Performance of Ziehl-Neelsen Microscopy, Light Emitting Diode – FM and Xpert MTB/RIF in the Diagnosis of Tuberculosis in People with Presumptive TB from EAPHLNP study sites in Kenya

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Summary

INTRODUCTION

In Kenya, sputum smear microscopy, especially Ziehl-Neelsen (ZN) method has been the cornerstone for tuberculosis (TB) diagnosis at most public health facilities. Recently, Led Emitted Diode (LED) fluorescent microscopy (FM) and Xpert MTB/ RIF (GeneXpert), have been introduced in selected health facilities for diagnosis of TB and Drug Resistant TB. This study was undertaken to determine and compare the performance (sensitivity, specificity, positive and negative predictive test values) of these two new TB diagnostics with ZN microscopy as a benchmark, LED/FM and GeneXpert using either LJ or MGIT culture, whichever was available or if one was positive while the other negative, as a gold standard.

METHODOLOGY

A cross-sectional study was conducted between February 2013 and August 2014 in nine selected public health, facilities in Kenya. People with presumptive TB aged 18 years and above, both new and re-treatment cases attending the facilities with symptoms suggestive of TB (including cough of two or more weeks) were eligible for the study and consecutively recruited. Two sputum specimens (spot and early morning) were collected over two consecutive days. A total of 3073 sputum samples were collected from 1891 people with presumptive TB.

The specimens from the study sites were appropriately packaged and shipped to the TB research laboratory in KEMRI, Nairobi, whereby samples were received and processed for ZN, LED, GeneXpert, LJ and MGIT culture in accordance with standard procedures. Culture was used as a gold standard. The study was approved by the Ethical Review Committee of KEMRI.

RESULTS

A total of 639 specimens from 390 patients with culture results were included in the analysis. GeneXpert showed significantly higher sensitivity (83.7% (95%CI: 76.6-90.8)) than ZN

(65.4% (95% CI: 56.3-74.5)) and FM (68.3% (95% CI: 59.4-77.2)) microscopy methods in the diagnosis of TB.

On the contrary, specificity of GeneXpert (87.9% (95% CI: 85.1-90.7)) was significantly lower than that of ZN (93.5% (95% CI: 91.4-95.6)) and FM (93.3% (95% CI: 91.295.4)) microscopy. GeneXpert sensitivity in smear positive culture positive was (95.6% (95% CI: 90.7-100.0)) and (97.2% (95% CI: 93.4-100.0)) for ZN and FM, respectively, it was significantly lower in smear negative culture positive specimens with (61.1% (95% CI: 45.2-77.0)) and (54.5% (95%CI: 37.5-71.5)) for ZN and FM, respectively.

Sensitivity rate was significantly higher in specimens from non-previously treated with presumptive TB (71.1% (95%CI: 61.4-80.9)) for ZN, (73.5% (95% CI: 64.0-83.0)) for FM and (89.2% (95% CI: 82.5-95.9)) for GeneXpert than those from retreatment cases (42.9% (95% CI: 21.7-64.1)), (47.6% (95% CI: 26.2-69.0)) and (61.9% (95%CI: 41.1-82.7)), respectively. Overall, HIV status did not affect the performance of GeneXpert.

However, Sensitivity of GeneXpert (84.4% (95% CI: 71.8-97.0)) was significantly higher in HIV positive than that of ZN (53.1% (95% CI: 35.8-70.4)) and FM (56.3% (95% CI: 39.1-73.5)) microscopy. There were no significant differences in sensitivity of ZN (70.8% (95% CI: 60.3-81.3)) and FM (73.6% (95% CI: 63.4-83.8)) in HIV negative specimens compared to sensitivity of ZN (53.1% (95% CI: 35.8-70.4)) and FM (56.3% (95% CI: 39.1-73.5)) in HIV positive specimens. A small proportion (6.2%) of specimens with ZN and culture negative results was positive by GeneXpert.

CONCLUSIONS

Performance of GeneXpert was higher than both ZN and FM microscopy for diagnosis of TB in Kenya and is comparable with performance indicated in a few previous studies in Africa. Despite the low sensitivity in smear negative culture positive specimens, GeneXpert has potential to increase diagnostic yield in smear and culture negative specimens, especially from HIV positive people with presumptive TB. Further studies are required to ascertain its specificity and application in specific patient population. This will be possible when patients' clinical details are linked with respective laboratory data as a result of combination of tests to improve diagnostic yield.

