DIAGNOSIS OF ACTIVE PULMONARY TUBERCULOSIS USING THE XPERT MTB/RIF ASSAY IN SMEAR NEGATIVE TUBERCULOSIS SUSPECTS AT KENYATTA NATIONAL HOSPITAL AND MBAGATHI DISTRICT HOSPITAL.

DR. NKENGASONG AJUA ALEMANJI.

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A DISSERTATION SUBMITTED IN PART FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE: MASTER OF MEDICINE IN INTERNAL MEDICINE OF THE UNIVERSITY OF NAIROBI.

DECLARATION

This dissertation is my original work and has not been presented for a degree at any other university.

Signed……

DR. NKENGASONG AJUA ALEMANJI
SENIOUR HOUSE OFFICER, INTERNAL MEDICINE, UON.

APPROVAL BY SUPERVISORS

This dissertation has been submitted with approval by my supervisors, namely:

Prof. Adam M. Adam,
Professor of Medicine,
Department of Clinical Medicine and Therapeutics,
University of Nairobi.

Signed_____ Date_____

Dr. Jared O. Mecha,
Lecturer, Department of Clinical Medicine and Therapeutics,
University of Nairobi.

Signed_____ Date_____

Dr. Marybeth C. Maritim,
Lecturer, Department of Clinical Medicine and Therapeutics,
University of Nairobi.

Signed_____Date_____

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I wish to thank the almighty God, for his abundant blessings have brought my family and I through this step in life successfully.

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TABLE OF CONTENTS

LIST OF TABLES:

LIST OF FIGURES:

LIST OF ABBREVIATIONS:

1. AFB- Acid fast bacilli.
2. CCC- Comprehensive Care Center.
3. CI- Confidence Interval.
4. HIV- Human immunodeficiency virus.
5. KNH- Kenyatta National Hospital
6. LED- Light emitting diode
7. MDH- Mbagathi District Hospital
8. MDRTB- Multi-drug resistant tuberculosis.
9. Mtb- Mycobacterium tuberculosis.
10. NAAT- Nucleic acid amplification tests.
11. PI- Principal investigator
12. PTB- Pulmonary tuberculosis.
13. PLWHIV- People living with HIV.
14. TB- Tuberculosis
15. UON- University of Nairobi.
16. WHO- World Health Organization.
17. Xpert MTB/RIF assay- Genexpert Mycobacterium tuberculosis/Rifampicin assay.
18. Zn stain- Ziehl-Neelsen stain.

ABSTRACT

Background: Pulmonary tuberculosis (PTB) is a major global health problem and currently it stands as the second leading cause of mortality from a single infectious disease worldwide according to the WHO world TB report 2012. Over the past century, diagnosis of PTB has relied almost exclusively on smear microscopy which has been shown to have a low sensitivity especially in patients co-infected with HIV. Novel diagnostic methods, such as the recently developed Xpert MTB/RIF assay are bound to be essential tools in the global fight against TB.

Objectives: In this study, we sought to determine the yield of this novel assay in the microbiological diagnosis of PTB in patients suspected to have PTB who are sputum smear negative.

Design: It was a cross sectional descriptive study

Setting: The Kenyatta National Hospital (KNH) and Mbagathi District Hospital (MDH).

Methods: Files of prospective patients seen at the Tb clinics of both hospitals and the Tb wards in MDH were perused. Those which met the inclusion criteria were recruited with consent into the study and the study questionnaire administered. Spot sputum samples from each patient which were of proper quality were tested for active mycobacterium tuberculosis and rifampicin resistance using the Xpert MTB/RIF assay. Data from the study questionnaire and results of the assay were entered and analyzed using SSPS version 17.0 software. Measured outcomes included the proportion of active PTB in smear negative PTB suspects; the prevalence of rifampicin resistance in this group and the association between the Xpert MTB/RIF assay result and patients presenting symptoms.

Results: A total of 179 sputum samples were run from 179 eligible participants. The Xpert MTB/RIF assay yield was high at 19% (95%CI 13.5-25.5) with a 0% rifampicin resistance rate. Presence of night sweats on its own, or both night sweats and weight loss were weakly associated with Xpert MTB/RIF assay positivity (OR 10.1; 95%CI 1.3-76.7, p=0.007) and (OR 7.1; 95% CI 2.1-24.3, p<0.001) respectively.

Conclusion: The additional yield of the Xpert MTB/RIF assay in patients who are sputum smear negative PTB suspects in our study is 19% which when taken in the context of a 34% notified

smear negative rate in Kenya, is high. This has important public health implications wherein patients who can transmit Tb are not being identified by smear microscopy.

1.0 INTRODUCTION:

Tuberculosis continues to be one of the major global health problems. The greatest burden is borne by the developing countries which continue to suffer high rates of morbidity and mortality. According to the WHO global TB report 2012, there were nine million new TB cases in 2011, of which 1.4 million deaths were recorded. In Kenya, the estimated prevalence of TB in 2011 was 291 per 100,000 populations in both HIV positive and negative patients (1).

The HIV epidemic contributes greatly to the disease burden. Globally at the end of 2011, there were 34 million people living with HIV (PLWHIV). 69% of PLWHIV and almost 80% of people living with both HIV and TB were in Africa. The Incidence rate worldwide stood at 2.5 million people and there were 1.7 million deaths related to AIDS. Sub-Saharan Africa accounted for 70% of all AIDS related deaths witnessed worldwide. In Kenya the estimated incidence of HIV/TB co-infection in the same period was 113 per 100,000 populations (2). HIV co-infection is the strongest documented risk factor for developing active TB, increasing the risk by over 20 times. (3) In a similar manner, TB has been shown to worsen HIV progression. (4)Post mortem studies carried out in various countries with high HIV burden such as South Africa (5), Ivory Coast (6) and Kenya (7) showed TB to be the leading cause of death in HIV co-infected patients. Furthermore, it has been noted that the accuracy of definitive Tb diagnosis pre-death in HIV co-infected patients is poor (8, 9).

Conventionally over the past century, smear microscopy for acid fast bacilli (AFB) has been the initial diagnostic tool. Its simplicity and low cost made it ideal especially in the developing nations and most national TB control programs continue to rely on it despite its low sensitivity (10). Automated liquid culture is the recommended gold standard diagnostic test for tuberculosis. It is highly sensitive and specific. However, it delays time-to-treatment with a range of two to three weeks from sample collection to results. It is also quite expensive as it requires specialized equipment and a bio-safety level 3 facility (11).

Recently, methods based mostly on nucleic acid amplification for direct organism detection have reduced the diagnostic time while increasing sensitivity (12-14). The Genexpert mycobacterium

tuberculosis/rifampicin assay (Xpert MTB/RIF assay) is one of such methods which has recently been developed and approved for TB diagnosis (81, 90).

