be progressing rapidly	40	Disabled,requires special care and assistance.
	30	Severely disabled;hospital admission is indicated although death is not imminent.
	20	Very sick;hospital admission necessary;active supportive treatment necessary.
	10	Moribund,fatal processes progressing rapidly.
	0	Dead.

APPENDIX II

Laboratory form

Participant Code: _____

Interview Date (DD/ Month/ Year): _____

1.

	Level
Hemoglobin	
CD 4+ count	
Viral load	
Hepatitis B status	
Hepatitis C status	

2.HIV RESULTS

NEGATIVE ☐ POSITIVE ☐

3.SPUTUM ANALYSIS RESULTS

NEGATIVE ☐

POSITIVE

+		++		+++	

4.TB LAM RESULTS

NEGATIVE ☐

POSITIVE

Grade 1		Grade 2		Grade 3		Grade 4		Grade 5	

5.CULTURE STATUS:

No Growth		MTB detected		NTM detected	

6. CLASSIFICATION OF TB STATUS:

TB STATUS	
I-Culture confirmed TB.	
II-Positive TB.	
III-Not TB.	
IV-Not classifiable.	

I)MICROBIOLOGICALLY CONFIRMED TB (PRIMARY GROUP FOR SENSITIVITY)

- MTB cultured from any specimens.

II)POSSIBLE TB.

- No culture positive plus one or more of:
- Positive sputum smear.
- Started on TB treatment,clinical improvement documented
- Diagnosis of TB by non-study clinician
- Death reported to be due to active TB per medical source.

III)NOT TB:

- No feature of group I) or II) plus each of:
- No TB treatment given
- At follow up,confirmed to be alive or to have died from non-TB cause.

IV)NOT CLASSIFIABLE.

- Does not meet criteria for any of the other groups mentioned above.

APPENDIX III

LABORATORY TESTS

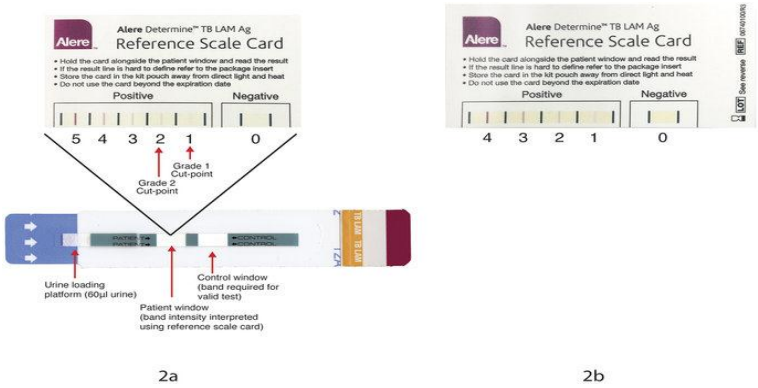
Lateral flow LAM assay

This is an immunochromatographic assay that is produced as a urine strip.
Capture antibodies are adsorbed onto the nitrocellulose membrane of the test strip and the detection antibody is labeled by conjugation to colloidal gold particles. When unprocessed urine is added to the test strip, the LAM present in the urine is bound by the gold labeled antibodies. The LAM-LAM antibodies complexes are immobilized in a line on the nitrocellulose membrane. The visible purple line that results is due to the colloidal gold particles to which the antibodies are attached. The control bar included in the sample test strip ensures validity of the test. The test strip is read between 25 and 35 minutes of incubation at r oom temperature.

<u>Interpretation.</u>

The intensity of any visible band is compared with the faintest positive
line on the reference card. Any line with similar or greater line intensity is scored as testing positive for LAM. It is therefore a qualitative test with the results being read as negative or positive. The urine LAM strip used in this study had 4 grades on the reference card. This represented the bands of varying intensities expected from urine samples tested for individuals with TB. This is the newer version of the LF-LAM strip. The previous strip manufactured (up to 2014), had 5 bands on the reference card. The grade 2 on the older version corresponds to grade 1 on the current strip. This adjustment made it easier to conduct and compare studies as there previously had been confusion as to whether the grade 1 or 2 cut-off for postive results provided optimal results. The intensity of the band however does not reflect the burden of mycobacteria and any visible band is reported as positive.[59]

Figure 9. Lipoarabinomannan urine strip and reference scale card for interpretation.2a LAM strip in use before 2014,2b LAM strip in use since January 2014.