Key words: ZN, LED-FM; GeneXpert: Performance; Tuberculosis Diagnosis;

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Introduction

In Kenya, sputum smear microscopy has been the cornerstone for TB diagnosis at most health services. This method was rapid inexpensive and highly specific for Mycobacterium tuberculosis (MTB). However, the main limitation was its low and variable sensitivity, exacerbated in high HIV prevalence settings [1]. High TB - HIV co-infection rates and consequent low TB case detection rates impede disease control in many TB endemic settings notably sub-Saharan Africa [2]. In addition, sensitivity was largely determined by the duration of microscopic examination. Where workloads were high and the amount of time spent examining smears was low and sensitivity also correspondingly low [3].

An earlier systematic review had shown that Fluorescent Microscopy (FM) was on average 10% more sensitive than conventional Light Microscopy in

detecting AFB in clinical{what is clinical specimens} specimens [4]. FM had also comparable specificity and took significantly less time to read smears but required technical proficiency [4].

Light Emitting Diodes (LEDs) for FM, commonly referred to as Optimized Sputum Smear Microscopy (OSSM) have been identified as an alternative to conventional FM for screening of MTB [5,6].

LED lamps do have several advantages over the mercury vapor lamps.
These include:

1. The life expectancy of LED lamps averages around 10-20 years of use and contrary to mercury vapor lamps
2. They do not explode after excessive usage.
3. Since they require less power, they are also able to run on batteries [6,7,8].
4. Several commercial LED systems are now available, either as stand-alone microscopes, or as add-on adapters to conventional microscopes [9].
5. Data published so far on LED microscopy for TB show that, results in terms of sensitivity or specificity are comparable or even better with LED lamps than mercury vapor lamps [5-12].
6. The life expectancy of LED lamps averages around 10-20 years of use and contrary to mercury vapor lamps.
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8. Since they require less power, they are also able to run on batteries [6,7,8].
9. Several commercial LED systems are now available, either as stand-alone microscopes, or as add-on adapters to conventional microscopes [9].
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Although LED microscopy is now widely used for TB diagnosis in Kenya, the methodology has only been evaluated in one site in Nairobi findings of which may not be a replica of the situation in Kenya [10].

Tuberculosis culture methodology which is the gold standard takes long (4-8weeks) before results are available and despite advancement in rolling out this method in some designated centers, it was still not readily available in most settings. The most traditional approach in most countries, particularly in developing countries, was the use of solid Löwenstein-Jensen (L-J) medium. The total turnaround time, including primary culture isolation followed by an indirect susceptibility test can be as long as 2 and even 3 months in many laboratories. There was urgent need for laboratory infrastructure strengthening, development and evaluation of more sensitive and rapid TB diagnostics [Stop TB Partnership Retooling Task Force, 2008] to mitigate the spread of TB and specifically Multi Drug Resistant Tuberculosis (MDR-TB) [12].

The BACTEC - 960 (Becton - Dickinson Diagnostic Instrument System, Sparks, MD) was the first semi-automated system introduced for Drug Susceptibility Testing (DST) of MTB in liquid medium. The major advantage of that technique was that it was rapid, with the ability to detect growth earlier than by any other means [13-15].

Xpert MTB/RIF (GeneXpert), (Cepheid, Sunnyvale CA, USA), a fully automated molecular test for TB case detection and rifampicin (RIF) drug resistance testing was developed through collaboration in a public–private partnership. The GeneXpert diagnostic test for MTB has recently been shown to have sensitivity and specificity comparable to culture in the diagnosis of pulmonary TB (92.2% and 99.2%, respectively) and MDR TB (99.1% and 100%, respectively) in a multi-country evaluation [16].

When consideration was given on the above description of the new technologies, together with the recommendations from the World Health Organization (WHO) TB/HIV Working Group on Priority research questions for TB and TB/HIV in HIV-prevalent and resource-limited settings [17], there is need to conduct diagnostics evaluations.

