UTILITY OF GENEXPERT AND CYTOMORPHOLOGY IN THE DIAGNOSIS OF EXTRA PULMONARY TUBERCULOSIS -AT KENYATTA NATIONAL HOSPITAL

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DECLARATION FORM

I hereby declare that this proposal is my original work under the guidance of the supervisors listed below and has not been submitted to the University of Nairobi or any other higher learning institution.

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DEDICATION

I dedicate this dissertation to my wife,Mwape Kunda Manyepa, for their great support and encouragement throughout the study period, my Son, Chelela Manyepa, who I left one month old when I came for this study and the great joy he brought to the family

ACKNOWLEDGEMENT

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ACRONYMS

AAFB   - Acid Alcohol Fast Bacilli

AIDS   -Acquired Immunodeficiency Syndrome

DPX   -Diethylpthylene xylene

EPTB   -Extrapulmonary tuberculosis

ERC   -Ethics and Research Committee

FNAC   - Fine Needle Aspiration Cytology

H/E   - Hematoxylin and Eosin

HIV   -Human immunodeficiency virus

KNH   -Kenyatta National Hospital

MAC   -*Mycobacteria aviumintercillulare* complex

MDR   -Multi-Drug Resistant

MOT   -mycobacterium other than tuberculosis

MTB   -Mycobacterium Tuberculosis

NAAT   -Nucleic Acid Amplification Technique

NAOH   -Sodium hydroxide

PAP   -Papanicolou staining technique smear

TB   -Tuberculosis

UON   - University of Nairobi

WHO   -World Health Organization

ZN   - Ziehl Neelsen Staining

ABSTRACT

BACKGROUND: Tuberculosis (TB) is one of the most common public health challenges in Kenya. Both pulmonary and extrapulmonary TB (EPTB) is an issue of concern. The advent of Human Immunodeficiency Virus (HIV) in Kenya has resulted into increased cases of extra pulmonary tuberculosis and it remains a diagnostic challenge. Cytology and conventional Ziehl Neelsen (ZN) smear have shown low specificity and sensitivity in diagnosis of EPTB respectively. Mycobacteriological culture and drug susceptibility testing are not always available in limited resource settings, hence the need for more rapid and reliable methods.

Objective: In this study, we evaluated the performance of Cytomorphology, GeneXpert and ZN, for the diagnosis of cases suspected of EPTB refered to Fine Needle Aspirate Clinic (FNA) at Kenyatta National Hospital (KNH), Nairobi Kenya.

Methods: FNA was collected from presumptive EPTB cases. Two smears were prepared from each aspirate and processed for cytology and conventional ZN microscopy smear. The remaining aspirate was treated with 2 mls of 20%NaOH and allowed to stand for a minimum of 30 minutes. The bacilli were then floated using 0.5mls of xylene and scooped, and transferred to 2mls of Normal saline for GeneXpert test.

The Performance of the tests was calculated using R software version 3.2.2. [1] Bayesian method, the software is used in the absence of a gold standard or reference standard test

Result: There were 50 TB suspected cases recruited in this study, but only 43 were analyzed. The mean age was 26.74 years (SD ± 14.146) with the range of 2 to 56 years. The majority of them (25.6%) were aged between 21 to 40 years and 55.8% were male. Out of the 43 TB suspected cases analyzed, 10(23.3%) were confirmed by GeneXpert, 25(58.1%) were suggestive for TB by cytomorphology and one, 1 (2.3%) was positive by ZN microscopy. Sensitivity and specificity of tests in this study sample size were: cytomorphology 25(56.9%) and 18(36.7%), ZN 1(4.9%) and 42(94.4%) and GeneXpert 10(25.6%) and 33(66.2%). Cytomorphology showed the lowest specificity (36.7%) while ZN microscopy smear showed the lowest sensitivity of 4.9%. Among the 10 positive by GeneXpert, 10 were also suggestive for TB by cytomorphology and one was positive by ZN microscopy.

Conclusion: Our findings indicated that GeneXpert MTB/RIF test is a useful tool for the detection of MTB with reasonable sensitivity and specificity on EPTB fine needle aspirate with good performance as compared to cytomorphology and ZN smear microscopy.

1.0 INTRODUCTION/ BACKGROUND.

Tuberculosis (TB) is a global health problem. One-third of the world's population is said to be infected with *Mycobacterium tuberculosis* with an incidence of 9 million cases per year. [2] A major increase has been experienced in developing countries with an emergence of multi-drug-resistant tubercle bacilli (MDR). [3] Kenya is one of the twenty two high TB burden countries. The trend is still rising with an average annual increase of 16% cases. [4]

Tuberculosis is currently said to be the leading cause of death among the curable infectious diseases. [3,4] In 2004 World Health Organization (WHO), estimated 8.9 million new TB cases worldwide and Sub-Saharan Africa had highest incidence estimated at 356 cases per 100,000 populations per year. [1, 2, 3] In 2007, an estimated 13.7 million chronic cases were active globally, while in 2013, the incidence was estimated to be 9 million with an associated death of 1.3 and 1.5 million, most of which occurred in developing countries. [2,5] In developing world people contract tuberculosis because of a poor health immune system, largely due to HIV infection and malnutrition. [3, 4, 5]

Pulmonary tuberculosis which occurs in the lungs is the most common, extra pulmonary TB, occurs outside the lungs, is less common and at times they co-exist. Most affected parts by extra pulmonary TB are lymph node, pleural cavity, bone/joint, Genitourinary, Meningeal and peritoneal. General signs and symptoms include fever, chills, night sweats, loss of appetite, weight loss, and fatigue. [6]

Extra-pulmonary tuberculosis (EPTB) remains a major health problem in developing countries, with tuberculous lymphadenitis being the most common. [7, 8] There is an increased frequency seen in patients with human immunodeficiency virus (HIV). [8] Traditionally, the diagnosis of EPTB is established by histopathology and smear microscopy or by mycobacterial culture. Recently, fine-needle aspiration (FNA) cytology has assumed an important role in the evaluation of peripheral adenopathy and other lesions suspicious for TB as a possible non-invasive alternative to excision biopsy. [8, 9]

Despite various attempts to improve TB diagnosis, EPTB diagnosis is still a challenge, especially in sub-Saharan Africa, [10] where the rate of human immunodeficiency virus (HIV) infection is high. [11] The conventional Ziehl-Neelsen (ZN) staining method for acid- alcohol fast bacilli (AAFB) plays a key role in the diagnosis and monitoring of treatment in TB.

However, its major disadvantage is low sensitivity, ranging from 20% to 43 %. [12] Mycobacterial culture is the gold standard method for detection of tubercle bacilli, but it is time consuming and requires specialized safety procedures in laboratories. Serological techniques have the disadvantage as it lacks sensitivity and specificity. [13] Polymerase Chain Reaction has been found to be the most sensitive technique for rapid diagnosis of *mycobacterium tuberculosis,*(MTB) however it is not applicable in routine use because of cost implications. [13, 14] There have been alot of attempts to come up with a method which is cheap, rapid , affordable, and accurate in the diagnosis of TB. Gene-xpert, Nucleic Acid Amplification, has proven to satisfy all the required factors and has been successfully used in pulmonary TB diagnosis, due to the affordability, rapid and acurancy, W.H.O recommends the use of Gene-Xpert in the diagnosis of MTB. [14, 15] In view of the above, this study airms to establish the utility of Gene-xpert and cytopathology in the diagnosis of EPTB using FNA material.

2.0 LITERATURE REVIEW

2.1 TUBERCULOSIS

Tuberculosis, an airborne infectious disease, is the major cause of morbidity and mortality among adults. One-third of the world's population is infected and more than 1.5 million people die of TB each year.   Only about 5–10% of this infected population progress to active disease, with higher rates among those co-infected with HIV. [7, 8, 15]

2.2 CAUSATIVE ORGANISM AND MORPHOLOGICAL CHARACTERISTICS

 Mycobacteria are aerobic and slender rods which grow in branching and straight chains. They have a waxy mycolic cell wall that makes them retain carbolcfuschin stain when decolourised with 30% acid alcohol hence called alcohol acid fast bacilli (AAFB). The species responsible for Tuberculosis infections is *Mycobaterium Tuberculosis*. Other species are *mycobacteria bovis* found in unpasteurised animal milk, *mycobacteria leprae* which causes leprosy and *mycobacteria avium intracellulare complex* (MAC) which is found in soil, water, dust and domestic animals and is thought to cause variety of diseases in immune compromised people.[8, 16]

2.3 EPIDEMIOLOGY

TB has a world-wide distribution, with the majority of cases in developing continents such as Asia and Africa. An estimate of 9 million people have developed TB globally in 2013, 56% of them were from south East Asia and Western Pacific region. One quarter was from Africa which had high death rate. India accounted 24% while China had 11%. In 2013, 510,000 women died globally and more than one third of those who died had HIV infections. About 80,000 children died of TB in the same year. About 1.1 million people from 9 million infected were HIV positive globally. [3, 16] Globally the TB rate has decreased by 45% from 1999 to 2013 and the prevalence rate reduced by 41% in the same period. [4, 6]

African region contributes about 28% of TB globally, second from Asia which contributes 59%. In Africa, four out of five HIV related deaths cases are Tuberculosis positive. [3, 4, 16]

Twenty-two countries world-wide considered "high-burden countries" and account for approximately 80% of new TB cases each year. Most of them are in Africa and Asia.
 India, China, Indonesia, South Africa, and Nigeria have the highest number of new TB cases in the world. TB incidence (206 per 100,000) and death rates (27 per 100,000) are declining globally. [10, 16] In Sub-Saharan Africa, up to 80% of TB patients are co- infected with HIV. The incidence of tuberculosis has increased at least tenfold due to HIV infection. [16]

Kenya is among the 22 high TB burden countries. The trend is still rising with an average annual increase of 16% cases. [12, 16] In 1990, the incidence rate was below 50 per 100,000 and increased to 320 per 100,000 in 2008. The HIV infection has increased the TB cases and the prevalence of TB-HIV related infections was rated at 35% in 2014. Also the trend of multi drug resistant strains of TB has been on rise in Kenya but the incidences of MDR is not yet established though estimated at 10%. [4, 16]