2.0 LITERATURE REVIEW

2.1 SMEAR MICROSCOPY IN DIAGNOSIS OF PULMONARY TUBERCULOSIS:

2.1.0 BACKGROUND:

According to the WHO, TB is the second leading cause of mortality from an infectious disease worldwide after HIV. The 2011 estimates show that there were approximately 9 million new cases worldwide with 1.4 million reported TB related deaths. (1)

Worldwide in 2011, notification rates of smear negative disease were 24.2%. In Kenya the smear negative rate in notified cases where smear was done stood at 32% (1). Smear microscopy for AFBs is the mainstay of diagnosis for PTB especially in the developing nations which shoulder over 90% of disease burden. (15-17). Microscopy is cheap, fast, comparatively easy to carry out and highly specific especially in high burden countries (18, 19).

2.1.1 SMEAR MICROSCOPY TYPES:

Conventional microscopy has been in use for over a century worldwide. It uses the carbol fuchsin Ziehl-Neelsen or Kinyoun acid-fast stains and is viewed with a conventional artificial light source or reflected sunlight (20).

Fluorescence microscopy was introduced in the 1930's. It uses the affinity of fluorochromes like auramine with or without rhodamine for mycolic acids found in the mycobacterial cell wall. (21, 22) The edge of fluorescence microscopy over conventional microscopy is the use of a lower power objective lens system (25xs versus 100xs). This allows the viewer to assess an area of a

slide in considerably less time (23,24). An added advantage is the simplicity of the fluorochrome staining technique as compared with the ZN staining method. (25,26) Fluorescence microscopy is commonly used in high-income countries but is more costly than conventional microscopy. (27). However it may be cost effective in certain low-income countries especially due to the time saved to view slides as compared to conventional microscopy. (23,24,28) Furthermore, fluorescence microscopy has been shown to be more sensitive than conventional microscopy and of equal specificity. A systematic review comparing fluorescence versus conventional microscopy in 45 selected studies, showed an average 10% higher sensitivity of fluorescence microscopy. The specificity of fluorescence microscopy was on average similar to that of conventional microscopy. (29) However there is a likelihood of false positive results because inorganic objects can incorporate fluorochrome dyes. (30-31)

Recently, light-emitting diode (LED) microscopy was introduced and endorsed by the WHO for use in resource limited settings (32). In a recent study in TB/HIV co-infected patients, LED microscopy was cheaper, faster and was equally as sensitive as conventional microscopy and fluorescence microscopy. (33)

2.1.2 FEATURES OF SMEAR NEGATIVE PTB:

Before the HIV epidemic, smear positive TB was noted in majority of cases and there was a strong association between the extent of cavitation and sputum smear positivity. (34,35) In this period, smear negative PTB was thought to be a less severe form of infection and was a less commonly occurring phenomenon than it is today. (36). Also in this period, patients smear negative for PTB were noted to be less infectious and had a lower morbidity and mortality than smear positive cases. (37-39) The proportion of smear negative PTB in the setting of the HIV epidemic has increased over the past 20 years. (40,41) In HIV infected patients, the presentation of PTB is atypical, resembling primary PTB. Smears tend to be negative due to the limited pulmonary inflammation and decreased cavitation. (42-44). Co-infection with HIV is shown to decrease the sensitivity of sputum microscopy and specificity of chest x-ray for the diagnosis of PTB. (45-48).

Tshibwabwa et al in a 3 centre, 3 year comparative study on adults with diagnosis of PTB with or without HIV, showed there was significantly less cavitation (33% vs 78%;p=0.001) and less atelectasis (12% vs 24%;p=0.001) in the HIV infected group compared to the HIV negative group (48). Pitchenik et al and Lee MP et al, also showed that abnormal chest radiographic findings are common in immune-suppressed PTB patients. (49, 50)

HIV co-infected PTB patients are also noted to have considerably lower concentrations of bacteria in their sputum (36). However, a significant amount of transmission of PTB has been shown to occur via smear negative cases (51-53). Behr et al found that, of 71 clusters of patients infected with mycobacterium tuberculosis strains with matching DNA fingerprints, there were 183 secondary cases in these 71 clusters. Of these, a minimum 32 were directly traced to infection by smear negative patients (17%[95%CI, 12%-24%]) (51). Alma Tostmann et al analyzed 394 clusters with a total of 1285 patients. Based on molecular linkage, 12.6% of the secondary cases were attributable to transmission from a patient with smear negative TB. (52)

2.1.3 SENSITIVITY OF SMEAR MICROSCOPY:

Smear microscopy sensitivity has been shown to be relatively low in several studies. On average, the sensitivity rate in immuno-competent cases is less than 60% (36, 54-59). The sensitivity of smear microscopy is even lower still in HIV co-infected patients (47, 60-64). Monkongdee et al, in a performance evaluation study of smear microscopy and mycobacteria cultures for diagnosis of PTB among people co-infected with HIV in Thailand and Vietnam, found that smear microscopy diagnosed only 29% of culture positive TB cases (64). A retrospective analysis by Gupta et al showed that laboratory confirmation of PTB by smear microscopy or culture in HIV infected and non HIV infected adults was 53.9% and 74.3% respectively (53.9% vs 74.3%; $p<0.001$). They also showed that HIV infected cases had a higher proportion of lower grade sputum smear positivity even among patients with CD4 counts>500 cells per micro-liter (63).

2.1.4 OUTCOMES OF SMEAR NEGATIVE PTB

The WHO algorithm for diagnosis of PTB in smear negative HIV prevalent, resource-constraint settings, recommends performing a chest X-ray before antibiotic trial course (65). Evaluation studies on this algorithm have shown low sensitivity ranging from 23% to 59% (66-70). Huerga et Al, in a prospective study carried out in Homa Bay, Kenya, showed a clinical-radiological algorithm sensitivity of 55% and specificity of 72.9% (70).

Smear negative TB has been shown to be associated with poor treatment outcomes especially in high HIV prevalence areas (19, 71). Mortality rates in patients with smear negative PTB have been shown to be higher than those with smear positive PTB. This has been attributed to a number of factors. More advanced immune suppression, inaccurate diagnostics and incorrect therapy all account for higher mortality. (72-75) Complicit in poor outcomes is delays in initiating appropriate therapy due to trial of antibiotic therapy and repeat smear exams. (76-78)

2.2 THE XPERT MTB/RIF ASSAY.

2.2.0 Background:

A number of nucleic acid amplification tests (NAATs) have brought improvements to the detection of MTB as well as to drug susceptibility testing. However the inability of these earlier methods to simultaneously detect MTB and Rifampicin resistance has limited their efficiency and use. Furthermore they have been reported to be too complex and prone to operator errors and sample cross contamination (79, 80).