These, will assess the impact of new diagnostics on patient important outcomes, including the incremental value of new diagnostics and appropriateness of the treatment regimen offered on the basis of the diagnostic test especially in areas where the new diagnostics are currently in use. This study was undertaken to determine and compare the diagnostic test values (sensitivity, specificity, positive and negative predictive test values) of ZN, LED/FM and GeneXpert using either LJ or MGIT culture, which ever was available or if one is positive while the other was negative, as a gold standard.

Methodology

A cross-sectional study was conducted between February 2013 and August 2014 in nine selected public health facilities in Kenya. People with presumptive TB aged 18 years and above both new and retreatment cases attending the facilities with symptoms suggestive of TB such as anyone with cough of two or more weeks and who had given written informed consent were eligible for the study and consecutively enrolled. Two sputum specimens (spot and morning) were collected over two consecutive days.

The specimens from study sites were appropriately packaged and shipped to the TB research laboratory in KEMRI, Nairobi. Macroscopic assessment of sputum specimen was performed and quality sputum specimens [18] were processed for direct sputum smear microscopy. Smears stained with ZN method, were examined with light microscope while LED was used for FM. GeneXpert, LJ and MGIT culture was done in accordance with standard procedures. Specimens for LJ and MGIT liquid culture (Bactec MGIT; BD Microbiology Systems, Cockeysville, MD, USA) were processed with N-acetyl-Lcysteine (NALC) NaOH (2%) decontamination. All ZN positive cultures were subjected to an identification process using *Immuno Chromomatogenic*

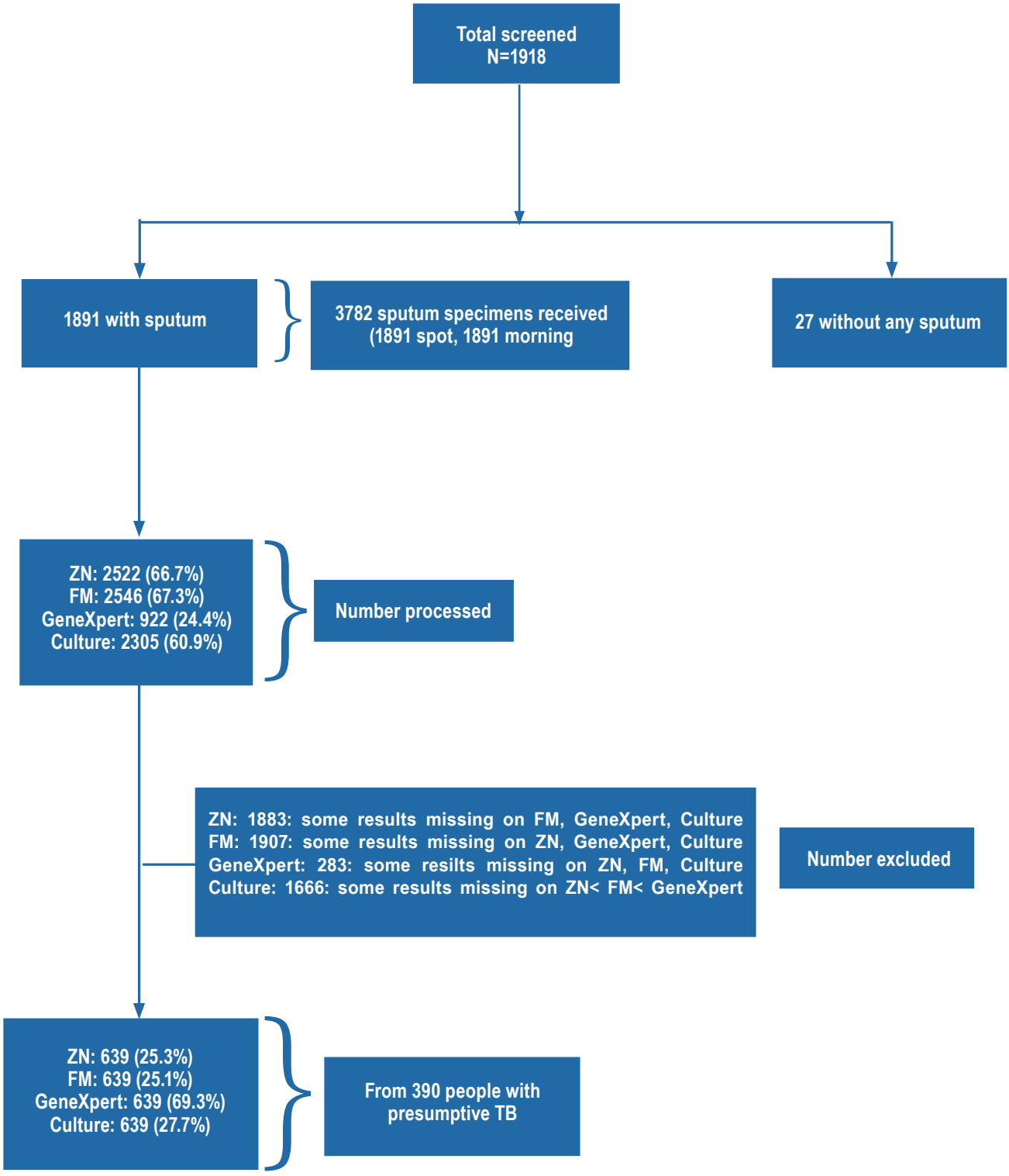
Assays (ICA) identification kit (BD MGIT TM TBc identification test) to confirm for MTB. A positive culture for MTB was used as a gold standard for positivity. Specimens for GeneXpert were transferred into respective cartridges and immediately loaded in the GeneXpert machine located at the National TB Reference Laboratory within the same building with KEMRI. HIV results were obtained from respective health facility clinical records.