2.4 PATHOGENESIS

Mycobacterium Tuberculosis is the causative agent of tuberculosis. [13, 16] Most cases are acquired after inhalation of droplets of MTB from an active case to susceptible host. [16] Development of the disease has different manifestation of clinical signs as the organisms affect different organs on the body. After inhalation of the infected aerosols, the pathogen gets deposited in the lungs, this result into the formation of Ghon focus of inflammation and then spread to the lymphatics leading to the formation of primary ghon complex. At this stage, there is conversion of tuberculin reactivity. Haematogenous circulation follows which seeds the bacteria in the various organs and results into milliary disease, pleurisy, tuberculous meningitis among others. [16, 17] Cervical lymph node gets affected when inhaled tubercle bacilli deposited on the tonsils or the pharynx and spread via the lymphatics to the regional cervical nodes. Likewise, pulmonary infection can be complicated by haematogenous seeding from cervical nodes. [18]

The rising HIV pandemic has led to the resurgence of tuberculosis. The infected immunocompetent individuals may not progress to the development of the disease, while two thirds may progress to development of the disease but only 5% get primary tuberculosis. Latency disease may be seen in pulmonary and extra pulmonary sites such as soft tissue and lymph nodes. [7, 18] Pulmonary tuberculosis is the most common form of Tuberculosis but extra-pulmonary tuberculosis is also on the rise, it constitutes 15 to 20% in the immunocompetent persons and 50% in the immunosuppressed individuals. [14, 18]

2.5 LABORATORY DIAGNOSIS

Globally, diagnosis of extrapulmonary tuberculosis still remains a challenge especially in developing countries. [4, 19] In most Laboratories, diagnosis of extrapulmonary tuberculosis is same as the diagnosis of pulmonary tuberculosis. The most used techniques include direct demonstration of organisms by Ziehl Neelson and Fluorescence microscopy smears, Isolation of the organism by culture remains a gold standard, but it requires alot of sophisticated equipment and takes alot of time which may delay commencement of medication. Other tests which can be used are; Antibody detection –Enzyme Linked Immunosobernt Assay, immunochromatography which also have a challenge of specificity as it can detect even those who have been cured from the disease. [20] Detection of Mycobacterial deoxyribonucleic acid or ribonucleic acid (DNA or RNA) using polymerase chain reaction (PCR) is very acurate but very expensive which can be another challenge in poor resource settings . The cytomorphology test, which depends on the cytological changes lacks specificity and requires skills and experience to interpret it. Diagnosis of Pulmonary TB and extra pulmonary TB is the same, the only difference is the type of specimen used, EPTB include biopsies, fine needle apsirates, body fluids while pulmonary uses sputum. The cases of extrapulmonary tuberculosis is said to be higher than diagnosed due to difficulties in sampling appropriate specimens and this results into increased number of false negative results. [21] which can be corrected by the use of culturing method as it is the gold standard and can detect the bacilli at the lowest concentration. [20, 21]

2.5.1 CULTURE

Despite being time consuming, culture remains a gold standard in the isolation of MTB. Many mycobacterial species, including *Mycobacterium tuberculosis*, grow extremely slowly in the laboratory and require 3 to 8 weeks of incubation on solid medium(LJ media) or at least 2 weeks in a radiometric liquid culture system (BACTEC) and about 3 to 7 on MIGIT. This slow growth results into a delay in TB diagnosis. However, culture has a higher sensitivity than microscopy and can increase the detection rate. Culture can be used to identify mycobacteria species and to perform drug sensitivity in suspected drug resistant cases. [13, 21]

2.5.2 SMEAR MICROSCOPY

The detection of acid fast bacilli in the fine needle aspirate smears is directly related to the concentration of bacilli in the specimen. The microscopy detection requires a concentration

of at least 1000-10,000 microorganisms/ml of aspirates. At a concentrations below 1000/ml of aspirates, the chance of detecting it is less than 10% While, in a properly performed culture, the detection limit is about 100 organisms/ml. [22]

ZN staining is the most commonly used method in developing countries despite having low sensitivity. [22] Because of low sensitivity, it means that about half of all TB cases cannot be detected, namely, smear-negative cases and extra-pulmonary cases that make up an estimated 55% of all cases in developing countries. [23] The low sensitivity of smear microscopy has resulted into delayed treament at an early stage of disease, the likelihood of having smear-negative TB (and thus miss diagnosis) is higher. [24]

2.5.3 DIRECT DETECTION OF MTB (MOLECULAR BASED TECHNIQUES)

 Nucleic acid amplification technique (NAAT) methods detect Mycobacterial DNA or RNA directly from the specimens. [25] Several molecular methods have been developed for direct detection, identification, and susceptibility testing of Mycobacteria. These methods can reduce the diagnostic time from weeks to days. [26] Examples of these include the Enhanced Mycobacterium tuberculosis Direct Test (E-MTD; Gen-Probe, San Diego, CA) and the Amplicor Mycobacterium Test (Amplicor; Roche Diagnostic Systems, Inc, Branchburg, NJ) Gene-xpert, produces rapid, accurate and reliable TB results. This molecular technique employs the nucleic acid amplification technique. They are specific to DNA of MTB. In South Africa, where it was used on extra-pulmonary specimens, gene-xpert showed high sensitivity of 98.2% and specificity of 89.9%) in both pulmonary and extrapulmonary tuberculosis. [25, 27] Gene-Xpert can also detect multidrug resistant MTB, which is an important aspect in TB patient management. [2, 26] Its reliability and reproducibility of results cannot be compared to microscopy which has both low sensitivity and specificity. [13, 27] World Health Organizations recommends the use of Gene-Xpert on both pulmonary and extra-pulmonary specimens due to its high sensitivity and specificity. [28]

2.5.4 CYTOMORPHOLOGY USING H/E AND PAP STAIN

Diagnostic methods used in the diagnosis of extrapulmonary tuberculous in our local setting (KNH) are Hematoxylin and Eosin, PAP stains and routine smears (conventional ZN). It has been noted that only 10% of the routine Ziehl Neelson stain done on extrapulmonary specimens are positive for mycobacteria despite suggestive features of TB by Cytomorphology. [29, 30] Demonstration of epithelioid granulomas with or without necrosis suggestive of TB and may result into commencement of treatment. This means that other

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causes of granulomatous inflammation like sarcoidosis, leprosy, cat scratch disease and Toxoplasmosis are treated for tuberculosis without confirmation. [30]

2.5.5 DIAGNOSIS BY GENE -XPERT

Gene-xpert is an automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance. [31] It is used in the diagnosis of pulmonary and extrapulmonary TB in both adult and Children. [3, 31] *Mycobacterium tuberculosis* causes more preventable adult deaths than any other infectious disease. Its diagnosis in developing countries still depends on 19th century technology: Ziehl-Neelsen (ZN) staining of a smear. Since this method is cheap, rapid, specific and reasonably easy to perform, WHO recommends its use for screening patients with cough lasting more than two weeks. However, the technique has low sensitivity, detecting only 20-43% of culture-positive cases. Due to the limitation of this method, studies have been conducted which aim at coming up with the method with high senstivity and specifcity in the diagnosis of MTB. In 2015, after ther review of some studies done world wide on the use of Gene Xpert on EPTB, WHO recommended the use GeneXpert for extrapulmonaly tuberculosis instead of conventional microscoy smear. [32]

A series of recent sytematic review has demonstrated that Gene-Xpert can be used in extra pulmonary TB detection using well processed samples: The difference between detection of pulmonary and extrapulmonary TB is sample preparation. Other methods of sample preparations for Gene-Xpert involves the use of sodium hypochlorite and centrifuged samples which uses the deposits. [32] This method of sample preparations (Extrapulmonary samples) for GeneXpert under study, involves sample treament with 20% sodium hydroxide for a minimum of 30 minutes, which digests the tissue fragments to ensure that no bacilli is trapped in the tissue fragments. This is then followed by floating the bacilli using xylene and scoopping the bacilli using pasteur pipette. [29, 32] About 0.2mls of the activated sample – reagent mixture was transferred to the GeneXpert cartridge. The cartridge was then inserted into the GeneXpert Device Model 4. Results were automatically generated after 90 minutes.

GeneXpert, (NAAT) which is rapid, accurate and reliable in TB diagnosis is not being used in EPTB at KNH cytology laboratory despite demonstrating sensitivity of 98.2% and specificity of 89.9%). [7, 32] This method, GeneXpert, if properly utilized can enhance rapid and accuracy diagnosis of extrapulmonary TB which is a challenge at the moment in Kenya and other developing countries.

2.6 STUDY JUSTIFICATION

Despite the increase in the extra pulmonary cases of mycobacterium Tuberculosis in Kenya, the prevalence in Kenya is not known. The guidelines for *mycobacterium tuberculosis* management recommend accuracy in diagnosis and treatment of the disease to effectively break the cycle of TB transmission but diagnosis of extra pulmonary TB remains a challenge in Kenya. Many health facilities in Kenya including Kenyatta National Hospital, the largest teaching and referral hospital in East Africa, currently use the routine H&E and Pap smear which demonstrate presence of epithelioid granulomas with or without necrosis suggest TB diagnosis. Ziehl Neelsen demonstrates the presence of tubercle bacilli. This results into initiation of treatment despite the fact that there are other causes of granulomatous inflammatory conditions such as sarcoidosis, Cat scratch disease, Leprosy, Syphilis, and Toxoplasmosis. The current confirmation method used (ZN) is less sensitive and specific. Gene-Xpert has demonstrated high specificity and sensitivity in both pulmonary and extra-pulmonary tuberculosis. Despite its accuracy in detecting tubercle bacilli in extra-pulmonary specimen, it is not being used in cytology Laboratory at KNH; hence this study was set out to evaluate the value of GeneXpert and cytomorphology at KNH cytology laboratory.

2.7 HYPOTHESIS

The Performance characteristics of Cytomorphology and Gene-Xpert in the diagnosis of extrapulmonary Tuberculosis at Kenyatta National Hospital have no differences.