The Xpert MTB/RIF assay was co-developed by the Foundation for Innovative New Diagnostics, (Sunnyvale, California, USA), and the University of Medicine and Dentistry of New Jersey. It is a self-contained, fully automated cartridge-based system that incorporates microfluidic technology and nucleic acid analysis to purify, concentrate, detect and identify

specific nucleic acid sequences. It amplifies a sequence of the RNA polymerase β subunit (rpoB) gene which is specific to Mtb complex, while simultaneously probing for mutations within the 81-bprifampin resistance determining region (RRDR) of the rpoB gene using molecular beacon technology (81,82).

The Xpert MTB/RIF assay has been shown to have ease of use with minimal training required which is important in the low income countries setup. (83). It has also been shown in some studies to be cost effective for diagnosis of Mtb in the setting of low income countries bearing the highest burden of disease (84-88). The Xpert MTB/RIF assay has also been shown to generate no viable aerosolized pathogens in either the manual sputum-sample reagent processing stage, or the automated cartridge processing stage. This allows for operation of the Xpert MTB/RIF assay in laboratories without costly bio-safety cabinets. This is in contrast to the routine smear preparation process for microscopy where infectious bacterial aerosols are generated (89). In December 2010, the WHO recommended the use of the Xpert MTB/RIF assay as the initial diagnostic test for the diagnosis of pulmonary tuberculosis in patients with HIV co-infection or those suspected to have multi-drug resistant tuberculosis (MDR-TB) (90).

2.2.1 DIAGNOSTIC ACCURACY OF THE XPERT MTB/RIF ASSAY.

Several evaluation studies have been carried out on the Xpert MTB/RIF assay. The first analytic studies were carried out by Blakemore et al. In this validation study, the Xpert MTB/RIF assay correctly identified all 79 Mtb isolates and correctly excluded all 89 non-tuberculosis isolates. Rifampin resistance was also accurately identified in all 37 resistant isolates and in none of the 42 rifampin susceptible isolates. They also demonstrated the unlikely-hood of a false positive result occurring (91).

Helb et al carried out two clinical validation studies on the Xpert MTB/RIF assay. In suspected Tb cases in Vietnam, the sensitivity of the assay was 100% in smear-positive culture-positive cases and 84.6% in smear-negative solid culture-positive cases. Overall specificity of the assay was 100% as no culture-negative sample was detected by the assay. Analysis of 64 smear-positive culture-positive Tb retreatment cases in Uganda revealed a 98.4% sensitivity of the

assay. 100% sensitivity of rifampin resistance was observed with 100% specificity in this group (83).

Boehme et al evaluated the performance of the Xpert MTB/RIF assay in 1730 patients with suspected drug-sensitive or multi drug-resistant pulmonary tuberculosis in 4 regions: Peru, Azerbaijan, South Africa and India. The overall sensitivity was 97.6% with 98.1% specificity. Among HIV positive patients with PTB, the sensitivity of the assay was 93.9%. The sensitivity and specificity of the assay in smear negative-culture positive cases in the various regions was as follows: In Lima, Peru, the sensitivity was 83.3% with specificity of 100%; In Baku, Azerbaijan, the sensitivity was 92.8% with specificity of 97.1%; In Cape Town, South Africa, the sensitivity was 90.4% with specificity of 98.4%; In Durban, South Africa, the sensitivity was 86.7% with specificity of 97.3%; In Mumbai, India, the sensitivity was 88.5% with specificity of 97.2%. The sensitivity and specificity of the assay in detection of rifampicin resistance was as follows: In Lima, Peru, the sensitivity was 100% with specificity of 98.4%; In Baku, Azerbaijan, the sensitivity was 95.9% with specificity of 95.7%; I Cape Town, South Africa, the sensitivity was 93.8% with specificity of 100%; In Durban, South Africa, the sensitivity was 100% with specificity of 100%; In Mumbai, India, the sensitivity was 98.3% with specificity of 95.3%. Overall the assay achieved a sensitivity of 97.6% and specificity of 98.1% in the detection of rifampicin resistance. Of note was the finding that a single direct Xpert MTB/RIF test identified a greater proportion of culture positive cases than did a single Lowenstein-Jensen culture. Furthermore, in a group where non-tuberculous mycobacteria were isolated post-culture, the Xpert MTB/RIF assay was 100% specific in returning a negative result in all samples (92).

In a hospital based evaluation study done in Zambia, where HIV and TB are highly endemic, the Xpert MTB/RIF assay had an overall sensitivity of 86.1%(95% CI, 80.3%-90.4%) and specificity of 95.7%(955 CI,93.3%-97.3%). The sensitivity of the assay in smear-negative cases who were HIV positive was 78.9% (95% CI, 67.8%-87.1%) compared to 55.6% (95% CI, 31.3%-77.6%) in the HIV negative group (P=.0407) not withstanding that the number of HIV negative patients in the cohort was low. The sensitivity of the assay in detecting rifampin resistance was 81.3% (95%CI, 53.7%-95.0%) with a specificity of 97.5% (95% CI, 90.4%-99.6%) (93).

In another high HIV and TB endemic area of Tanzania, a hospital based clinical validation study was done. The Xpert MTB/RIF assay achieved a sensitivity of 88.4% (95% CI, 78.4%-94.9%) among patients with positive culture and 99% (95%CI, 94.7%-100%) specificity. Amongst a group of 77 patients classified as having clinical PTB (culture negative), the Xpert MTB/RIF assay detected a further 9.1% who went on to show clinical improvement and hence were considered as true positive. Drug sensitivity testing revealed no rifampicin or isoniazid resistant Mtb strains by either liquid culture or the Xpert MTB/RIF assay, giving the assay a specificity of 100% (94).

In a hospital based study in Spain on smear negative samples, Moure et al found that the Xpert MTB/RIF assay detected sixty four of the eighty five culture positive samples and none of the culture negative samples. This represented a sensitivity of 75.3% and specificity of 100% for the assay. In addition, the assay identified all six liquid culture rifampicin resistant strains and none of the rifampicin sensitive strains giving the assay a 100% sensitivity and specificity in rifampicin resistance testing (95).

A recent meta-analysis of 16 Xpert MTB/RIF assay studies revealed a pooled sensitivity of 90% (95% CI, 89%-91%) and specificity of 98% (95%CI, 96%-98%). In a 7 study subset of the meta-analysis, the pooled sensitivity of rifampin resistance detection was 94% (95%CI, 92%-96%) and specificity of 97% (95% CI, 96%-98%) (96).