The KEMRI Mycobacteriology Research Laboratory is quality assured through the WHO-based Quality Assurance Programme for Drug Susceptibility Testing with National Mycobacterium Reference laboratory, Public Health England. The study was approved by the Ethical Review Committee of KEMRI. Statistical analysis was performed using IBM SPSS version 21. Comparison of performance of ZN and FM microscopy, GeneXpert was done using data from techniques using homogenous specimens descriptive statistics such as frequency and proportions were done for categorical variables. Mean, standard deviation and ranges were computed for continuous variables. In order to assess performance of the diagnostic tools; sensitivity, specificity, positive and negative predictive values of ZN, LED and GeneXpert for specimens were determined.

Results

A total of 3073 sputum specimens (of 3782 received) from 1891 people with presumptive TB were accepted for analysis using ZN and FM microscopy, GeneXpert and culture. In this paper we report results of six hundred and thirty nine (639) specimens, obtained from 390 patients, each of which had been tested using the three diagnostics and had culture results for MTB *(Figure 1). next page*

Figure 1: *Study Profile for The Enrolled People with Presumptive TB*



Demographic characteristics of all the enrolled females (44.6%) was comparable. Mean age People with presumptive TB are presented in (+SD) of the People with presumptive TB was *Table 1.* The proportion of males (55.4%) and 42 (+ 16) ranging between 18 and 93 year.

Table 1: *Selected Demographic Characteristics of The Enrolled People with Presumptive TB*

Variables	n=390	%
Gender Male	214	55.4
Female	172	44.6
Not documented	4	
Age in years <30		
	113	30.1
30-39	94	25.1
40-49	68	18.1
50 or more	100	26.7
Not documented	15	

Out of 386 people with presumptive TB with known treatment history, 17.4% had a history of previous treatment while 28.7% were found to be HIV positive (Table 2).

Table 2: *History of TB treatment and HIV status*

Variables	Number of People with presumptive TB=390	%
Previous treatment for TB Retreatment	67	17.4
New	319	82.6
Not documented	4	
HIV status Positive	112	28.7
Negative	278	71.3

Key: *TB=Tuberculosis; HIV= Human Immunodeficiency Virus; %=Percentage*

Table 3 indicates sensitivity, specificity, and contrary, specificity of GeneXpert (87.9% predictive values in different TB diagnostic (95%CI: 85.1-90.7)) was significantly lower tools. GeneXpert showed significantly higher than that of ZN (93.5% (95%CI: 91.4-95.6)) sensitivity (83.7% (95%CI: 76.6-90.8)) than and FM (93.3% (95%CI: 91.2-95.4)) ZN (65.4% (95%CI: 56.3-74.5)) and FM microscopy. Combination of FM microscopy and (68.3% (95%CI: 59.4-77.2)) microscopy GeneXpert improved sensitivity of GeneXpert by methods in the diagnosis of TB. On the only 1.9%.