2.8 STUDY OBJECTIVES

2.8.1 BROAD OBJECTIVE

To establish the utility of Gene-Xpert and cytomorphology in the diagnosis of extra-pulmonary TB from suspected cases at KNH**.**

2.8.2 SPECIFIC OBJECTIVES
1. To determine performance characteristics of GeneXpert in the diagnosis of extra pulmonary tuberculosis
2. To compare Gene-Xpert, Cytomorphology and ZN for the diagnosis of extra pulmonary tuberculosis

3.0 METHODOLOGY

3.1 STUDY DESIGN

This was a descriptive cross sectional study.

3.2 STUDY SITE

The study was conducted at Kenyatta National Hospital FNA clinic, Cytology and Microbiology Laboratories and was done for the period of two months. The clinic operates on Mondays and Thursdays from 08:00 hours to 13:00 hours. It serves an average of 25 to 35 patients per day.

3.3 STUDY POPULATION

All patients suspected of clinically having TB with lymphadenopathy and other benign lesions on the body, males and females, children and adults, referred for Fine Needle Aspirate-clinic or cytology Laboratory at Kenyatta National Hospital.

3.4 INCLUSION CRITERIA

Patients with benign lesions suspected of extra-pulmonary TB who met any of the following criteria;

i. Previous treatment on tuberculosis

ii. Exposure to Tuberculosis (living or nursing a tuberculosis patient)

iii. Clinical history of night sweats and loss of weight

iv. Having a history of inflamed lymph node for more than 2 months

v. Patients with accessible lesions to fine needle aspirate and suspected of extrapulmonary Tuberculosis.

vi. Age groups 2 and 60 years.

vii. Patients willing to provide written consent or assent.

3.5 EXCLUSION CRITERIA

i. Patients who decline to give consent or assent

ii. Patients with confirmed cases of malignancy

3.6 SAMPLE SIZE DETERMINATION

The sample size in this study was calculated using the R software version 3.2.2 [1] and the formula (Connor R. J. 1987) [33] is shown below. This software is used to calculate sample

size needed for testing differences in proportions for the paired-sample design, which in this study are smears for cytomorphology and GeneXpert.

$$n = \left\{ \frac{Z_{\alpha} * \sqrt{Pdisc} + Z_{\beta} * \sqrt{Pdisc - Pdiff^2}}{Pdiff} \right\}^2$$

Where

n = required sample size.

Z_{α} = Critical value.

Z_{β} = Power.

P $disc$ = Proportion of discordant.

P $diff$ = Proportion difference.

Based on a study by Tadessa, the discordant rate was 0.1 and 0.4. [34] At 95% confidence level and a power of 80%, a minimum of 42 samples was calculated as shown below:

n = required sample size.

$Z\alpha$ = 1.96.

$Z\beta$ = 80% or 0.80

P disc = 0.1 + 0.4 = 0.5

P diff = 0.4 − 0.1 = 0.3

$$n = \left\{ \frac{1.96 * \sqrt{0.5} + 0.80 * \sqrt{0.5 - 0.3^2}}{0.3} \right\}^2$$

$n = 41.296$

3.7 SAMPLING METHOD

Consecutive sampling method was used. Patients who met the inclusion criteria and willing to give consent were recruited in the study until the desired sample size was obtained.

3.7.1 RECRUITMENT

Before recruitment and consenting, the Principal investigator gave a talk to the identified patients where the procedure, possible expectations, information about the study, risks and benefits were clearly explained to legible participants.

3.7.2 ADMINISTRATION OF THE QUESTIONNAIRE AND CONSENTING

Patients who accepted to participate in the study were administered with a pre-designed questionnaire by a principal investigator. Those who qualified were asked to give consent. The questionnaire had only identification numbers. The register or log book was used to capture the name and address of the patient for the purpose of communicating results.

3.7.3 SPECIMEN COLLECTION

The specimens was obtained by performing FNA of the accessible lesion. The procedure was carried out by a consultant pathologist/reident in Pathology Department at Kenyatta National Hospital after obtaining consent from the patients. Fourty three (43) patients were sampled. Two smears were prepared and fixed then, the remaining sample in the hub of the needle was rinsed into 20% sodium hydroxide for GeneXpert. The slides and tubes were labelled with patient serial number and study numbe derived from laboratory request forms. Samples were transported to microbiology laboratory for GeneXpert analysis.

3.7.4 SAMPLE PROCESSING FOR GENEXPERT, ZN AND CYTOMORPHOLOGY

The sample for GeneXpert were prepared by rinsing the needle and the syringe in 2mls of 20% sodium hydroxide in 5 ml conical tubes, samples were allowed to stand for a minimum of 30 minutes and were mixed at regular intervals for complete tissue digestion. About 0.5 mls of xylene was added into the conical tubes to float the bacilli. The mixtures were allowed to stand for 15 minutes undisturbed. The top creamy layer was carefully collected using a Pasteur pipette and then rinsed into 1 ml of normal saline . It is this 1 ml of saline which was added to the sample reagent supplied with the test kit (1.5 ml). The mixture was then vortexed and allowed to stand for 15 minutes. The slides were examined by the principal investigator, the pathologist on duty and the supervisors.

3.8 GENEXPERT REPORTING AND INTERPRETATION OF RESULTS

The results were interpreted by the GeneXpert system from measured fluorescent signals and embedded calculation algorithms. Results were displayed on the results window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template.

Where MTB target DNA is detected- the MTB result were displayed at High, Medium, Low or Very Low depending on the Ct value of the MTB target present in the sample. Ct stands for cycle threshold. (Table below)

The Ct value ranges for the displayed MTB results

MTB result	Ct range
High	<16
Medium	16-22
Low	22-28
Very Low	>28

1. Where the result was indicated MTB not detected, it meant that MTB target DNA was not detected in the sample.

2. GeneXpert gives Rifampicin status to whether resistance or not which indicate multidrug- resistance or indetermined if the CT for mutated DNA is too low to be detected.

3. Also the GeneXpert can give Error message where the sample is processed but no results given out, instead, an error results are given, and in this case, the test must be repeated using a new cartridge. The causes of error mainly attributed to insufficient amount of reagent in the cartridge and in rare cases spoiled sample.

3.9 CYTOMORPHOLOGY REPORTING

Cytomorphological features consistence with TB such as granuloma, epithelioid histiocytes and necrosis was considered as suggestive for TB

Chronic granulomatous inflammation was defined by the presence of epithelioid histiocytes in cohesive clusters, Langhan giant cells with or without necrosis. Acute suppurative necrotising inflammation was defined by the presence of Neutrophils, necrosis with or without histiocytes. Non-specific reactive inflammations was defined by the presence of

polymorphic populations of inflammatory cells showing lymphoid cells in maturation spectrum which include plasma cells, occasional polymorphs and tissue cells. [29, 32].

3.10 BIOSAFETY MEASURES

During collection of samples, the Consultant/resident pathologist in pathology ensured that aseptic procedures were employed. To avoid cross contamination, Personal protective clothing and sterile equipment were used. Sample processing were done in microbiology and cytology laboratory under the supervision of a qualified technologist. All procedures were performed according to cytopathology and microbiology laboratory biosafety guidelines SOPs and biohazard waste disposal.

3.11 QUALITY ASSURANCE

A trained Laboratory Technologist was recruited to run the already prepared samples. All standard operating procedures were followed during specimen collection, handling and analysis. Slides were fixed immediately after preparation for a minimum of 15 minutes. A total of 10% of both positive and negative slides for ZN and cytology were identified and reviewed by an independent pathologist.

GeneXpert samples were processed as per SOP, no expired reagent was used, and samples were kept at the required temperature (room temperature).

3.12 DATA MANAGEMENT

Data was collected and stored in hard cover register and Microsoft excel. To maintain confidentiality, only the researcher had access to the data. Information on soft copy was protected from unauthorized persons by password. All records were identified by study identification number. Data will be stored for minimum period of 5 years.

3.12.1 STATISTICAL ANALYSIS

Data were analyzed using SPSS version 23 (StataCorp, College Station, TX, USA) at the significance level of $P \leq 0.05$. Descriptive statistics frequency (%), mean, standard deviation and medium (interquartile ranges at 25% and 75%) were used to present the quantitative data. Cohen's kappa coefficient (k) analyses to test the agreement of tests against a reference standard.

The sensitivity, specificity, NPV and PPV were calculated using R software version 3.2.2 [1] Bayesian method, Latent class analysis model. The software is able to calculate the sensitivity, specificity, negative predictive value and positive predictive value of the tests in the absence of a gold standard or reference standard test. [35, 36, 37, 38]

3.12.2 DATA PRESENTATION

Data were presented in tables, charts, graphs and percentages. Photomicrographs representing these patterns were displayed.

3.13 ETHICAL CONSIDERATION

Permission to conduct the study was obtained from KNH/UON Ethics and Research Committee assigned number P78/02/2016, and permission to conduct research in the unit was sought from the manager in charge of FNA clinic. Informed consent was obtained from all the participants in the study. In cases of children, assent was obtained. All GeneXpert, ZN and cytology results were communicated to the requesting physician to guide treatment as soon as they were available. Information obtained in the study was treated as confidential throughout the study by making sure it was locked. On the soft copy, password was changed from time to time.

The benefits of participating in the research was explained to the participants such as; the machine used in the study was expected to have a high sensitivity and specificity, therefore could detect the TB at the lowest concentration and the results was to be used to guide treatment. No costs would be incurred by the participant and results would improve EPTB diagnosis in Kenya.