3.0 RATIONALE:

TB is the second leading cause of death from an infectious disease after HIV with 1.4 million deaths recorded in 2011. Smear negative PTB rates are on the rise with 24.2% worldwide and 32% in Kenya of total smears reported with a growing MDRTB burden (1).

Smear microscopy and chest x-ray, which is the main diagnostic tool worldwide and especially in high burden countries, have been shown to have a low sensitivity and specificity respectively for diagnosis of PTB especially in patients co-infected with HIV (45-48).

Xpert MTB/RIF assay has shown a higher sensitivity and equal specificity to smear microscopy in evaluation studies (83, 91-96) and the WHO recommends its use as the initial diagnostic tool for diagnosis of PTB in HIV co-infected cases (90).

There is a paucity of data in Kenya regarding the additional yield of the Xpert MTB/RIF assay in patients who are suspected to have PTB but are sputum smear-negative.

4.0 RESEARCH QUESTION:

What is the diagnostic yield of the Xpert MTB/RIF assay in the diagnosis of active PTB in sputum smear negative PTB suspects at KNH and MDH?

5.0 OBJECTIVES

Broad Objective:

To describe the diagnostic yield of the Xpert MTB/RIF assay in the diagnosis of active PTB in sputum smear negative PTB suspects.

Primary Objective:

1) To determine, by Xpert MTB/RIF assay, the proportion of active PTB in smear negative PTB suspects at KNH and MDH.
2) To determine the prevalence of rifampicin resistance in this group of patients.

Secondary Objectives:

3) To describe the association between the Xpert MTB/RIF assay result and patients presenting symptoms.

6.0 MATERIALS AND METHODS

6.0.1 Study Design:

This was a cross-sectional descriptive study.

6.0.2 Study Area:

It was carried out at 2 proximal hospitals: the KNH outpatient TB clinic and Mbagathi district hospital (MDH) TB clinic and medical wards.

The KNH is a teaching and referral hospital situated at the Upper Hill area in Nairobi County, Kenya. It has since its inception offered referral services for all specialties from across the country. It is however not a major center for cases of tuberculosis which tend to be treated at the peripheral facilities. The TB clinic is located within the medical outpatient unit of clinic 17 and runs from Monday to Friday. Records perused at the clinic indicate that on average 12 outpatients are seen per week, all from the Nairobi area. Only 8 of these patients complete sputum microscopy testing of which 3 are smear negative. We thus envisaged a greatly lower recruitment rate from this facility.

The Mbagathi District hospital is a level 4 health facility in Nairobi County. It has served as the Tb referral treatment center for Nairobi for the past 58 years. Despite being upgraded to a general medical, surgical and paediatric hospital in 1995, it has continued to be the major Tb treatment center for Nairobi and its environs. There are 4 medical wards at MDH (2 male and 2 female), two of which are specialized wards for suspected PTB cases. Patients found not to have PTB are subsequently transferred to one of the other 2 wards. The TB clinic is located within the special clinics building and runs between Monday and Friday.

6.0.3 Study Population:

Subjects suspected to have PTB at both KNH and MDH, who were smear negative on light microscopy.

6.0.4 Case Definition.

Elizabeth Corbett et al showed that cough for more than two weeks, drenching night sweats and weight loss were each independently predictive of PTB. In combination, they had a negative predictive value of over 99% in HIV infected patients (97).

Thus, participants in this study referred to as 'smear negative PTB suspects', were those with recorded complaints of cough for more than two weeks, plus either night sweats or weight loss or both and who had 2 recent smear microscopy results negative for AFBs.

6.0.5 PATIENT SELECTION

Inclusion Criteria:

1) Patients who were 18 years of age and older who were willing to provide a written consent.

Exclusion Criteria:

1) Patients who could not produce sputum.
2) Patients who declined informed consent.
3) Patients who were on treatment for any form of tuberculosis.

6.0.6 Sample Size calculation:

The appropriate formula for calculating the sample size was as follows:

$n = Z^2 \times P(1-P) / d^2 . (98)$

Where

n = sample size.

Z = Z statistic for 95% level of confidence – 1.96.

P = estimated sensitivity of theXpert MTB/RIF assay in smear negative patients.

d = margin of error.

Using part of a multi-centre study done by Boehme et al in the Durban, South Africa setting (92) with an 86.7% sensitivity of the Xpert MTB/RIF assay in smear-negative patients (P) and using a margin of error (d) of 5%, a sample size of 177 was sufficient to estimate the proportion of active PTB in smear-negative patients at KNH and MDH using the Xpert MTB/RIF assay.

6.0.7 Sampling Method:

Consecutive sampling was used to recruit patients from the TB clinic of KNH and both the wards and TB clinic of MDH over a period of 4 consecutive months until the sample size of 177 was attained.

6.0.8 PATIENT RECRUITMENT

Suspected PTB cases seen at the TB clinic in KNH and MDH are sent for sputum microscopy and results are availed within 2 hours. The patient's on rare occasion may return for the results the following day. Medical records of prospective patients with suspected PTB were perused. Suspected PTB patients as per case definition with recorded smear microscopy results negative

were informed about the study by the principal investigator and 2 trained assistants and consent sought. The 2 trained assistants were qualified clinical officers.

The study questionnaire (Appendix 4) was then administered to the enrolled study participants by the principal investigator and his trained assistants. Instructions on proper sputum submission (99) were given to the patients by the principal investigator and 2 trained assistants. Patient recruitment and sputum sample collection were done on the same day with no patient having to be re-called at a later date.

6.0.9 LABORATORY METHODS

6.0.9.1 SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION

The principal investigator and his assistants were trained by qualified laboratory technicians at the comprehensive care center (CCC), KNH on patient instruction for proper sputum submission (99).

Enrolled participants were given instructions on proper sputum submission lasting about three minutes as follows: Rinse your mouth thoroughly twice with water then take three deep breaths, followed by a deep cough to bring up sputum from your lungs. Expectorate the material into the sterile screw-cap specimen bottle and close it tightly. They were then provided with one 50ml sterile sputum bottle (falcon tube) for sample collection. Participants at the TB clinics were directed to a designated sputum collection point for sample production. Ward participants had their samples taken at the bedside. The sputum bottle was labeled with the patient identification code and hospital name. One spot sample of 5-10ml was provided by the participant and the bottle closed. Visual confirmation of the sputum quality using the rating system of Bartlett (100) was done by the principal investigator and his assistants. Specimens which were visually purulent, mucoid or slightly blood stained were considered good quality sputum, while those which were clear and watery in appearance were considered saliva. Saliva samples were discarded following the waste management protocols at KNH and MDH and the participants requested for a repeat sample. The sputum bottle was then wrapped in gauze and placed into a

biohazard zip lock bag and closed. The biohazard bag and its contents were then placed in a cooler box. A sample request form (APPENDIX 5) was filled for each patient sample and placed in a carrier folder.