Table 3: Sensitivity, Specificity, and Predictive Values in Different TB Diagnostic Tools

Diagnostic tool	Sensitivity% (95% CI)		Specificity% (95% CI)		ppv	npv	n
ZN	68/104	65.4(56.3-74.5)	500/535	93.5(91.4-95.6)	66.0	93.3	639
FM	71/104	68.3(59.4-77.2)	499/535	93.3(91.2-95.4)	66.4	93.8	639
GXpert	87/104	83.7(76.6-90.8)	470/535	87.9(85.1-90.7)	57.2	96.5	639
FM & GXpert	89/104	85.6(78.9-92.4)	468/535	87.5(84.7-90.3)	57.1	96.9	639

Key: ZN= Ziehl-Neelsen; FM= Fluorescence Microscopy–LED; GXpert=GeneXpert; 95% CI= Confidence Interval; ppv=positive predictive value; npv=negative predictive value; n= Number of specimens.

Table 4: Represents Sensitivity, Specificity,and Predictive Values Of ZN, FM and GeneXpert In Different HIV status. Sensitivity of ZN and FM microscopy was higher in HIV negative (70.8% (95%CI: 60.3-81.3) and 73.6% (95% CI: 63.483.8), respectively) than HIV positive (53.1% (95% CI: 35.8-70.4) and 56.3% (95% CI: 39.1-73.5), respectively) while sensitivity of GeneXpert was not significantly higher in HIV positive (84.4% (95%CI: 71.8-97.0) than HIV negative (83.3% (95%CI: 74.7-91.9)) but was significantly higher in HIV positive than that of ZN and FM microscopy, respectively.

Table 4: Sensitivity, Specificity, and Predictive Values of ZN, FM and GeneXpert According to HIV Status

Diagnostic tool	Sensitivity%(95% CI)		Specificity%(95% CI)		ppv	npv	n
ZN							
HIV positive	17/32	53.1(35.8-70.4)	147/159	92.5(88.4-96.6)	58.6	90.7	191
HIV Negative	51/72	70.8(60.3-81.3)	353/376	93.9(91.5-96.3)	68.9	94.4	448
FM							
HIV positive	18/32	56.3(39.1-73.5)	147/159	92.5(88.4-96.6)	60.0	91.3	191
HIV Negative	53/72	73.6(63.4-83.8)	352/376	93.6(91.1-96.1)	68.8	94.9	448
GXpert							
HIV positive	27/32	84.4(71.8-97.0)	134/159	84.3(78.7-90.0)	51.9	96.4	191
HIV Negative	60/72	83.3(74.7-91.9)	336/376	89.4(86.3-92.5)	60.0	96.6	448

Key: ***ZN*** = Ziehl-Neelsen; ***FM*** = Fluorescence Microscopy–LED; ***GXpert*** = GeneXpert; ***HIV*** = Human Immunodeficiency Virus; ***95%CI*** = Confidence Interval; ***ppv*** = positive predictive value; ***npv*** = negative predictive value; ***n*** = Number of specimens

Table 5 indicates sensitivity, specificity, and predictive values of GeneXpert in smear results (ZN and FM microscopy). A relatively high proportion of specimens (61.1% (95% CI: 45.277.0)) with ZN negative and culture positive results were positive by GeneXpert. Similarly, smear Results.

Table 5: *Sensitivity, Specificity, and predictive values of GeneXpert in Positive and negative smear results*

Diagnostic tool	Sensitivity% (95% CI)		Specificity %(95% CI)		ppv	npv	n
GXpert ZN Positive	65/68	95.6(90.7-100.0)	1/35	2.9(0.0-8.5)	65.7	25.0	103
ZN Negative	22/36	61.1(45.2-77.0)	469/500	93.8(91.7-95.9)	41.5	97.1	536
GXpert FM Positive	69/71	97.2(93.4-100.0)	2/36	5.6(0.0-13.1)	67.0	50.0	107
FM Negative	18/33	54.5(37.5-71.5)	468/499	93.8(91.7-95.9)	36.7	96.9	532

Key: ZN = Ziehl-Neelsen; ***FM*** = Fluorescence Microscopy–LED, ***GXpert*** = GeneXpert,
95%CI= Confidence Interval; ***ppv***= positive predictive value, ***npv***= negative predictive value, ***n***= Number of specimens.