2a 2b

Sputum microscopy

Direct Ziehl-Neelsen Smear Microscopy.

Two sputum samples will be collected (at least one of these should be an early morning sample).After collection of the sputum, the sample will be registered.The sputum will then be processed according to standard guidelines.The sample will be fixed thinly on a glass slide in preparation for staining by Ziehl Neelsen .The sample will be allowed to air dry and then will be heat fixed. After this, the sample will be flooded with 1% carbol fuschin and heated for 5 minutes before washing with water and decolorizing using 3% acid-alcohol followed by a brief wash with water. 0.3% methylene blue will be used to stain the sample for 30-60 seconds. Water will be used to wash before allowing the sample to air dry. The slide will then be visualized under a light microscope (CX21 Olympus microscope) at a magnification of 1000. A positive sample is one that will show acid-fast bacilli in a 300 fields of the slide as recommended by the World Health Organisation. A negative sample will be one showing a less number of the acid fast bacilli.

Direct Fluorescence Smear Microscopy.

The same sputum sample preparation as for ZN staining will be used but the smears will instead be stained with Auramine O/ rhodamine stain and counterstained with potassium permanaganate for 60 seconds.
The bacilli shine fluorescent yellow against a blue background making them easier to visualize and count.
The slides will be read using the same microscope equipped with the LED FluoLED fluorescence illuminator (magnification times 400). Light source will be a light emitting diode.The number of acid fastbacilli read per standard length of 2 centimetres long will be reported. A length corresponding to 100 fields under 1000 magnifications was estimated to be equivalent to 20 fields under 400 magnifications. Two experienced laboratory technologists will read the smears. . The bacillary load will then be established.

Bacillary load is a measure of culture forming units as seen with the aid of staining dye and is used to quantify the number of bacilli in a sample. If a sample contains 10-99 acid fast bacilli per 100 fields it is categorized as 1+, if it contains 1-10 acid fast bacilli per field in 50 fields this is 2+, if it contains 10 acid fast bacilli per field in 20 fields it is categorized as 3+.

Sputum culture

The sputum culture method to be used in this study will be the BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 method. This is a liquid culture medium.

The MGIT Mycobacteria Growth Indicator tube contains 7millilitres of modified middlebrook 7H9 broth base usually with OADC enrichment and PANTA antibiotic mixture.

The sputum sample is processed by the N-acetyl L-cysteine (NALC)-sodium hydroxide (NaOH) method. NALC is a mucolytic agent and it allows the decontaminating agent , sodium hydroxide, to be used at a lower final concentration. Sodium citrate is included to

bind the heavy metal ions that might be present in the specimen and that could inactivate NALC.

Procedure.

2-5 millilitres of sputum is added to the centrifuge tube. An equal volume of the NALC-NaOH solution is added and the screw cap tightened.The mixture is vortexed for not more than 20 seconds then kept at 20-25 degrees Celsius for decontamination. The tube is then filled to within 2 centimetres of the top (for example to the 50 mL mark on the tube) with phosphate buffer, and vortexed again. The tube is centrifuged at 3000g for 15 minutes. The supernatant is then carefully poured through a funnel into a discard can containing5% phenol or other mycobacterial disinfectant. The deposit is resuspended in approximately 0.8 mL phosphate buffer. The deposit is then inoculated using a pipette into a vial of liquid medium labeled with the ID number. Each vial is inoculated with 3-4 drops (approximately 0.1-0.15mL). Smear one drop on a slide, marked with the ID number, for microscopic examination.

The MGIT tube must be continuously monitored either manually or by automated instruments until positive or the end of the testing protocol.

A fluorescent compound is embedded in silicone on the bottom of 16*100mm round bottom tubes. The fluorescent compound is sensitive to the presence of oxygen dissolved in the broth. Little fluorescence can be detected initially due to large amounts of dissolved oxygen in the broth. Later, actively respiring microorganisms consume the oxygen and allow fluorescence to be detected.