Samples were transported in the cooler box to the CCC Xpert MTB/RIF laboratory at KNH compounds within 6 hours. Once at the CCC laboratory, samples were unboxed and inspected to ensure sample and request form concurrence. Sample registration was done using a laboratory registration booklet for purposes of accountability. Samples were then subjected to the Xpert MTB/RIF assay as per specifications (APPENDIX 1). Samples which were not tested on the same day were frozen at -30 degrees Celsius for testing at a later date. Results of positive assay were immediately relayed to the respective facilities for initiation of treatment protocols. Participants whose samples tested negative for the assay continued management according to the medical facilities instruction. Rifampicin resistant results were also relayed to the various facilities for initiation of multi drug resistant TB treatment protocols.

6.0.9.2 SPECIMEN ANALYSIS

See Appendix 1.

6.0.10 VARIABLES

DEPENDENT VARIABLES

1) Xpert MTB/RIF assay results.
2) Rifampicin resistance results

INDEPENDENT VARIABLES

1) Age.
2) Sex.

3) Level of education.

4) Night sweats.

5) Weight loss.

6) Previous treatment for PTB.

7) HIV sero-status.

8) CD4 count.

6.0.11 Data Management and Analysis:

Data from the questionnaires were coded, entered and analyzed in SSPS version 17.0 software. The population was described using age, sex, level of education, HIV sero-status, CD4 count and previous history of TB treatment. Categorical variables (Sex, Xpert MTB/RIF, HIV status, previous history of TB treatment, night sweats and weight loss) were summarized into percentages while continuous variables (Age, CD4 count) were analyzed and presented using means or medians.

Proportion of active TB was summarized and presented as percentage of smear negative participants with a positive Xpert MTB/RIF result. Prevalence of rifampin resistance was calculated out of all active TB participants and summarized as a percentage. 95% confidence interval was calculated for both proportions. Xpert MTB/RIF results were associated with patient symptoms (night sweats, weight loss) using chi square test. All the statistical tests were measured at 5% level of significance.

Study findings were presented using tables and graphs.

6.0.12 Quality Assurance:

The CCC Xpert MTB/RIF laboratory operates in accordance with standardized protocols of quality control and quality assurance.Through the internal quality control (IQC), the quality of the results were verified. This helped in the detection and rectification of various procedural processes that introduce errors.

7.0 ETHICAL CONSIDERATIONS

The study was undertaken after approval by University of Nairobi's department of Clinical Medicine and Therapeutics, Mbagathi District Hospital and the Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee. Only patients who gave informed consent were recruited into the study. Information gathered from the study was kept confidential, and the study results disseminated to health care providers to aid in patient care. Participants were assured that participation was voluntary and that no medical attention would be denied had they declined to participate. The subjects were also informed of the medical benefits and also physical harms to their satisfaction prior to being included in this study. The participants were assured of full and free access to their results and that therapeutic interventions would be recommended where the need arises, according to accepted standards of practice.

Following the full explanation and acceptance by the patient of the above, the subjects were requested to sign the consent form (appendix 3).

8.0 RESULTS:

8.1 PARTICIPANTS

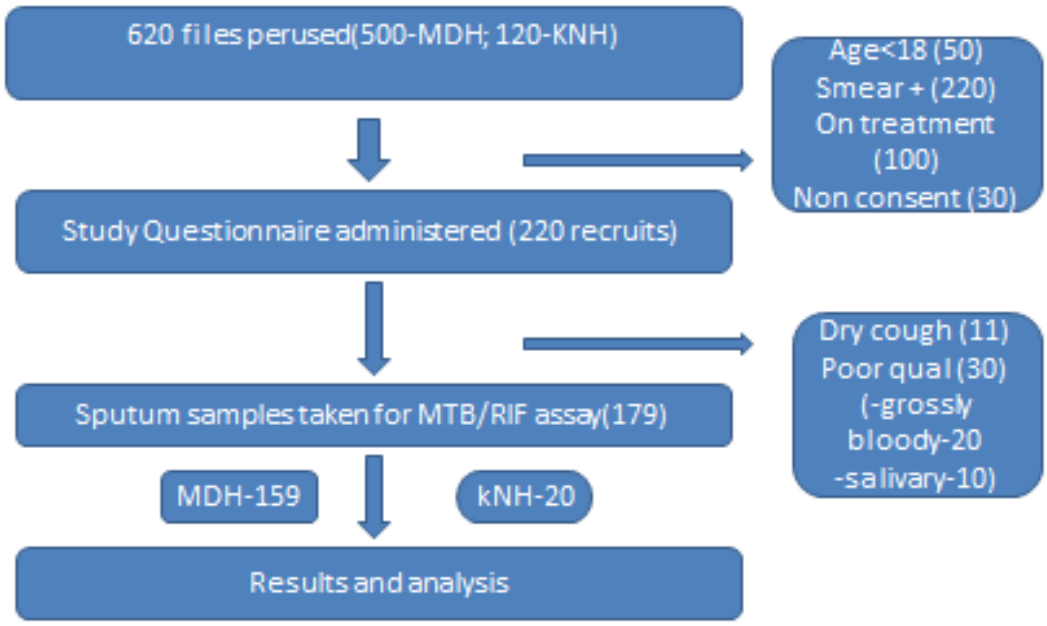

Figure 1.

Between January 2014 and April 2014, the records of 620 patients with suspected PTB on follow up at the TB clinic and wards of MDH and TB clinic of KNH were consecutively perused. Four hundred were excluded on basis of: Age less than 18 years- 50, smear positive- 220, on current TB treatment- 100, non-consent- 30. The remaining 220 were recruited into the study and the study questionnaire filled. Of those recruited, a further 41 were excluded on basis of: Non-productive cough- 11, Poor quality sputum specimens- 30. The quality of the specimens ranged

from salivary- 10 to grossly blood stained- 20. Thus 179 sputum samples were fully tested and analyzed.

8.2. Population Characteristics:

Table 1: Socio-demographic characteristics of study population (N=179)

Variable	**Frequency (%)**
Age in years	
Mean (SD)	37.7 (13.5)
Min-Max	18-83
Sex	
Male	94 (52.5)
Female	85 (47.5)
Education Level	
None	9 (5.0)
Primary	77 (43.0)
Secondary	71 (39.7)
Post-secondary	22 (12.3)

As illustrated above in table 1, the study population was relatively young with a mean age of 37.7 years. The sex distribution was almost even with 52.5% of participants being male and 47.5% being female. Our study population was relatively educated with 95% of participants having attained a minimum primary level of education. Only 5% of participants did not have any formal education at all.