Table 6 indicates sensitivity, specificity, and predictive values of ZN, FM and GeneXpert in different treatment categories. Sensitivity of ZN microscopy, FM microscopy and GeneXpert was higher in non-previously treated with presumptive TB (71.1% (95% CI: 61.4-80.9), 73.5% (95% CI: 64.0-83.0) and 89.2% (95% CI: 82.5-95.9), respectively) than in treatment categories retreatment (42.9% (95% CI: 21.7-64.1), 47.6% (95% CI: 26.2-69.0) and 61.9%
Similarly, specificity and ppv of GeneXpert were lower (82.2% (95% CI: 74.3-90.1) and 44.8%, respectively in retreatment cases than in the new category.

Table 6: *Table 6: Sensitivity, Specificity, and predictive values of ZN, FM and GeneXpert in different treatment categories*

Diagnostic tool	Sensitivity% (95% CI)		Specificity %(95% CI)		ppv	npv	n
ZN							
Retreatment	9/21	42.9(21.7-64.1)	83/90	92.2(86.7-97.7)	56.3	87.4	111
NPT	59/83	71.1(61.4-80.9)	417/445	93.7(91.4-96.0)	67.8	94.6	528
FM							
Retreatment	10/21	47.6(26.2-69.0)	81/90	90.0(83.8-96.2)	52.6	88.0	111
NPT	61/83	73.5(64.0-83.0)	418/445	93.9(91.7-96.1)	69.3	95.0	528
GXpert							
Retreatment	13/21	61.9(41.1-82.7)	74/90	82.2(74.3-90.1)	44.8	90.2	111
NPT	74/83	89.2(82.5-95.9)	396/445	89.0(86.1-91.9)	60.2	97.8	528

****Key: ZN*** = Ziehl-Neelsen; ***FM*** = Fluorescence Microscopy–***LED; GXpert*** =GeneXpert;
95%CI = Confidence Interval; ***ppv*** =positive predictive value; ***npv*** =negative predictive value; ***n*** = Number of specimens.

Analysis of sensitivity, specificity, and predictive values of GeneXpert by smear results and HIV status is presented in Table7. A relatively high proportion of specimens from HIV positive patients 66.7% (95%CI: 42.9-90.6) with ZN negative and culture positive results were positive by GeneXpert. Similarly, 64.3% (95%CI: 39.2-89.4) of specimens from HIV positive patients with FM negative and culture positive results were positive by GeneXpert. Seventeen specimens of 353 (4.8% (95%CI: 2.6-7.0) from HIV negative patients with ZN negative and culture negative results were negative results were positive by GeneXpert. positive by GeneXpert. Fourteen of 147 (9.5% However, the difference was not statistically (95%CI: 4.8-14.2) specimens from HIV significant positive patients with FM negative and culture

Table 7: *Sensitivity, Specificity, and Predictive Values of GenXpert By Smear Results and HIV Status*

Diagnostic tool	Microscopy	Sensitivity% (95%CI)		Specificity %(95%CI)		ppv	npv	n
GXpert HIV Pos.	ZN Pos.	17/17	100.0	1/12	8.3(0.0-23.9)	60.7	100.0	29
HIV Pos.	ZN Neg.	10/15	66.7(42.9- 90.6)	133/147	90.5(85.8-95.2)	41.7	96.4	162
HIV Neg.	ZN Pos.	48/51	94.1(87.6-100)	0/23	0.0	67.6	0.0	74
HIV Neg.	ZN Neg.	12/21	57.1(35.9-78.3)	336/353	95.2(93.0-97.4)	41.4	97.4	374
GXpert HIV Pos.	FM Pos.	18/18	100.0	1/12	8.3(0.0-23.9)	62.1	100.0	30
HIV Pos.	FM Neg.	9/14	64.3(39.2-89.4)	133/147	90.5(85.8-95.2)	39.1	96.4	161
HIV Neg.	FM Pos.	51/53	96.2(91.1-100)	1/24	4.2(0.0-12.2)	68.9	33.3	77
HIV Neg.	FM Neg.	9/19	47.4(25.0-69.9)	335/352	95.2(93.0-97.4)	34.6	97.1	371