Tubes are filled with samples in the broth and continuously incubated at 37 degrees Celsius. The tubes are monitored for increasing fluorescence to determine if the tube is instrument positive i.e.,the test sample contains viable organisms. Fluorescence can be recorded by automated instruments such as Becton Dickinson's BACTEC MGIT 960 System.

This system is specially designed to accommodate MGIT and incubate them at 37 degrees Celsius. The instrument scans the MGIT every 60 minutes for increased fluorescence. Analysis of the fluorescence will determine if the tube instrument is positive, i.e., the test sample contains viable organisms. Approximately 100,000-1,000,000 colony-forming units per milliliter (CFUs/mL) are contained in an instrument-positive tube. A culture tube that remains negative for a minimum of 42 days (up to 56 days) and which show no visible signs of positivity is removed from the instrument as negative. All negative tubes are discarded.(Siddiqi, Salman H.;sabine Rüsch Gerdes(2006).Procedure manual for BACTEC MGIT 960 TB System).

Xpert® MTB/RIF

This is an automated nucleic acid amplification test (NAAT). It is a self-contained and cartridge-based technological platform that integrates sputum processing, DNA extraction and amplification and TB and MDR-TB diagnosis. The system is closed therefore reducing the risks of contamination meaning biosafety facilities are not required. Test results can be obtained in around 90 minutes.

Procedure.

Sputum is liquefied and inactivated 2:1 with the sample reagent. 5 milliliters of this material is transferred into the test cartridge. The cartridge is then inserted into the MTB-RIF test platform. The sample is automatically filtered and washed. Ultrasonic lysis of filter-captured organisms occurs releasing the DNA. The DNA is mixed with dry PCR reagents. Next, seminested real-time amplification and detection occurs in the integrated reaction tube and the results are then printed.

Results

1.Detection or no detection of MTB is reported.
2.Rifampicin (RIF) resistance is noted as present or absent.

HIV Testing

HIV rapid tests

The HIV testing algorithm currently used is in three stages, that is, screening,confirmation test and a tie breaker. Screening will be done using the KHB diagnostic kit for HIV 1 and 2 (Colloidal Gold).The first response HIV 1-2-0 card test will be for confirmation. Unigold HIV-1/HIV-2 will be used as the tie breaker. These are immunochromatographic assays also know as lateral flow assays. Detection of antibodies against HIV is the most common method for the diagnosis of HIV infection in adults and children more than 18 months old.

Colloidal gold
The device is removed from the pouch and placed on a flat dry surface.The device is labeled with the client's identification. The specimen of blood is collected after cleaning the client's finger and allowing it to dry. A sterile lancet will be used to prick the client's finger.The first drop of blood will be wiped off with a sterile gauze. The second drop of blood will then be allowed to form and will be collected. The blood is collected using the provided disposable capillary tube. Two drops of whole blood will be added to sample port of the KHB device. The amount of blood to be added should cover the sample port. One drop of running buffer is then added to the same port. The buffer contains reagents and provides optimal conditions for the test to develop. The used pipettes and capillary tubes will be disposed off appropriately after use. Wait thirty minutes to read the results. The results should be read exactly after the 30 minutes. A timer will aid with this timing.

Interpretation.

The control line will be the first to show; this indicates that the kit is working correctly and that the result is valid. A reddish purple line of any intensity on both control and test windows or a reddish purple line at the test area with none at the control area is read as positive. Proceed to the confirmatory test for positive test. A reddish purple line at the control area and no reddish purple line at the test area indicates a negative result. If there is no reddish-purple line at control or test areas, the test is invalid.

First Response HIV 1-2.0 card test,

This is the confirmatory test. It is done when the result of the screening test done is found to be positive. The testing procedure and interpretation of results is similar to the one for colloidal gold. The results are read immediately after 15 minutes have elapsed.

Interpretation.

If there is presence of a colour band at the control window plus a colour band at test regions 1,2 or both, the test is reported as positive. Presence of only one colour band at the control window, the result is reported as negative. If there is no colour line in any of the windows, or if there is appearance of one colour band at the HIV-1 window only; or appearance of one colour band at window 2 only; or appearance of two colour bands in 1 and 2 only, the result should be declared invalid.