4.0 RESULTS, ANALYSIS AND INTERPRETATION

4.1 CHARACTERISTICS OF STUDY PARTICIPANTS

Table 1 describes the baseline characteristic of the study participants

Referring clinic

A total of 43 EPTB suspected patients consented and gave samples for this study was analyzed. The majority 18 (41.9%) of the study sample size were from Surgical outpatient clinic (SOPC), followed by 15(34.9%) from Fine needle aspiration clinic (FNAC), 8(18.6%) from Medical clinic and 2(4.7%) from Comprehensive care clinic (CCC) as shown in figure 1.
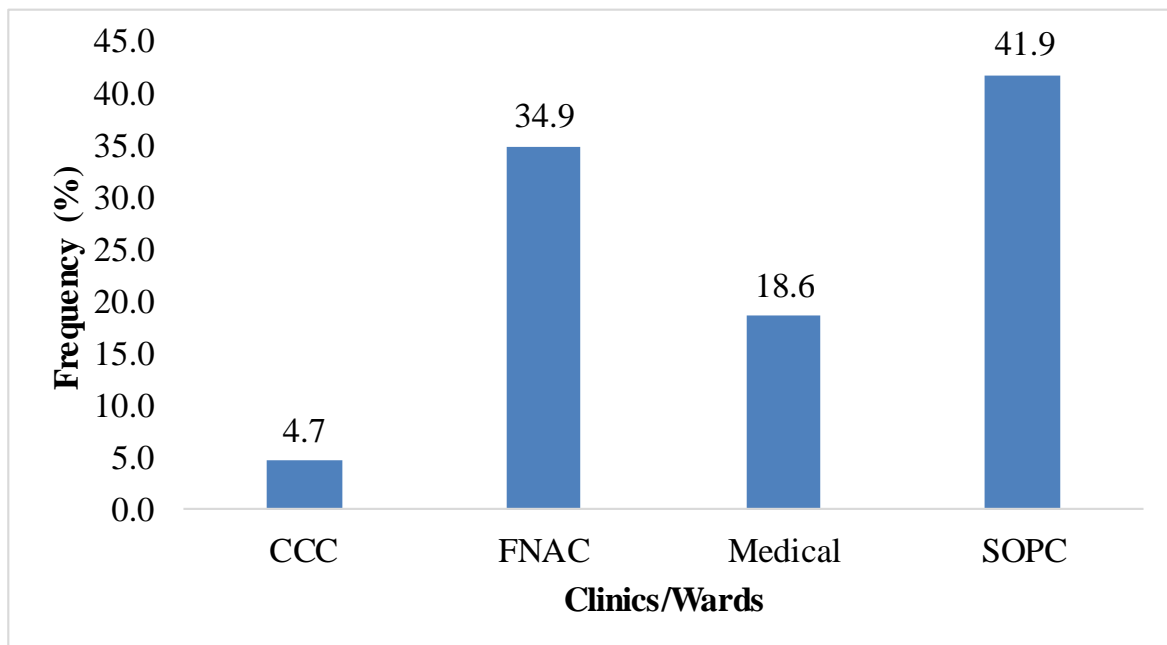
Figure 1: The distribution of the origin of study participants

4.2 AGE

The mean age of the 43 study participants was 26.74 years (SD ± 14.146) with the range of 2 to 56 years. There were two age peaks of 11 (26%) each aged between 21 to 30 and 31 to 40 respectively. Others included 8(19%) aged 1 to 10 years, 6 (14%) aged 11 to 20 years, 5(12%) aged 41 to 50 years and 2(5%) aged above 51 years as shown in figure 2.
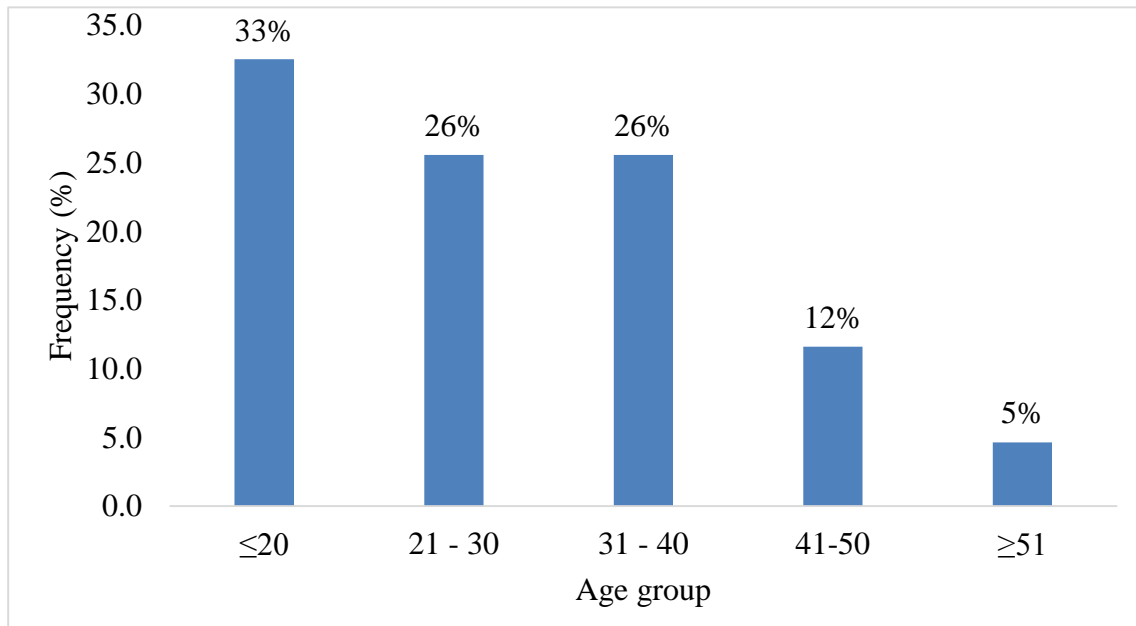
Figure 2: The age distribution of study participants.

4.3 GENDER

The study patients included 24(56%) male verses 19(44%) females (Table 1). The distribution in gender was not significantly different across study participants.

4.4 PAST TB TREATMENT

Majority of the study participants 38(88.4%) had never been treated for TB while only 5(11.6%) confirmed of having had a history of TB treatment (Table 1).

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4.5 SPECIMEN SITE

Majority of the specimen in this study 30(69.8%) were obtained from posterior cervical node, other sites included; 3(7%) from anterior cervical, 3(7%) from axillary and 2(4.7%) from inguinal area. About 5(11.6%) were obtained from other sites as shown in figure 3
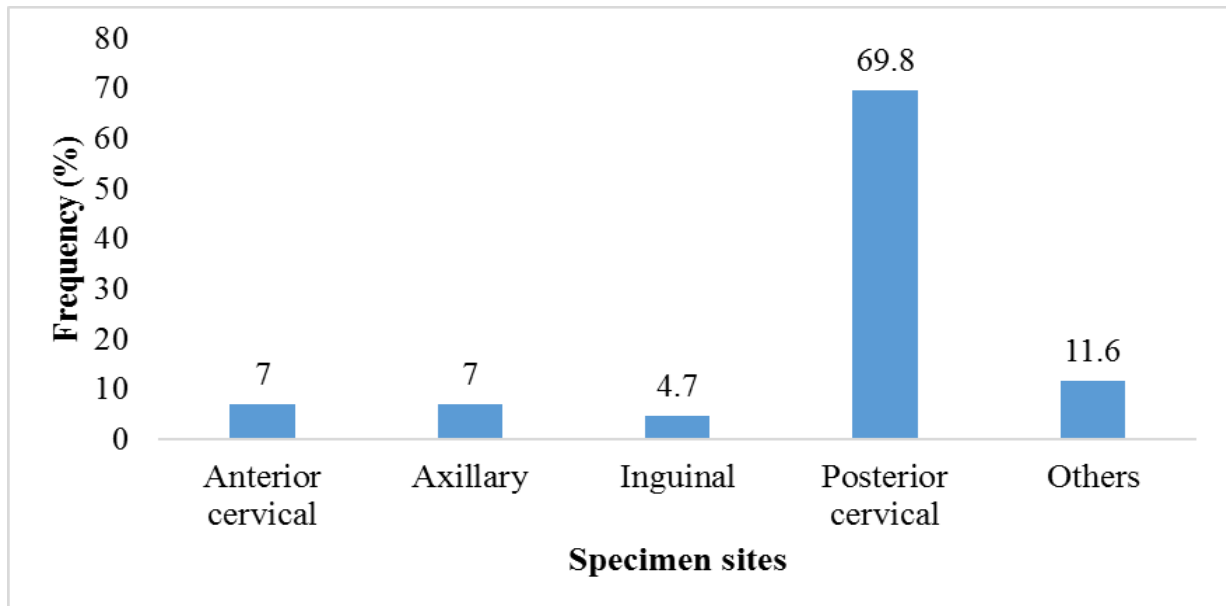
Figure 3: The distribution of TB specimen collection site

Table 1: Demographic and Characteristics of Study Participants

Parameters	Total (N = 43) N	%	Chi-square	df	P value
Referring clinic					
Comprehensive care clinic	2	4.7			
Fine needle aspiration clinic	15	34.9	14.395	3	0.002
Medical clinic	8	18.6			
Surgical out patient Clinic	18	41.9			
Total	**43**	**100**			
Age					
Mean (SD) (Years)	26.74	± 14.146			
Median (IQR)	28	17 to 37			
Range (Years)	54	2 to 56			
1 - 10	8	18.6			
11 - 20	6	14.0			
21 -30	11	25.6	8.767	5	0.124
31 - 40	11	25.6			
41 - 50	5	11.6			
≥51	2	4.7			
Total	**43**	**100**			
Gender					
Female	19	44.2	0.581	1	0.542
Male	24	55.8			
Total	**43**	**100**			
Past TB Treatment					
Never	38	88.4	25.326	1	0.001
Past	5	11.6			
Total	**43**	**100**			
TB contact					
First	6	14	22.349	1	0.001
Dont know	37	86			
Total	**43**	**100**			
Specimen site					
Anterior cervical	3	7			
Axillary	3	7	67.116	4	0.001
Inguinal	2	4.7			
Posterior cervical	30	69.8			
Others	5	11.6			
Total	**43**	**100**			

N-Number; %-Percentage; SD-Standard deviation; IQR-Interquartile range; df-degree of freedom;
P-level of significance; P<0.05 is significant

4.6 PROPORTIONS OF TB POSITIVITY BY DIFFERENT TESTS

Proportions of TB in this study cohort varied widely depending on the test used: as low as 1/43 (2.3%) using Ziehl-Neelsen (ZN) staining, 10/43 (23.3%) using GeneXpert and 25/43 (58.1%) using Cytomorphology.