Key: ***ZN*** = Ziehl-Neelsen, ***FM*** = Fluorescence Microscopy–*LED,* ***GXpert*** = GeneXpert, ***HIV*** = Human Immunodeficiency Virus, ***95% CI*** = Confidence Interval, ***ppv*** = positive predictive value, ***npv*** = negative predictive value, ***n*** = Number of specimens

Discussion

As per our knowledge, that was the first time in Kenya, to document performance of GeneXpert despite the tool being in use in several health facilities. In that study, sensitivity of GeneXpert (83.7% (95%CI: 76.6-90.8)) was higher than both ZN (65.4% (95%CI: 56.3-74.5)) and FM (68.3% (95%CI: 59.4-77.2)) microscopy for diagnosis of TB in Kenya,

These findings were comparable with those indicated in a few previous studies in Africa. A study in Tanzania indicated sensitivity of (88.4% (95% CI: 78.4%- 94.9). In a multicentre study on the feasibility, diagnostic accuracy, and effectiveness of decentralized use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance, sensitivity of GeneXpert in Uganda was (83·4% (95% CI: 76·6–88·6) while in south Africa it was (86·3% (95% CI: 81·3–90·1) [19].

On the contrary, in our study specificity of GeneXpert was lower (87.9% (95% CI: 85.1-90.7)) than ZN (93.5% (95% CI: 91.4-95.6)) and FM (93.3% (95% CI: 91.2-95.4)) microscopy respectively, also lower than that observed in previous African studies (100·0% (95% CI: 97·4–100·0)) and (99·7% (95% CI: 98·9–99·9) in Uganda and South Africa, respectively and 99.0% (95% CI: 94.7%-100.0) in Tanzania [19, 20].

In 2010, WHO recommended the use of GeneXpert for diagnostics in individuals suspected of having TB, MDR-TB, or HIV associated ailments [21, 22].

However, this recommendation did not exclude the use of conventional microscopy, culture as well as drug susceptibility testing (DST) for diagnostics. The findings are in agreement with that recommendation on the use of conventional microscopy, FM in particular and culture due to high specificity and sensitivity, respectively.

Alternatively, GeneXpert was not recommended for retreatment monitoring as it detects live and dead *bacilli*. Two possible reasons that may have contributed to low specificity in GeneXpert compared to that of microscopy, in our study, were:

1. The presence of nonviable organisms in the specimens due to delayed transportation from the health facilities to the KEMRI laboratory resulting into no viability of the tubercle bacilli in the sputum.
2. The presence of very low bacillary load, especially in HIV positive, in the sputum that may have been lost in the process of decontamination for culture but would still be detected by GeneXpert [23].

Therefore, the fourteen specimens (9.5% of 147) from HIV positive patients with microscopy negative and culture negative results but positive by GeneXpert in our study were likely to be ”true” MTB positive in as was reported in a study in Tanzania.Such patients were confirmed to have clinical TB. However, this aspect requires further elucidation [19].

GeneXpert sensitivity was close to 100% in smear positive culture positive specimens with (95.6% (95% CI: 90.7-100.0)) and (97.2% (95% CI: 93.4-100.0) for ZN and FM, respectively. Only 5 (0.8%) specimens that were smear positive (3 on ZN and 2on FM) and culture positive were missed by GeneXpert.

However, GeneXpert sensitivity was significantly lower in smear negative culture positive specimens with (61.1% (95% CI: 45.2-77.0)) and (54.5% (95% CI: 37.5-71.5)) for ZN and FM, respectively. Despite similar observations made in a study in Tanzania [19], these results are in contrast with findings of a previous multicounty studies which included Uganda and South Africa [20].

Presence of very low bacillary load in sputum specimens, resulting in smear negativity, could have been a potential contributing factor coupled with presence of PCR inhibitors in sputum that degraded the DNA. Studies are needed to ascertain this.