Unigold

This is the tie breaker test. It is used when the screening test is found to be positive and the confirmatory test negative. The kit will be allowed to reach room temperature (15-27 degrees Celsius), approximately 20 minutes if previously stored in the refrigerator. Once at room temperature, the required number of UniGold Recombigen HIV-1/2 devices will be removed from their pouches. Only one test will be performed at a time. The device will be placed on a clean flat surface in a well illuminated room. The device will be labeled with the appropriate patient information/ identification code. The blood sample will be collected using a similar method to the one used for the other two rapid tests and add to the device using the disposable pipette. Two drops (approximately 60 microlitres) of blood will be collected and added in the sample port. (Ensure air bubbles are not introduced into the sample port). Two drops of the buffer solution will be added to the sample port using the dropper of the buffer solution bottle. The results will be read after 10 minutes incubation time but not more than 12 minutes. Since whole blood is being tested it has to be ensured that there is full red colour in the sample port for the test to be valid. A pink line must appear adjacent to the the word control. A pink/red line may appear adjacent to the word test (pink/red line of any intensity is taken in to account). If no red colour is seen in the sample port, the test will be repeated with a fresh kit.

Interpretation of the results:

Invalid report will be given to samples where the test line is present but control line is absent with a full red colour at the sample port. If there is no test line and no control line but there is full red colour at the sample port, the result is also reported as invalid. Any kit that has no full red colour at the sample port is reported as invalid as well. Where the test line and control lines are present in the presence of full red colour at the sample port, the test is reported as positive. A negative result is one in which no test line is present, the control line is present and there is a full red colour at the sample port.

HIV ELISA testing.

A common feature of all varieties of ELISA is the use of enzyme conjugates that bind to specific HIV antibody, and substrates/chromogens that produce colour in a reaction catalysed by the bound enzyme conjugate. The most popular ELISA involves an indirect method in which HIV antigen is attached to a well of a 96-well microtiter plate. The dish is washed with the client's blood sample. Antibody in the sample is allowed to react with the antigen-coated solid support, usually for 30 minutes at 37 or 40 degrees Celsius. After a wash step to remove unbound serum components, addition of a conjugate (an antihuman immunoglobulin with a bound enzyme) binds to the specific antibody that is attached to the antigens on the solid phase. Following another wash, addition of an appropriate substrate results in colour development (due to the enzyme that is bound to the second antibody). This colour change is detected by a spectrophotometer and is proportional to specific HIV antibody concentration in the sample. Optical density values are produced as the coloured solution absorbs transmitted light, and provide an indication of the amount of colour, which is proportional to the amount of antibody bound that is, antibody concentration. ELISA tests that detect HIV antigen are also available, these are known as sandwich ELISA. For this, the HIV antibodies are the ones secured to the bottom of the petri-dish. The client's blood is added to the dish and allowed to interact with the HIV antigen. If the sample contains HIV, the p24 antigen will attach to the antibodies in the petri-dish. If the blood sample is negative this will not occur. A solution with an antibody specific to the p24 antigen is then added, the antibodies attach to the antigen that had been bound to the antibody in the petri-dish initially. If there is no p24 antigen, these antibodies are washed away. Another solution containing an anti-human antibody to which an enzyme (marker) is bound is added. This antibody binds the first antibodies that had been added. Finally the dish is washed with a dye. The enzyme (marker) on the second antibody results in a colour change in the solution added. Where there are no antihuman antibodies, the solution remains clear. Just as with the ELISA method above the optical density based on the intensity of the solution will be used for interpretation of the results. Several indirect ELISA tests incorporate polyvalent conjugates (anti-IgG and anti-IgM) and antigen-sandwich configurations in order to increase sensitivity for detecting early infection (during seroconversion). Third generation ELISA tests detect antibodies only whereas fourth generation ones,use both of the methods described above detecting both antibodies and antigens. The fourth generation test will be used in this study.

Interpretation.