8.3. Clinical Characteristics of study participants:

Table 2: Presenting symptoms of study population (N=179)

Variable	Frequency (%)
Night sweats	
Yes	144 (80.4)
No	35 (19.6)
Weight loss	
Yes	150 (83.8)
No	29 (16.2)
Both symptoms	
Yes	117 (65.4)
No	62 (34.6)

As illustrated in table 2 above, the commonest presenting symptom among our study population was weight loss (83.8%). This was closely followed by night sweats (80.4%). A considerable number of participants were shown to have both symptoms of night sweats and weight loss (65.4%).

Table 3 : Prior PTB treatment (N-179)

Variable	Frequency (%)
Prior treatment	
Yes	43 (24.0)
No	136 (76.0)

A vast majority of participants (76%) did not report history of past treatment for any form of tuberculosis as shown in table 3 above.

Table 4: HIV status (N-179)

Variable	Frequency (%)
HIV status	
Positive	40 (22.3)
Negative	101 (56.4)
Unknown	38 (21.2)
CD4 cells count (n=40)	
Available results	16 (40.0)
Median (IQR)	152.5 (74.0-460.0)

Of the 179 participants, 40 had recorded HIV status positive (22.3%) while 101(56.4%) were HIV negative. A fair number of these participants did not have a recorded HIV status (21.2%). The median CD4 cell count of the 40 HIV positive patients with documented CD4 count, was 152.5cells/microliter.

8.4 Xpert MTB/RIF assay yield.

Table 5: Xpert MTB/RIF assay and rifampicin resistance result (N-179)

Variable	Frequency (%)	95% CI
MTB Detected		
Yes	34 (19.0)	13.5-25.5%
No	145 (81.0)	74.5-86.5%
RIF resistance(n-34)		
None	34 (100.0)	

The Xpert MTB/RIF assay in this study yielded 34 cases or 19% (95% CI 13.5-25.5) of active PTB out of the 179 suspected cases. Of these 34 cases with MTB detected by the assay, none was found to be rifampicin resistant, giving a 100% rifampicin sensitivity rate in this group.

8.5. Xpert MTB/RIF assay yield in association to symptoms and HIV status

Table 6: Associations between Xpert positivity and HIV status and presenting symptoms

Variable	MTB Detected		OR (95%)	P value
	Yes(n-34)	No(n-145)		
HIV status				
Negative	17 (50.0)	84 (57.6)	1.0	
Positive	10 (29.4)	30 (20.7)	1.6 (0.7-4.0)	0.266
Unknown	7 (26.6)	31 (21.4)	1.1 (0.4-2.9)	0.825
Median CD4 (IQR)	119 (88-180)	177 (67-543)	-	0.871
Night sweats				
Yes	33 (97.1)	111 (76.6)	10.1 (1.3-76.7)	0.007
No	1 (2.9)	34 (23.4)	1.0	
Weight loss				
Yes	32 (94.1)	118 (81.4)	3.7 (0.8-16.2)	0.070
No	2 (5.9)	27 (18.6)		
Both symptoms				
Yes	31 (91.2)	86 (59.3)	7.1 (2.1-24.3)	<0.001
No	3 (8.8)	59 (40.7)	1.0	

The prevalence of HIV in participants in whom MTB was detected by the assay (34) was 29.4% while 50% were HIV negative. A substantial number of participants in this group (26.6%) did not have a documented HIV status result and hence were classified as unknown.

The symptoms of night sweats, weight loss or both, were very prevalent in this Xpert assay positive group at 97.1%, 94.1% and 91.2% respectively. On univariate analysis, the presence of night sweats alone as well as the presence of both night sweats and weight loss were associated with Xpert assay positive yield; p=0.007 (OR 10.1; 95%CI 1.3-76.7) and p<0.001 (OR 7.1; 95%CI 2.1-24.3) respectively. While attaining statistical significance, the wide confidence intervals noted indicate that our study sample size of 179 was insufficient to power this outcome.

9.0 Discussion

Tuberculosis remains a major global health problem with approximately 9 million new cases in 2011, the majority of which were in the developing nations. The rate of smear negative disease among notified cases in Kenya is relatively high at 32%, as of 2011 (1). A substantial amount of PTB transmission occurs via smear negative cases as shown by Behr et al and Tostmann et al (51, 52). It is important that active disease is rapidly established in smear negative cases to prevent such transmission and disease spread. This is even more imperative in a setting of high HIV and TB burden like Kenya (2). This study was an opportunity to assess the additional yield of the Xpert MTB/RIF assay in a high burden setting of both HIV and smear negative PTB.

The yield of the assay in our study was 19%. This is comparable to published data on yields of the assay on smear negative suspected cases in 9 countries (101) which revealed an overall yield of 16.8% with a rifampicin resistance rate of 13.6%. These comparative results were from high burden TB countries (1). Our study employed the use of passive case finding strategy which entails waiting for patients who have developed symptoms to present to the health facilities as opposed to an active case finding strategy whereby healthcare professionals systematically interrogate all high risk groups at every visit to assess for active or latent Tb infection. These comparative studies utilized either case finding strategy with some combining both. The sites utilized in all except 2 countries (Bangladesh, Pakistan) were public referral, district or sub-district facilities similar to this study. 3 sites (Kenya, Mozambique, DRC) of the overall 9 carried out Xpert assay testing on single smear negative sputum samples without the use of a chest x-ray as a screening test. This was similar to our study. The yields of the Xpert assay in these 3 sites were: 10.2%, 15.3% and 10.7% respectively. These lower yields could be due to the fact that the study sites in these 3 countries were district hospitals with none being a major center for TB diagnosis unlike Mbagathi district hospital in our study. In the other 6 sites, patients who were smear microscopy negative but had a suggestive chest x-ray were excluded from Xpert assay testing. This resulted in relatively higher yields of the assay as follows: Bangladesh- 21.5%, Cambodia-23.6%, Malawi-11.7%, Moldova-18.2%, Nepal-22.2% and Pakistan-20.9%.