Table 2: TB proportions across different tests

Outcome	ZN		Cytomorphology		GeneXpert	
	N	%	N	%	N	%
Negative	42	97.7	18	41.9	33	76.7
Positive	1	2.3	25	58.1	10	23.3
Total	**43**	**100**	**43**	**100**	**43**	**100**

N-Number; %-Percentage
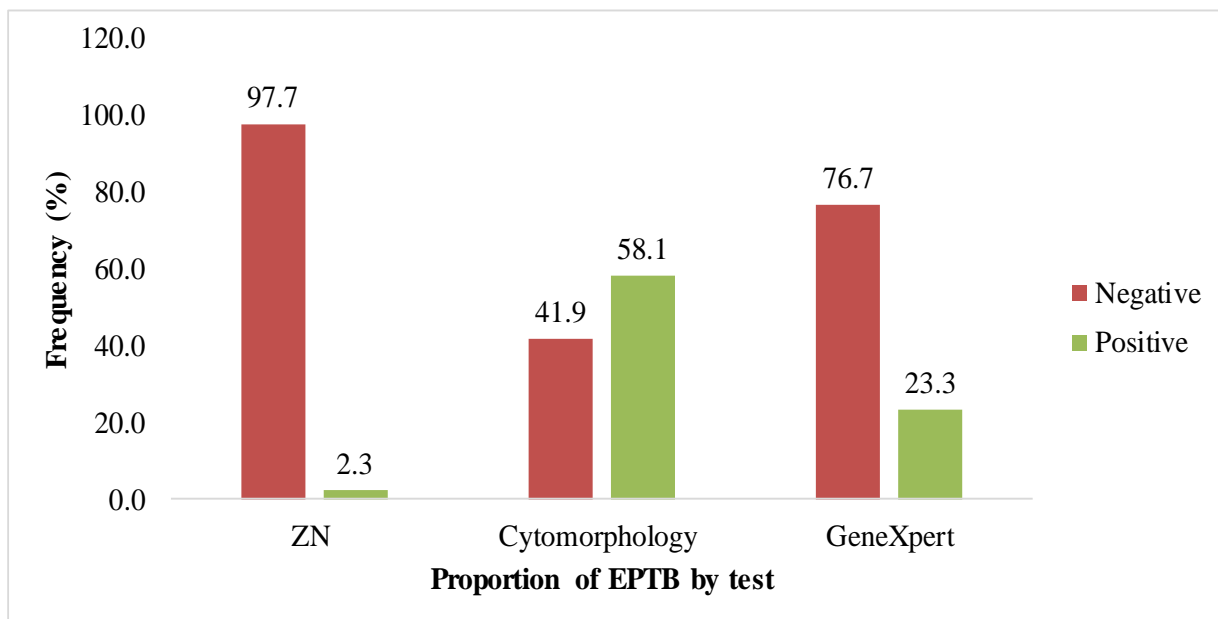
Figure 4 Proportions participants suggestive or diagnosed for tuberculosis by different tests

Comparing the distribution of TB by reference clinic showed that, the patient suggestive or diagnosed with TB by ZN 1(5.6%) was from surgical outpatient clinic; 10(66.7%) by Cytomorphology were from fine needle aspiration clinic while 1(50%) by GeneXpert was from comprehensive care clinic.

Comparing the distribution of TB by age group using different tests shows that the patient suggestive or diagnosed with TB by ZN 1(9.1%) was aged 31 to 40 years. Two peaks of 11(72.7%) by Cytomorphology were aged 21 to 30 and 31 to 40 years. Two peaks of 11(36.4%) each detected by GeneXpert were aged 21 to 30 years and 31 to 40 years.

Across gender, the patient infected with TB by ZN 1(4.2%) was male. About 14(58.3%) by Cytomorphology were male, 5(26.3%) by GeneXpert were female.

Concerning past TB treatment the patients with TB 1(20%), 4(80%) and 4(80%) by ZN, Cytomorphology and by GeneXpert respectively had history of TB infection.

Current suggestive or diagnosed TB patients; 1(16.7%) 6(100%) and 4(66.7%) using ZN, Cytomorphology and GeneXpert respectively had a history of TB case contact.

Patients 1(3.3%) infected with TB by ZN had their specimen obtained from posterior cervical, Patients 3(100%) suggested to have TB by Cytomorphology specimen were from anterior cervical and axillary sites.

4.7 TEST PERFORMANCE FLOW CHART FOR GENEXPERT, CYTOMORPHOLOGY AND ZIEHL NEELSEN.
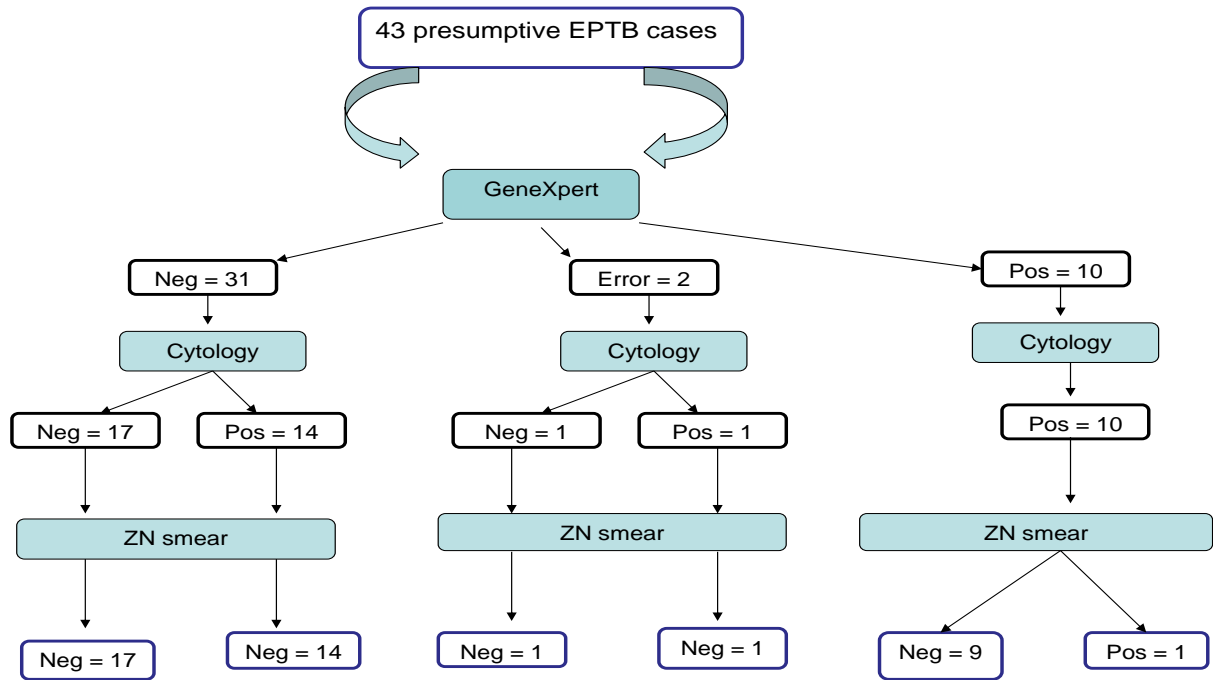
From the total number of 43 cases analyzed, of which GeneXpert detected 10 positive cases, two (2) errors and 31 negative cases.
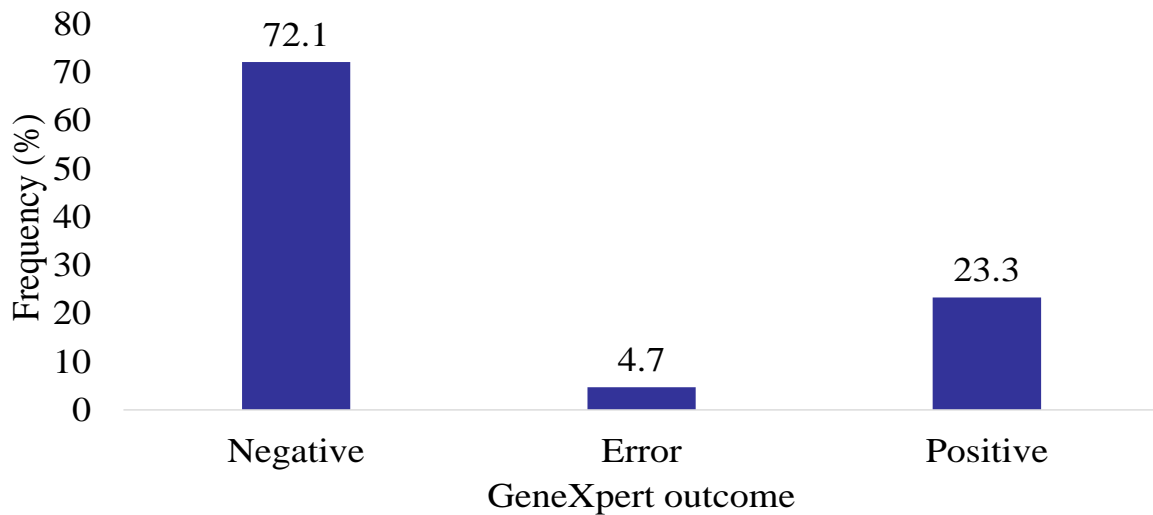
Figure 5 Gene Xpert performance

4.8 TEST PERFORMANCE ON DETECTION OF EPTB

Performance of different test on MTB detection was calculated using R software version 3.2.2 [1] Bayesian method, Latent class analysis model. The software is able to calculate the sensitivity, specificity, negative predictive value and positive predictive value of the tests in the absence of a gold standard or reference standard test. The results were obtained as indicated in Table 3 below.

Table 3 Tests performance on detection of EPTB using R software version 23.3

| Test | N | Latent class analysis | | | |
		Sensitivity (%) 95% CI	Specificity (%) 95% CI	NPV (%) 95% CI	PPV (%) 95% CI
GeneXpert	43	25.6 (0.8 - 96.1)	66.2 (32.8 - 99.1)	44.4 (0.5 - 99.3)	46.5 (1.3 - 98.6)
Ziehl-Neelsen (ZN)	43	4.9 (0.1 - 47.3)	94.4 (48.1 - 99.8)	31.5 (0.4 - 99.5)	48.7(2.4 - 97.6)
Cytomorphology	43	56.9 (8.7 - 98.9)	36.7 (1.1 - 90.5)	47.7 (1.9 - 97.3)	50.3(2.5 - 97.7)

N-Number; %-Percentage; CI-Confidence interval;

The test sensitivities were as follows: 25.6% by GeneXpert, 4.9% by ZN, 56.9% by Cytomorphology changes.