A mathematical calculation, usually based on the optical density (OD) of the negative controls multiplied by a factor, produces a cut off value on which the OD of the sample is compared to determine the antibody status; samples with OD cutoff values >1.0 are considered positive thise with values less than 1.0 are considered negative. An inconclusive test result is one whose OD value is close to one, a confirmatory test is recommended.

CD4 counting

CD 4 T lymphocyte occupy the central position in regulating immune functions of the human body, they coordinate B lymphocytes that produce antibodies and the T lymphocytes in their cellular immune response. The HIV primarily targets the CD 4 T lymphocytes progressively causing a decline in the number of these cells. The loss of these T-helper cells results in loss of ability to mount an appropriate immune response to any pathogen and increases

vulnerability to opportunistic pathogens characteristic of AIDS. The estimation of peripheral CD4 T lymphocytes count is used to make a decision on when to start ART, to monitor disease progression (surrogate marker of the degree of suppression of the immune system) and to monitor the effectiveness ART.

CD4 counting will be done by flow cytometry,which is immunofluorescent analysis of T-helper lymphocytes . This is the gold standard for CD4 T-lymphocyte measurements.
Flow cytometry refers to a technology that simultaneously measures and analyses multiple physical and chemical characteristics of single cells or other biological particles. This is done as they flow in a fluid stream past optical and or electronic sensors.

It provides information about their relative size, relative granularity or internal structure and fluorescence in several spectral regions emitted by fluorochrome labeled probes which bind specifically and stoichiometrically to cellular constituents such as proton antigen and nucleic acids for example the CD4 receptor on T-lymphocytes.[56]

Principle.

The whole blood obtained from the participants and stored in EDTA vaccutainers will be used for CD4+ T-lymphocyte enumeration. A sample of the whole blood will be mixed with a solution of the fluorescent antibody specific for the cell population to be studied.

Flow cytometers are generally made up of three systems, that is the fluidics, optics and the electronics.

Fluidics: purpose is to transport cells in a fluid stream through the laser for interrogation.Any cell ranging from 0.2-100 micrometres in size is suitable for flow cytometer analysis.

Optics: composed of a complex system of lenses which consist of two major parts, that is the excitation/illuminator optics and collection optics/ photodetectors. The former is composed by the light sources and lens. The collection optics consist of a collection lens to collect signals emitted from the cells that is the forward scatter, sidescatter and fluorescence.

Electronics: electrical pulses originating from light detected by the photomultipliers are then processed by a data processoir using a series of linear and log amplifiers. This allows for the events that occur in the cytometer to be plotted graphically and then stored.

Individual cells will be stained with fluorescent labels or absorption dyes and then will be suspended in physiological solution. The solution will be introduced under slight pressure through a flow chamber in to the centre of a stream of cell-free sheath fluid. The cells will scatter the light and also emit fluorescence based on the fluorescent antibody on the cell surface. The visible light undergoes deflection based on the size and internal structures of the cell. Forward scatter (FSC), correlates with the cell volume whereas side scatter (SSC) depends on the inner complexity of the particle –the shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughnesss). These properties will be used for analysis and sorting cells based on the fluorescent antibody.

Single platform approach in flow cytometry enables CD4+ counts to be derived directly without the need for a hematological analyser.

Dual platform approach estimates the absolute CD4+ T cell counts by a mathematical formula using two independent parameters, that is, the percentage of CD4+ T cells multiplied by the absolute lymphocyte count. The CD4+ T lymphocyte percentage is obtained by flow cytometry and a haematology analyser (or haemocytometer) is used to estimate the total white blood cell count and differential lymphocytes.[56].

Bead-based single platform technology on conventional flow cytometer is employed by the FACScalibur machine which will be used for CD4 counting in this study. This cytometer uses trucount tubes and is manufactured by Becton Dickinson (BD).It is compatible with various reagent systems and flow cytometers. Absolute CD4 counts are determined using the ratio of CD4 to a known quantity of fluorescent beads. It measures the absolute CD4 and CD percentage. The machine can run up to 250 samples per day. It requires up to 100 microlitres of whole blood collected in an EDTA vaccutainer. The blood can be stored for up to 24 hours before testing (after blood has been drawn) and the test can also be carried out on fixed/stabilized blood. The machine requires uninterrupted supply of electricity. Climate control is recommended for the machine to work optimally. The reagents require refrigeration. Significant training is required for the operator. [58]

Interpretation.