Our sample collection method could have negatively impacted on the yield of the Xpert assay. We utilized a ‘spot’ sample collection method whereby a sputum sample was requested from participants once they were enrolled into the study. The use of a morning sputum sample has

been shown to have a higher sensitivity with the use of the Xpert assay with 88.4% sensitivity versus 84.1% using a spot sample technique (94). The utilization of an early morning sputum sample or both early morning and spot samples would likely have improved the yield of the assay in this study. Furthermore, the subjective assessment of sputum samples using the rating system of Bartlett (100) could have also negatively influenced the yield of the assay in this study. In a prospective study in South Korea, Yoon et al showed that gross appearance of purulent or blood stained sputa was more associated with smear positive yield than mucoid or salivary samples {OR 2.05,95%CI(1.21-3.47) (102). In similar fashion, Gounder et al in an audit of smear examination in Fiji showed good quality sputa positively correlated with smear positivity for AFBs (103). The effect of sputum quality, while not yet assessed in the yield of the Xpert MTB/RIF assay is likely to influence it in similar way to smear microscopy. Besides, the Xpert MTB/RIF assay does not analyze grossly blood stained sputum samples. In our study, we excluded 20 samples from analysis due to the grossly blood stained appearance of the sputum samples. We also excluded a further 10 samples which were salivary in appearance despite the participants having associated symptoms of active disease. We cannot conclusively comment on the effect of these excluded samples on our results had they been analyzed.

The effect of freezing and thawing of the sputa could also have influenced the yield of the assay in this study by altering the nucleic acid stability of the Mtb, Moure et al in an evaluation study of the assay, tested 125 samples which had been frozen for over 10 years. The sensitivity of the assay was 75.3% with 100% specificity (95). This sensitivity while being within the range of other evaluation studies was comparatively lower. In our study, we froze 100 samples for later testing.

The quality of laboratory personnel preparing the samples for the assay as well as the calibration of the Xpert MTB/RIF assay console could also negatively impact assay yield. However there are so far no studies to identify inter-laboratory variability in the assay results.

The yield of the assay in this study could be statistically lower than it otherwise would, assuming an estimated sensitivity of 86.7% and specificity of 97.3% in smear negative cases on which this study was based (92), the true estimate yield of the Xpert assay in this study could be 19.6%

(95% CI 12.7-26.5%); positive predictive value (PPV) – 88.6%; Estimated negative predictive value (NPV) – 96.7% (104).

The rifampicin resistance rate in this study was 0%. This is not surprising based on the fact that Kenya is currently rated in the World TB report 2012 as a low burden MDRTB country (1). A study by Ogaro et al in Nairobi found a 0.81% rifampicin resistance rate among new patients who made up 65% of that study. Furthermore, he found an MDRTB prevalence rate of 0.54% among these 65% new patients (105). In our study, 76% of participants were new PTB suspected cases and thus never been treated before for any form of TB. False positive rifampicin resistance results from the Xpert assay have been reported, leaving a substantial reduction in the positive predictive value of the assay for diagnosis of rifampicin resistance in this setting of low MDRTB prevalence (106).

In this study, the group HIV prevalence in those who tested positive for the assay was 29.4%. A high number (26.6%) of assay positive cases had unknown HIV sero-status due to a nationwide shortage of testing kits at the time of this study. Hence the absence of association between the HIV sero-status and assay positivity in this study cannot be conclusively put forward.

In relation to the symptoms of the study participants in association to the Xpert assay result, we found that the presence of night sweats alone was statistically significant ($p=0.007$), along with presence of both symptoms of night sweats and weight loss ($p<0.001$) in the participants who tested positive for the assay confirming active disease. This association is in keeping with the very definition of PTB 'suspects' which incorporates the symptoms of night sweats and weight loss. It is also comparable to findings by Elizabeth Corbett which showed a negative predictive value of both night seats and weight loss of over 99% in the diagnosis of active PTB in suspected cases in a setting of high HIV prevalence (97). However the wide confidence intervals noted in this analysis don't allow us to draw a definite conclusion from this due to a potentially underpowered sample size of 179.

The public health impact of the Xpert MTB/RIF assay in our setup could be quite substantial. An estimated 400,000 lives per year can be saved by making a diagnosis of active PTB using a sputum based assay with a sensitivity of 85% and specificity of 97% (108). The Xpert MTB/RIF assay has achieved these rates and indeed surpassed them in almost all evaluation studies done so

far. A rapid turnaround time, though not assessed in this study, will certainly reduce the rate of under treatment of patients with smear negative disease. The Kenya national algorithm for the diagnosis of smear negative suspected PTB advocates for an initial trial of antibiotics and eventual treatment for PTB should symptoms persist. This delay in definitive diagnosis and treatment of active disease in smear negative PTB suspects, will most certainly adversely affect the TB control at national level. The Kenya national genexpert algorithm limits the diagnostic use of the assay to smear negative HIV positive patients and children. In our study, we found that 50% of patients who were Xpert assay positive were HIV negative. As per this finding, a significant number of patients with smear negative active disease may be otherwise missed if the Xpert assay is not used. The resultant spread of infection will place increased burden on the public health systems.

9.1 CONCLUSION:

The additional yield of the Xpert MTB/RIF assay in smear negative PTB suspects is 19% which when taken in the context of a 34% smear negative rate in Kenya, is relatively high. The rate of rifampicin resistance in smear negative PTB suspects is very low at 0%. Our data suggests that smear negative PTB suspects could greatly benefit from the Xpert assay especially in areas of low culture availability regardless of HIV status. This benefit results from the fact that a high number of smear negative cases will be detected as having active disease by the assay, hence reducing the spread of the disease by avoiding the time delay in treatment which the national algorithm for diagnosis of TB allows for (107).

9.2 LIMITATIONS:

Our study does have some limitations. The vast majority of enrolled patients were from MDH as compared to KNH. This could have introduced a bias to the assay yield considering that MDH is the major referral hospital for patients suspected to have PTB. We did not obtain demographic data of the patients who did not meet the inclusion criteria of the study. Thus we cannot exclude the possibility of bias at enrollment. The absence of HIV testing in 26.6% of the participants who were positive by Xpert makes it difficult to ascertain the true yield of the assay in HIV positive individuals. Furthermore, the full effect of freezing and thawing on the assay yield has not been exhaustively assessed in literature so far, neither in this study. Finally confirmation of the positive Xpert assay results with culture would have been ideal as the assay is a novel technology. However, this is offset by the fact that several evaluation studies have indeed shown the assay to be highly specific.

9.3 RECOMMENDATIONS:

Future studies are recommended to assess the yield of the Xpert assay in various populations to enable plotting of the overall prevalence of active PTB in smear negative cases. This will in the long term help in defining the area of maximal utility of the Xpert assay. A recommendation for the expansion of the national Genexpert algorithm to include its use in smear negative HIV negative patients can also be drawn from the findings of this study.

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11.0 APPENDIXES.