The specificities of each test were: 66.2% by GeneXpert, 36.7% by Cytomorphology changes; 94.4% by ZN.

The positive predictive values (PPV) ranged from at 95% CI, 46.5% (1.3 - 98.6) GeneXpert, 48.7 %(2.4 - 97.6) by ZN and 50.3 %(2.5 - 97.7) by cytomorphology. The negative predictive values ranged from 31.5 %(0.4-99.5) by ZN, 44.4% (0.5 - 99.3) by GeneXpert to 47.7 %(1.9-97.3) by cytomorphology.

ZN microscopy smear had lowest sensitivity with 1(4.9%) with highest specificity of 42(94.4%). GeneXpert had better sensitivity of 10(25.6%) than ZN with a specificity of 33(66.2%) while cytology had the lowest specificity of 18(36.7%) with a sensitivity of about 25(56.9%).

When performance of GeneXpert was compared with ZN and cytomorphology, results were as follows; ZN and GeneXpert showed slight agreement which was however not significant (Cohen's kappa coefficient (k) = 0.146; P = 0.066). Cytomorphology had a fair agreement with GeneXpert which was significant (k = 0.358; P = 0.002). The results are as shown in the table below

Table 4 Evaluation of tests agreement for GeneXpert, ZN and Cytomorphology.

Test	N	Kappa	
		k	P
Ziehl-Neelsen (ZN)	43	0.146	0.066
Cytomorphology	43	0.358	0.002

N-Number; %-Percentage; k - Cohen's kappa coefficient; P - Level of significance

5.0 DISCUSSION.

Extra pulmonary Tuberculosis is on the rise globally and its diagnosis remains a challenge especially in poor resource settings or developing countries. The methods used to diagnose EPTB such as ZN as demonstrated by Rioki et al at KNH, had a low sensitivity ranging from 20 to 43%. [29] Cytology, fluorescence microscopy and cultures have their own challenges ranging from low sensitivity to long turnaround time. Therefore diagnosis remains a serious challenge which causes the need for more sensitive, rapid and easy methods in the diagnosis of EPTB and this was the aim of this study to come up with the rapid, accurate and reliable test for EPTB diagnosis. Okinyi et al conducted a study at KNH and found that ZN staining had a sensitivity of 1.4% which is similar to our results. [40]

In this study, there were more males than females patients that presented with cervical node, 24 out of 43, (58.8%) though the difference was not statistically significant. In another study done in Libreville Gabon, a slight predominance of men was observed which concurs with our study. [39] A similar study done in Kenya by Okinyi et al, (un published data) differs from other studies and showed more females, 52 out of 100 patients, (52%) than males. Okinyi et al cited the health seeking behavior of females as a contributing factor as females take health issues more serious than males. [40] The difference between the study by Okinyi et al and this study may be due to different sample sizes used in these studies. The majority of the participants were between 20 to 30 and 30 to 40 years, which are generally considered to be the most active age groups.

The distribution of tuberculosis in this study is more common among the active age group, which is also the most affected group by Human immunodeficiency virus. This concurs well with the Ministry of Health observations in Kenya. [31]

Majority of the specimens in this study 30(69.8%) were obtained from posterior cervical node, this corresponds well with the study again done in Libreville where they observed that the infected lymph nodes were most often located at the posterior triangle of the neck, which contributed 61%, the reason being that most of the head and neck parts such as the mouth, nasal pharyngeal, larynx are all drained by posterior cervical node which puts them at high risk of being infected by EPTB or other infectious agent. [39]

According to W.H.O recommendation the GeneXpert MTB/RIF rapid test should be used as the initial diagnostic test in individuals suspected of MDR-TB or HIV associated TB, and as a follow-up test for smear negative TB. [14, 15] Furthermore, the test is equally useful in the diagnosis of TB in extrapulmonary samples. However, there is limited data on the performance of GeneXpert test in high and drug resistant TB prevalence settings where EPTB remains diagnostic challenge. Out of the 43 patients with clinical presumptive EPTB, 25(58.1%) were suggested by cytology to have MTB, 1(2.3%) was positive by ZN microscopy and 10(23.3%) were positive by GeneXpert. Higher proportions have been recorded in other studies Southwest Ethiopia, in a sample size of 143, with a country prevalence of 61.5%, recorded a proportion of EPTB of 18.9% by smear microscopy, 60.1% on GeneXpert and 67.1% by cytology. [41] In a study done in India, 33.33% were positive by ZN and 41.94% were suggested by cytology out of 218 cases recruited with a country TB prevalence of 50%. [42] Another study in Ethiopia [43] reported lower proportions than this study namely 5.2% by smear microscopy, 38.9% by GeneXpert assay , this was out of 231 cases. In South Africa with a prevalence of 23.5%, out of 1175 samples, a proportion of 66.7% by cytology and 2.1 by GeneXpert were reported. [44] The variation in proportions by the GeneXpert could be due to the variation in the overall prevalence of TB and EPTB by countries. The different samples sizes used could have contributed too.

In the present study, the sensitivities of GeneXpert, ZN and cytomorphology were 25.6%, 4.9% and 56.9% respectively. Higher sensitivity of GeneXpert has been reported in other studies. A systematic review and meta-analyses conducted by Tadesse et al in southwest Ethiopia [45, 41]   showed that GeneXpert test has a sensitivity ranging from 50% to 100% with pooled sensitivity of 83%. More recently, reviewed 36 studies done by Denkinger et al [2014] in Canada, in their meta-analyses and confirmed GeneXpert pooled sensitivity of 87% that is far much higher than sensitivity found in this study. [46] A much higher sensitivity of 96.7% was reported in a study done by Ligthelm LJ. [44] In this study, sensitivity was calculated using Latent class analysis model, which calculates the sensitivity without using a gold standard. The other studies, unlike the Latent class analyses model, used reference standard test to calculate the test performances. [38]   There were 15 samples suggestive of TB by cytology and were negative on GeneXpert. The reason for the difference between cytology and GeneXpert test results may be due to the limited number of bacilli in the FNA sample which could not be detected by GeneXpert. The detection rate for GeneXpert is-from 34

bacilli per mls of an aspirate and above. Granulomatous inflammatory changes seen in Cytology is not specific, the same cytological feature can also be seen in other conditions such as sarcoidosis, fungal, Toxoplasma and many others. Also other mycobacteria other than TB can cause similar cytological changes. [47] All these may have contributed to the differences in sensitivity between cytology and GeneXpert.

The results of the study reveal that the GeneXpert test has a better sensitivity than ZN for AFB-smear, because it diagnosed a significant proportion of ZN negative cases and increased the relative proportion of diagnosed EPTB cases. Even though conventional ZN microscopy has played an important role in the diagnosis of EPTB in poor resource settings, GeneXpert detected MTB in a significant cases missed by smear microscopy. The single smear positive sample in this study was also positive by GeneXpert. This is in agreement with other studies, which have reported the higher sensitivity of GeneXpert than smear microscopy. [48, 44, 47, 41].

In the present study, the specificity of GeneXpert, ZN and cytomorphology were 66.2%, 94.4% and 36.7% respectively. The specificity (66.2%) of the GeneXpert in this study was found to be lower than previous studies done by Lighthelm et al in USA, where they were looking at the performance of GeneXpert on TB of mediastinal lymphadenopathy. [49] another study done by Denkinger CM et al, in Canada, where they were looking at GeneXpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis [45] and Penz E, et al. where they looked at Diagnostic accuracy of the GeneXpert1 MTB/RIF assay for extra-pulmonary tuberculosishad a specificity of 89–99%. The specificity in this study was similar to the study done by Mouba JF, et al. where they looked at Cervical lymph node tuberculosis in Libreville, which had a specificity of 69.2%. [39]

Previous Studies have shown the potential differences in specificity among cytology, ZN smear and GeneXpert. This could be due to several factors, including the type of specimen and the low volume of residual material used following FNAS. Furthermore, the scant number of organisms in the lymph node lesion with a tendency of MTB to form clumps that could lead to an uneven distribution of the bacilli. [49, 50] Another possible difference is that ZN smear detects live organisms while GeneXpert can detect both live and dead DNA of the MTB [45] Smear microscopy can detect the bacilli in FNA sample when the concentration is about 1000 to 10,000/ml of an aspirate, the concentration below that is almost impossible to

detect it and Gene-Xpert can detect the presence of the bacilli at a very low concentration ranging from 34 and above bacilli /ml of an aspirate. [28]

Although cytology is considered a guide, the outcome of its interpretation depends on the pathologist/ Cytologist. Cytological interpretation is often limited by a number of factors including the characteristic cells which may not be sufficient in many instances to yield a definitive diagnosis.

The other reason for the differences obtained on specificity between this study and other studies could be the method used to calculate the specificity. In this study we did not have a gold standard and used latent class analysis, with different sample sizes. Latent Class analysis method produces reliable results if a good sample size is used. Our sample size was small and may have contributed to the differences.

5.1 Limitation

1. In this study, we did not have a gold standard and calculated performances of the tests using Latent class analysis model. This method requires sufficient sample size to produce reliable results, our sample size was small which may have not given good estimations of the test performances as it is indicated be a wide range of sensitivity and specificity of 0.8 – 96.1 and 32.8 – 99.2 respectively.

5.2 Conclusion

1. Our findings indicated that gene Xpert MTB/RIF test is a useful tool for the detection of MTB with reasonable sensitivity and specificity on EPTB using fine needle aspirate with good performance as compared to ZN smear microscopy and cytology.
2. ZN staining showed low sensitivity of 2.3% which is unreliable as it is misleading.

5.3 Recommendations

1. Results by cytology and ZN staining maybe considered for confirmation with Gene-Xpert if available and a testing algorithm should be developed in KNH.
2. Further prospective studies are required to evaluate the performance of GeneXpert on FNA samples by using a more sensitive reference standard, the liquid culture as a gold standard with a large sample size.

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APPENDIX (I) ADULT INFORMED CONSENT EXPLANATION DOCUMENT

Title of study: utility of gene-expert and cytomorphology in the diagnosis of extra pulmonary tuberculosis at Kenyatta national hospital

Principal Investigator: Mespa Manyepa, MSc student, University of Nairobi/ KNH.