Despite the fact that previously treated people with presumptive TB are among the risk group for developing drug resistance, MDR-TB in particular and therefore recommended potential for GeneXpert testing, our findings indicate marginally lower sensitivity in specimens from people with presumptive TB with a history of previous treatment in all the testes used; (42.9% (95% CI: 21.7-64.1), for ZN, (47.6% (95% CI: 26.2-69.0)) for FM and (61.9% (95% CI: 41.1-82.7)) for GeneXpert than those from non-previously treated with presumptive TB (71.1% (95% CI: 61.4-80.9)) for ZN, (73.5% (95% CI: 64.0-83.0)) for FM and 89.2(82.5-95.9) for GeneXpert. To our knowledge, this was also the

first time to document performance of GeneXpert in previously treated people with presumptive TB using a reasonable number of sputum specimens (111 from this category of patients).

A recent study reported findings of only four patients with successful treatment for TB up to 5 years who presented with respiratory tract infection and were GeneXpert-positive, but had negative TB cultures and clinical improvement without anti-tuberculosis treatment. In their study, hypothesized that the GeneXpert results were false-positive due to the presence of dead MTB *bacilli* in lungs and sputum.

Although this hypothesis is based on a small number of patients, the results are in contrast with our findings in this category of patients. Some of the reasons that may contribute to this discrepancy include the small number of respondents studied as well as the fact that in our study, we did not have detailed clinical information to enable us make conclusive statement on that aspect. Further research is required for appropriate justification. In addition, there was no rifampicin resistance both in this category and in the entire study population [28].

HIV status did not affect the performance of GeneXpert. Inspide, sensitivity of GeneXpert 84.4% (95% CI: 71.8-97.0) was significantly higher in HIV positive than that of ZN 53.1% (95% CI: 35.8-70.4) and FM 56.3% (95% CI: 39.1-73.5) microscopy. Similar observations have been made in a previous study [19]. One of the main advantages of GeneXpert for diagnosis of TB is the shorter turn-around time (TAT) than culture. Despite the shorter period since its introduction, a significant number of articles have been written on the use of this test, especially in low income countries [15 - 27].

A multicenter study conducted in 2013 comparing the use of GeneXpert to microscopy, indicated that using GeneXpert, more patients had same day diagnosis [29] Similar observations were made in this study suggesting that when good quality specimens are used, followed by standard and meticulous specimen processing algorithm, more patients would be diagnosed faster with GeneXpert than microscopy, especially in HIV positive smear negative people with presumptive TB, allowing earlier treatment initiation as well as facilitating prompt and accurate decisions on provision of prophylaxis leading to prevention of monotherapy with isoniazid (INH) in active TB.

This will subsequently curb the chain of transmission, reduce the burden of TB and MDR-TB diseases as we strive towards accomplishing the targets for TB control in the Millennium Development Goal (MDG‖s). These include ―to halt and begin to reverse the spread of TB by 2015‖ as well as achieve the targets for Stop TB partnership including reduction of prevalence and death rates by 50%, when compared with their levels in 1990 by 2015 and to eliminate TB as a public health problem [16]. The main limitation of this study is the lack of linking patient clinical details with respective laboratory data because this study was focused on performance of the diagnostic tools rather than individual patient health outcomes.

Performance of GeneXpert, in terms of sensitivity, is higher than both ZN and FM microscopy for diagnosis of TB in Kenya and is comparable with performance indicated in a few previous studies in Africa. Despite the low sensitivity in smear negative culture positive specimens, GeneXpert has potential to increase diagnostic yield in smear and culture negative specimens, especially from HIV positive people with presumptive TB.

Further studies are required to ascertain its specificity and applicability in specific patient populations. In response to the findings presented here, our ongoing research is assessing the reliability, in terms of reproducibility of these diagnostic tools, GeneXpert in particular, using a larger number of sputum specimens from respective study sites as well as linking patient clinical details with respective laboratory data, an approach which is likely to shed some light on the based best fit algorithm for management of TB in Kenya. Early and increased detection will not only facilitate proper management of TB patients but also appropriateness of the treatment regimen offered on the basis of the diagnostic test result. This data is important for policy change of TB diagnostics as well as surveillance, both in Kenya and regionally.

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