11.0.1 Appendix 1

SPECIMEN TESTING BY XPERT MTB/RIF ASSAY:

1) Sputa were prepared and analyzed according to the manufacturer's specifications as follows:

a) Sample Reagent was added in 2:1 ratio (v/v)to sample and shaken vigorously 10 - 20 times.
b) Resulting specimen was then left to incubate at room temperature for 15 minutes.
c) Specimen was again shaken at mid-point of incubation.
d) The now liquefied specimen was then transferred into a sterile pipette until above the minimum mark.
e) A pre-labeled Xpert MTB/RIF cartridge was opened and the specimen introduced into the port slowly.
f) The cartridge was then shut and loaded into the Xpert module.
g) The sample ID was loaded into the Genexpert DX system software and automated testing commenced.
h) Results were interpreted by the Genexpert DX system and presented as; MTB detected/ MTB not detected/ Invalid. Same with Rifampin results.
i) Specimens which attained an Invalid reading were re-tested.
j) Frozen samples were thawed at room temperature and then prepared in similar fashion.

2) The Xpert system has an inbuilt quality control system characterized by:

a) Sample Processing Control (SPC) which ensures the sample was correctly processed. The SPC passed if it met the validated acceptance criteria and Probe Check Control (PCC) System which measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. PCC passed if it met the assigned acceptance criteria.

b) Failure of either QC was interpreted and presented as invalid by the Xpert system.

3) Xpert MTB/RIF results were printed out and recorded by the PI or his assistant.
4) Xpert MTB/RIF results are software based and hence independent of the user.

11.0.2 Appendix 2

INFORMED CONSENT EXPLANATION FOR PARTICIPATING PATIENTS

Title of Study:

Diagnosis of active pulmonary tuberculosis using the Xpert MTB/RIF assay in smear negative tuberculosis suspects at Kenyatta National Hospital and Mbagathi District Hospital.

Principal Investigator: *Dr. Nkengasong Ajua Alemanji.*

Introduction:

We are interested in finding out the magnitude of active PTB infection in patients who are sputum smear-negative.

Procedure to be followed:

Upon accepting to participate in this study, you will be requested to sign a consent form after which you will answer questions relating to your socio-demographics and past history of illness. You shall then be required to provide us with 1 sputum sample. We shall guide you on how to collect the sputum sample and a repeat sample may be required if the initial sample is not of proper quality. This sputum sample will be tested for presence of PTB bacilli by Xpert MTB/RIF assay.

Risk:

We do not anticipate any risk in the provision of a sputum sample. However you may experience mild chest discomfort from the coughing process while attempting to expectorate sputum for testing.

Benefits:

The above mentioned procedures will be done free of charge. A copy of the results will be availed to you and appropriate treatment initiated.

Confidentiality:

Strict confidentiality will be maintained and all data obtained will be securely stored and used for purposes of this study only.

Who is eligible to participate in this study?

Any person above 18 years of age.

Right to refuse or withdraw:

Your participation in this research is voluntary. You do not have to participate. If you do choose to participate, but prefer not to answer certain questions, you are free to do so. You are also free to terminate the interview and withdraw from the study at any time. You are free to ask questions before signing the consent form. If you agree to participate in the study, please sign on the consent form.

11.0.3 APPENDIX 3

Diagnosis of active pulmonary tuberculosis using the Xpert MTB/RIF Assay in smear negative tuberculosis suspects at KNH and Mbagathi District Hospital.

Consent Form (English)

I, ----------

do consent to participate in the study on: Active tuberculosis in patients who are smear negative at Kenyatta National Hospital/Mbagathi District Hospital. I do this with full understanding of the purposes of the study and the procedures involved which include filling out a study questionnaire and having laboratory tests, all of which have been explained to me by Dr. Nkengasong Ajua Alemanji.

Signature of patient----------

Signature of witness----------

Date----------

If you have questions during the course of the study, you may contact the following:

Dr. Nkengasong Ajua- 0722-657875; Dr. J.O Mecha – 0722-842741.

OR

The Chairman of Ethics and Research Committee, KNH-020 2726300 ext 44355, 726300-9.

IDHINI

Nambari ya hospitali……. Umri……..

Mimi……..
Natoai dhini mwenyewe bila aina yoyote yakushurutishwa au kulazimishwa kushiriki katika utafiti uliotajwa hapa kuhusuutafiti mpya utakaotumia kikohozi kupima kuwepo kwa ugongwa wakifua kikuu (TB). Nimeelezewa kikamilifu kuhusu madhumuni yake na naelewa kuwa nitaulizwa maswali kadhaa na nipimwe kikohozi.
Pia naelewa kuwa naweza kujiondoa wakati wowote iwapo nitabadilisha mawazo.

Sahihi ya mshiriki……

Sahihi ya shahidi…….

Tarehe……..

Ukiwa na maswali au jambo lolote unahitaji kuelezewa zaidi tafadhali wasilianana Dkt.Nkengasong Ajua Alemanji kwa nambari ya simu ifuatayo: 0722657875.

Asante

INVESTIGATOR'S STATEMENT

I, the investigator have educated the research participant on the purpose and applications of this Study entitled: Diagnosis of active pulmonary tuberculosis using the xpert MTB/RIF assay in smear negative tuberculosis suspects at KNH and Mbagathi District hospital.

Signed…… Date…….

11.0.4 APPENDIX 4

Diagnosis of active pulmonary tuberculosis using the xpert MTB/RIF assay in smear negative tuberculosis suspects at KNH and Mbagathi District hospital.

QUESTIONNAIRE

Patient File Number:_____

ID Code: _____

Part 1: Demographics.

Age:_____

Sex: _____

Level of education: _____

Part 2: Symptom screening.

1) Have you had night sweats over the past 2 weeks?

A) Yes. _____

B) No. _____

2) Have you noticed any weight loss over the past 2 weeks?

A) Yes. _____

B) No. _____

Part 3: TB History.

Tick correct response

1) Have you been treated for TB in the past?

A) Yes._____

B) No. _____

Part 4: HIV sero-status. (To be confirmed from patient records)

Positive:_____

Negative:_____

Unknown:_____

Part 5: Latest CD4 count. (To be confirmed from patient records)

A) _____ cells/Ul.

B) Not available. _____

Part 6: Investigations Results:

Tick correct response.

Xpert MTB/RIF assay result.

MTB detected _____

MTB not detected_____

RIF resistance detected _____

RIF resistance not detected_____

11.0.5 APPENDIX 5.

Diagnosis of active pulmonary tuberculosis using the xpert MTB/RIF assay in smear negative tuberculosis suspects at KNH and Mbagathi District hospital.

Xpert MTB/RIF Laboratory Request Form

ID Code: _____

Age: _____

Sex: _____

Facility: _____

Xpert MTB/RIF results:

MTB Detected _____

MTB not detected _____

RIF Resistant _____

No RIF resistance _____

Signed: _____