Introduction

I would like talk to you about the study being conducted by Mespa Manyepa, a clinical cytology student at the University of Nairobi/ Kenyatta National Hospital. This consent form serves to give you information which will help you to make a decision on whether to take part in the study or not. If you have any question concerning the purpose of the study, possible risks and benefits, as a volunteer feel free to ask and ensure that this consent form is clear to you. When you are satisfield with all your concerns, you may then decide to participate in the study or not. If you decide to participate, you will be given a copy of this form for your records.

Purpose of the study

This study is about the diagnosis of Tuberculosis in extrapulmonary specimens. Since your doctor is suspecting you could be suffering from tuberculosis, and is asking a sample to be taken from you for routine testing, I am kindly requesting you to give permission to use the residual specimen for my research which will be used to identify the most reliable and rapid method to use for the diagnosis of extrapulmonary TB. This research is not going to interfere at all with the routine processing of samples in this setting. Complete confidentiality of your test results will be ensured.

Specimen Collection

In this study, specimens will be obtained by performing fine needle aspiration of the accessible lesion. The procedure will be carried out by a consultant pathologist/ registra in Pathology Department at Kenyatta National Hospital after obtaining consent and assent from the patients. Slides will be prepared from the aspirated specimen for cytology and ZN test. The remaining sample in the hulb of the needle and syrigy will be rinsed in 20% sodium hydroxide for gene-xpert analysis.

Benefits

The benefits of participating in the research will be explained to the participants such as; the machine used in the study has a high sensitivity and specificity, therefore can detect the TB at the lowest concentration level and the results will be used to guide treatment. No costs will be incurred by the participant and the use of the research results will improve EPTB diagnosis in Kenya as it is still a challenge.

Risks

Minimal risks are involved in this procedure, a bit of pain during FNA procedure and slightly bleeding after the procedure.

Data dissemination

The data acquired will be presented to the Kenyatta National Hospital, Department of Human Pathology, cytology Laboratory and Fine Needle Aspiration Clinic. It will also be published in peer reviewed journals and presented in conferences and seminars.

Participant's rights

Your participation in this study is voluntary and if you decline to participate, you will not be denied any services that are normally available to you.

Assurance of confidentiality of volunteer's identity

Records relating to you or your patient's participation in the study will remain confidential. You will be given a signed copy of the consent form.

Contact information

If you have questions now or in future regarding your rights or this study, you may contact:

- Mespa Manyepa, MSc student at the University of Nairobi on 0737595285
- Chairperson, KNH/UON/ERC. P.O BOX 20723-00200 Nairobi. Tel#: 726300-9, Fax 725272.
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- Mrs. Josephine. N. Rioki Tel +25477531874

Ridhaa ya kukubali kuwa muhusika katika Kiswahili

Jina langu ni Mespa Manyepa. Mimi ni mwanafunzi wa chuo kikuu cha Nairobi. Nina fanya uchunguzi wa mbinu za kupima ugonjwa wa kifua kikuu. Kwa vile daktari wako anadhania yakwamba unaweza kuwa unaugua kutokana na ugonjwa huu, ameuliza   utolewe kiasi kidogo cha sampuli kutoka kwenye uvimbe wako ili ikapimwe kama ilivyo kawaida. Nami naomba unikubalie nitumie sehemu ya mabaki ya sampuli hii katika utafiti wangu. Matokeo haya pia yatasambazwa kwa serikali na wadau kama Wizara ya Afya/WHO, ambao watatengeneza miradi mbalimbali itakayolenga kupunguza maambukizi na kuboresha huduma za walioathirika katika jamii yako na nyingine hapo baadaye. Sampuli yako itawekwa katika mahabara ya hospitali hii kwa muda wa miaka tano iwapo kutakua na hitaji ikatumike kwa utafiti zaidi baadaye. Ruhusa ya kutumia sampuli hii itapeanwa na KNH/UON/ERC. Utafiti huu umepitishwa na kuruhusiwa na kamati inayohusika na utoaji wa vibali vya utafiti ya KNH/UON/ERC.

Faida/mapungufu

Hutapata malipo yoyote ya kifedha, na pia hutatumia pesa zako mwenyewe katika utafiti huu. Utafiti huu utakufaidi wewe na wakenya wote baadaye kwa sababu mbinu inayoaminika zaidi na iliyo ya haraka ya kupima ugonjwa huu inaweza kupatikana. Na hivyo basi itapunguza vivo vya watu wengi kutokana na maradhi haya. Matokeo ya utafiti huu yatazambazwa kwa wasimamizi wa KNH na pia WHO ambayo itapendekeza na kuunda mikakati mwafaka ya kuakikisha madhara ya ugonjwa huu umepungua.

Haki za mshirika

Ushiriki wako katika utafiti huu ni wa hiari kabisa. Ukikataa kushiriki, hutanyimwa huduma zinazotolewa kwa kawaida.

Usiri/utunzaji wa taarifa

Katika utafiti huu, tutahakikisha kuwa maelezo yako, jina lako ni siri kabisa. Majibu yako hayataonyeshwa kwa watu ambao sio waugusi.Utapata nakala ya fomu ya usiri ulioitia sahihi.

Mawasiliano

Iwapo utakuwa na swali kuhusiana na haki zako ama utafiti huu, unaruhusiwa kuwasiliana na Mkuu wa utafiti huu,

- Mespa Manyepa, mwanafunzi wa chuo kikuu cha masomo cha Nairobi nambari ya simu 0737595285
- au Mwenye kiti, KNH/UON/ERC P.O.BOX 20723 -00200 Nairobi; nambari ya simu: 726300-9, Fax 725272.
- Professor Emily Rogena Supervisor Tel +254721674647.
- Dr. Julius Oyugi Supervisor Tel +254 713 898564
- Mrs. Josephine. N. Rioki Supervisor   Tel +25477531874

APPENDIX (II) ADULT CONSENT FORMS
Consent from the patient

The above details about the study and the basis of participation have been explained to me and I agree to give permission for use of the residual material from my specimen in the proposed study.

I understand that I am free to choose to let my specimen be used in the study or not. I give my consent for my residual specimen to be tested for extra pulmonary TB.

Patient   signature/ Thumb mark: --

P I's signature: --

Date:

Ridhaa ya kutolewa sampuli ya kupimwa kwa Kiswahili

Nimekwisha elezewa juu ya utafiti unaokusudiwa kufanywa kwa mabaki ya sampuli yangu. Nimeelewa maelezo ya hapo juu yanayohusu utafiti huu, na ninakubali kushiriki katika zoezi hili. Naelewa kuwa ushiriki wangu ni wa hiari, na pia kama sitakubaliana sampuli yangu haitatumika katika utafiti huu.

Napeana ruhusa ya kutumika kwa mabaki ya sampuli yangu kutoka kwenye uvimbe wangu ili ipimwe iwapo itakua na ugonjwa wa kifua kikuu.

Sahihi/kidole gumba cha mgonjwa: ----------------------------------

 Sahihi ya mlinzi wa mgonjwa: ----------------------------------

Tarehe:

APPENDIX (III) MINOR ASSENT DOCUMENT

Title: Utility of gene-expert and cytomorphology in the diagnosis of extra pulmonary tuberculosis at kenyatta national hospital

Investigator: Mespa Manyepa, MSc student, University of Nairobi/ KNH

 I am doing a study on extrapulmonary tuberculosis diagnosis. The study will use the residual of your sample in the syringe and needle to identify the most rapid and accurate method of detecting EPTB. Since your doctor is suspecting you could be suffering from EPTB, am asking if you would allow me to use the residual of your specimen to test for TB.

Permission has been obtained from Kenyatta National Hospital-University of Nairobi Ethics and Research Committee to conduct the research. In this study, children will also participate and are free to participate or not, if you agree to participate, a fine needle aspirate will be done on you which will take few minutes, about 2 minutes maximum to get specimen for making slides and for GeneXpert

Benefits

The machine used is very sensitive and can detect TB at lower concentration. The results will be used to guide your treatment, no payment is involved and the test will not interfere with the routine testing procedure.

Risks

Minimal pain will be experienced during FNA procedure, a bit of bleeding and maybe haematoma too.

The information obtained from the study will be disseminated through workshops and seminars, but it will not bear your name or will it indicate that you were part of the study.

You are free to decide to be part of the study or not, if you decide not to be part of the study, no other health facilities will be denied from. Your Parents know about the study too.

If you decide to be part of the study, please write your name and sign and you will be given a copy of this document for your records.

APPENDIX (IV) MINOR ASSENT FORMS

To be signed by the parents/ guardian.

The above details about the study and the basis of participation have been explained to me on behalf of the patient and I agree to give permission for use of the residual material from the patient's specimen in the proposed study.

I understand that the patient is free to choose to let their specimen be used in the study or not. I give my assent for the residual specimen to be tested for extra pulmonary TB

Parents/Guardian's signature: --

PI s signature: ---

Date:

Ridhaa ya kutolewa sampuli ya kupimwa kwa Kiswahili

Nimekwisha elezewa juu ya utafiti unaokusudiwa kufanywa kwa mabaki ya sampuli yangu. Nimeelewa maelezo ya hapo juu yanayohusu utafiti huu, na ninakubali kushiriki katika zoezi hili. Naelewa kuwa ushiriki wangu ni wa hiari, na pia kama sitakubaliana sampuli yangu haitatumika katika utafiti huu.

Napeana ruhusa ya kutumika kwa mabaki ya sampuli yangu kutoka kwenye uvimbe wangu ili ipimwe iwapo itakua na ugonjwa wa kifua kikuu.