This combination of scattered and fluorescent light is picked up by the detectors in the flow cytometer. These detectors then produce electronic signals that are proportional to the optical signals received.

The data collected on each cell or event are stored in the computer. This data is then processed and analysed to provide information about cell populations within the sample. The CD4+ T-cell count will therefore given as an absolute count, that is, T-cell population (number of T-cells) per microliter.

Estimation CD4+ count from full blood count

Obtain full blood count with differentials then determine the absolute lymphocyte count. The lymphocyte count is approximately 30% of the total white cell count.

For example, total white blood cell count of 2.0×10^3/ uL (2000)

Absolute lymphocyte count = $2000 \times 0.3 = 600$

If the absolute lymphocyte count is less than 1900 cells/uL then the CD4+ count is most likely less than 200 cells/uL. *[60]*

APPENDIX IV

Data analysis

Dummy table for calculation of sensitivity and specificity.

		GOLD	STANDARD	
		DISEASE	NO DISEASE	TOTAL
TEST RESULT	+VE	a True positive	b False positive	a+b
	-VE	c False positive	d True negative	c+d
	TOTAL	a+c	b+d	
		Sensitivity $\frac{a}{a+c}$	Specificity $\frac{d}{d+b}$	

APPENDIX V

CONSENT INFORMATION

Title

The diagnostic value of the urinary TB-lipoarabinomannan assay in adults with pulmonary tuberculosis.

Introduction

I, Dr. Carole A.Okoth. am a postgraduate student at the University of Nairobi pursuing a Masters degree in Clinical Medicine and Therapeutics. As part of the requirement for attainment of the degree I am conducting a study on the diagnostic value of the lateral flow-urinary TB LAM assay. This form provides further information on the study to enable you decide whether or not to participate.

Once you have provided consent for participation in the study, I will proceed to obtain demographic and clinical information from you. I will also obtain clinical specimen from you for analysis.

Objectives of the study

This study aims to establish the diagnostic performance of the urinary TB-lipoarabinomannan assay in adults with pulmonary tuberculosis. This assay offers the opportunity of diagnosing t uberculosis within 25-35 minutes of providing a urine sample. This will in turn facilitate e arlier start of treatment and improve patient outcomes.

You have been chosen to participate in this study because you meet the inclusion criteria required for enrollment.

Benefits

There are no monetary benefits being provided in this study. However, the information obtained will be relayed to your primary physician to facilitate your treatment.
Any individual found to be HIV positive at the time of the study will be referred to the comprehensive care clinic for continued care as per the National guidelines for persons living with HIV/AIDS. For those already HIV positive and not on treatment, they will also be referred to the CCC for management. Those who are HIV positive and on treatment will be allowed to continue with their care at their CCC of choice.

Risks

It is expected that you may experience some pain and swelling at the site of venepuncture (drawing blood).
No other risk is expected in participating in this study.

Compensation mechanism

No monetary benefit will be provided.

Voluntarism
Participation in this study is voluntary. Participants are allowed to withdraw from the study at any time without compromise to their care.

Confidentiality
Confidentiality will be maintained throughout the study. Any information that is relevant to your management will be relayed to your primary care clinician. Your identity as a participant will not be revealed to the general public. Privacy will be maintained by using unique study ID. The information will be stored in a password protected computer file and will only be accessible to the investigators.

Type of specimens and amount to be obtained

Once consent has been provided, I will proceed to obtain demographic and clinical information from you. In addition I will obtain clinical specimen that is relevant to the study.

- 2 sputum samples, spot and morning-for sputum microscopy,culture and Xpert MTB/RIF where possible.
- Urine-for urine TB-LAM test.
- Blood will be drawn for HIV testing. Pre-test counseling will be done before blood samples are drawn for a rapid test and for a HIV ELISA test.For the rapid test a drop of blood will be drawn using a lancet and for the ELISA test, 3-5 millilitres of blood will be drawn into a plain vaccutainer. Post-test counseling will be done thereafter.For those found to be HIV positive, 3-5 millilitres of blood will be drawn into an EDTA vaccutainer to establish CD4+ count which will be used to determine the level of immunosuppression.