Sahihi/kidole gumba cha mgonjwa: ----------------------------------

Sahihi ya mlinzi wa mgonjwa: ----------------------------------

Tarehe:

APPENDIX (V) FNA TECHNIQUE

Fine needle aspiration cytology

Equipment

1. 3-4 glass slides
2. 22 gauge needle
3. 5 or 10 ml syringes
4. Gloves
5. Alcohol swabs

Procedure

1. The area or the lesion will be Localize
2. After localizing the lesion, the area will be clean with swabs.
3. Before pricking the localised lesion, air in the syringe will be expelled.
4. Then the needle will be attached to the syringe.
5. After fixing the needle to the syringe the needle will inserted into the mass
6. Sample in 360 degrees, short quick strokes in different directions will be done.
7. Then suctioning will be done and blood will be avoided.
8. Upon seeing material in the hub, suctioning will be released.
9. Detach the needle, air will be drawn into the syringe, pressure will be applied and the content will be expelled on the –slides
10. The smear will be spread on the slide using another slide , smear will be immediately fixed in 95% ethanol for pap and H/E stains
11. Sodium Hydroxide will be used to rinse the needle and syringe
12. The sample will be then taken to the microbiology Laboratory for Gene-Xpert analysis.

Complications

1. Pain,
2. Bleeding
3. Needle prick injury

APPENDIX (VI)   SPECIMEN PROCESSING PROCEDURE FOR GENE-XPERT

1. Sample will be digested using 2mls of 20% sodium hydroxide for minimum of 30 minutes with some shakings at regular interval

2. 0.5mls of xylene will be added to the sample and will be allowed to stand for 15minutes

3. The creamishy formed on the surface of the mixture will be scooped carefully with a loop wire and mixed with 2mls of normal saline.

4. 200 micrometre from the mixture will be added to the cartridge

5. The cartridge then will be inserted in the gene-xpert machine

6. Results will be generated after 90 minutes

7. Interpretation of the results

APPENDIX (VII) DUMMY TABLES

Dummy Table for Cytology

Characteristic	number	Percent(%)
Gross descriptions		
Purulent aspirate		
Caseous tissue fragment		
Blood tissue fragment		
Cytological diagnosis of pathological process		
Chronic granulomatous inflammation suspicious for MTB		
Acute necrotizing supurative inflammation		
Non-specific reactive and chronic inflammation		
Necrotizing inflammation		
Inadquate for evaluation		
Spectrum of cytomorphological features		
Epitheloid granulomas, inflammatory lymphocytes with necrosis		
Epitheloid granulomas without necrosis		
Necrosis with supurative, neutrophils, necrotic debri		
Non-specific inflammation		
Cancer cells		
Inadequate for evaluation		
Total		

Dummy Table for Gene-xpert

Gene-xpert results	Total Number	Percentage
Negative		
Low		
Medium		
High		
Undeterminant		

Dummy Tables for all Methods

Methods	Total negatives	Total positives
ZN		
Cytomorphology		
Gene-xpert		

APPENDIX (VIII) ZN PROCEDURE

Carbolfuchsine combined with Phenol binds to mycolic acid in the mycobacteria cell wall. After staining, acid decolorizing solution is applied. This removes the red dye from the background of cells, tissues fibers and any organism in the smear except mycobacteria which retains the dye and is therefore referred to as acid fast bacilli (AFB).

Procedure

1. Air dry or fix the smears in 95% alcohol
2. Cover the smear with carbolfuchsine stain for 30mins.
3. Wash off the stain with clean distilled water
4. Decolorize the smear with 3% v/v acid alcohol until the smear sufficiently decolorizes to pale pink.
5. Wash with clean water
6. Counter stain with malachite green or 1% methylene blue for 3 minutes
7. Wash off the stain with clean water
8. Wash the back of the slide clean and leave to air dry.
9. Examine the smear using 100 x oil immersion.

Interpretation and Reporting of ZN smears

>9 AAFBs seen per field......................++++	1
1-9 AAFBs seen per field 5 Fields…. + + +	2
1-9 AAFBs fields seen in 10 Fields…..+ +	3
1-9 AAFBs seen in 100 Fields …..... ...+	4
1-2 AAFBs fields seen in 300Fields……±	5
NO AFB seen in 300 Fields…………...... -	6

NB// CDC reporting format.

APPENDIX (IX) QUESTIONNAIRE

PROJECT TITLE: UTILITY OF GENE-EXPERT AND CYTOMORPHOLOGY IN THE DIAGNOSIS OF EXTRA PULMONARY TUBERCULOSIS -AT KENYATTA NATIONAL HOSPITAL

DATE…………….

LABORATORY NO (KNH)…………………………………

IP / OP NO……………………………STUDY NO…………..

Clinic/site or ward

WARD: SOPC [1] MEDICAL [2]

CLINIC: FNAC [3] CCC [4]

AGE (specify YR)…........

SEX:M [1] F [2]

Clinical History

1. TB treament recurrent [1] Past [2] Never [3]

2. TB Contact First [1] Rcurrent [2] dont know [3]

3. SPECIMEN SITE (specify)………………………….

Posterior cervical [1] Anterior cervical [2] Axillary [3]

Supraclavicular [4] ubmandibular [5] Inguinal [6] others [7]

4**. RESULTS**

Methods	Positive	Negative
Direct smear ZN	1	2
Cytomorphology	1	2
Gene-xpert	1	2

APPENDIX (X) MATERIALS

Equipments

Olympus microscope will be used in this study, DNA amplification will be done using Gene-Xpert.

Consumables

Consumables such as gloves, syringes (10ml), needles(gauge 23), staining racks, slide holders , falcon tubes and a wire loop to be used in this study will be sourced locally.
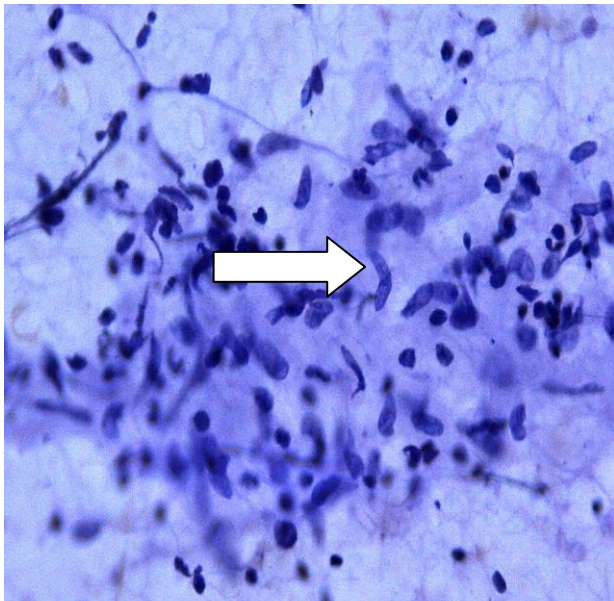
Reagents

Xylene, Alcohol, Hydrochloric acid, malachite green, carbolfuschin,sodium hydroxide, Gene-xpert catriadges will also be sourced locally.

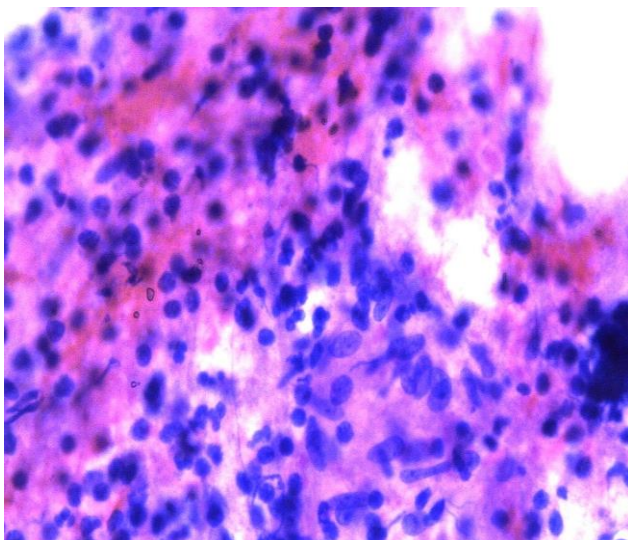**APPENDIX (XI) GENE-XPERT MACHINE**
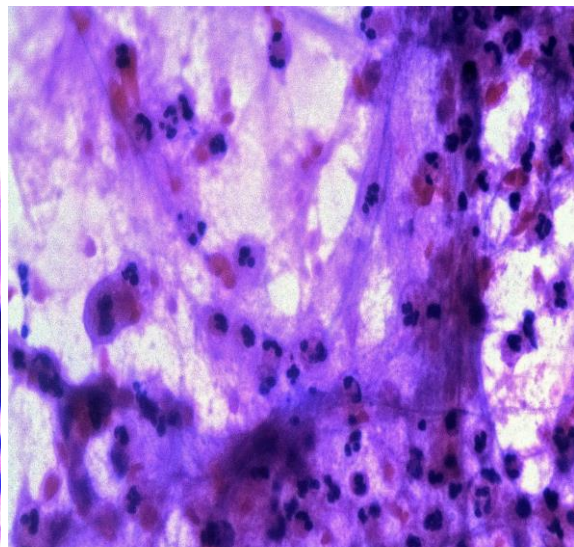
APPENDIX (XII) PHOTOMICROGRAPHS

Epithelioid histiocytes, lymph node FNA X40

Epithelioid histiocytes, lymph node FNA X40

Granulomatous and necrosis. Lymph node FNA X40

Necrosis from a lymph node FNA aspirate X40

| GeneXpert, version 4, model 2012, Cepheid Company | Sample preparation for GeneXpert by the PI |

Test Report

Patient ID:
Sample ID: 0529547
Test Type: Specimen
Sample Type: mass

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF Assay G4	5	In Vitro Diagnostic

Test Result: MTB NOT DETECTED

Test and Analyte Result

Analyte Name	Analyte Result	Probe Check Result
Probe D	NEG	PASS
Probe C	NEG	PASS
Probe E	NEG	PASS
Probe B	NEG	PASS
SPC	PASS	PASS
Probe A	NEG	PASS
QC-1	NEG	PASS
QC-2	NEG	PASS

User:	esther akalla		
Status:	Done	Start Time:	17/03/16 15:45:11
Expiration Date*:	26/03/17	End Time:	17/03/16 17:28:20
S/W Version:	4.4a	Instrument S/N:	802631
Cartridge S/N*:	243067090	Module S/N:	628399
Reagent Lot ID*:	19704	Module Name:	A1

Notes:

Errors
<None>

For In Vitro Diagnostic Use Only.

Page 1

Xpert© Dx System Version 4.4a

GeneXpert report print

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