Follow up schedules

Results will be relayed to the participants once available, the participants will be informed to report for collection of their respective results on the second day after collection of results. The results will then be presented to the primary care physician for appropriate management of the study participants.

Possible storage of specimen for further analysis with the permission from the KNH/UoN/ERC:

Specimen obtained from the participants will be appropriately stored should there be a need for retesting or further analysis of the specimen. This will be done with the permission of the KNH/UoN Ethics and Review Committee.

Ethical consideration.

This study is designed in accordance with the Declaration of Helsinki. It will be conducted after approval by the University of Nairobi/Kenyatta National Hospital ethics review committee. The ethics and review committee will ensure that the study will be conducted

properly and the participant's safety and rights will be respected. This can be verified via the addresses and contacts shown below

The secretary, KNH/UON-Ethics Review Committee, Kenyatta National Hospital, Hospital Rd, along Ngong Rd, P.O BOX 20723, code 00202 Tel 726300-9,Fax 725272

Email address: uonknh_erc@uonbi.ac.ke

You can also contact me in case of any issue as pertains to this study and procedure therein via P.O BOX 54628-00200 Nairobi or 0721556344 or my supervisors Prof. Munyao, Dr. Mecha and Dr. Oyugi via The University of Nairobi, College of Health Sciences, P.O.BOX 19676-00202, Kenyatta National Hospital, Nairobi or +254-020-2726300 Ext. 44355.

Consent forms will be given to each participant (interviewee) and those who agree to the terms will be recruited into the study.

I……of……have read the information provided and clearly understood its content. I understand the purpose of the study, the role of the investigator and my rights and obligations as outlined. I am also aware that my participation is voluntary and I can withdraw from the study at any time. I am aware that my care will not be compromised in any way during the study or should I withdraw from the study.
I consent to be recruited for the study

Signature……
Date…….
Name……
Participant

I, the principal researcher/ research assiatant, hereby confirm that I have adequately explained to the study participant the information indicated above and he/she has understood the information and has accepted to be recruited to the study.

Signature…….
Date……..
Name……..
Principal researcher/research assistant

Witnessed by
Name…….
Signature……....

APPENDIX VI

ETHICAL APPROVAL LETTER

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KENYATTA NATIONAL HOSPITAL APPROVED 09 MAR 2016 KNH/UoN-ERC P.O. Box 20723-00202 NRB.

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/92

9th March, 2016

Dr.Carole A. Okoth
H58/68931/2011
Dept.of Clinical Medicine & Therapeutics
College of Health Sciences
University of Nairobi

Dear Dr. Okoth

Revised research proposal: Diagnostic value of a lateral flow-urine Lipoarabinomannan Assay in Adults with Active Pulmonary Tuberculosis (P729/11/2015)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and **approved** your above proposal. The approval period is from 9th March 2016 – 8th March 2017.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

"Protect to discover"

Yours sincerely,

PROF. M.L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Chair, KNH-UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine,UoN
The Chair, Dept. of Clinical Medicine & Therapeutics, UoN
Supervisors: Prof.Titus Munyao, Dr. Julius Oyugi, Dr.Jared Mecha

APPENDIX VII

DECLARATION OF ORIGINALITY

Name of the student	Dr. Carole Mary Akinyi Okoth
Registration Number	H58/68931/2011
College	College of Health Sciences
School	School of Medicine
Department	Department of Clinical Medicine and Therapeutics
Course name	Master of Medicine in Internal Medicine
Title of the work	Diagnostic value of a lateral-flow urine lipoarabinomannan assay in adults with active pulmonary tuberculosis.

DECLARATION

I understand what plagiarism is and I am aware of the University's policy in this regard.

I declare that this dissertation is my original work and has not been submitted elsewhere for examination, award of a degree or application. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with University of Nairobi's requirements.

I have not sought or used the services of any professional agencies to produce this work.

I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own work.

I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University plagiarism policy.

Signature:…….. Date…….