

Evaluation of Diagnostic Accuracy of TB LAM Rapid Urine Antigen Screening Assay, GeneXpert and Smear Microscopy for TB and HIV Co-infected Population in the Guinea Savannah Zone of Nigeria

Article by A. A Ogundeji¹, I Ahmadu², J Awotoye³, J Ogwu⁴, S Laraban⁵, J Ajobiewe⁶, M Iwakun⁷, O.M Akinsola⁸

¹PhD Public Health, Texila American University Guyana

^{2, 3, 4, 5} National Tuberculosis and Leprosy Training Centre, Saye, Zaria, Nigeria

⁶Dept of Medical Microbiology & Parasitology, National Hospital Abuja, Nigeria

⁷Institute of Human Virology, Abuja Nigeria

⁸Dept of Animal Science, Ahmadu Bello University, Zaria, Nigeria

E-mail: damolaogundeji@gmail.com¹, kaydeji@yahoo.com

Abstract

Background: Report at the lunch of 1st Nigeria, National Strategic Plan for TB Control report opined that, at the current rate of transmission and development of Tuberculosis, over four million cases are forecasted to occur in Nigeria between 2015 and 2020 (WHO, 2015). **Objectives:** A study was conducted to examine diagnostic accuracy of GeneXpert, smear microscopy and TB LAM Rapid Urine Antigen Screening Assay for TB and HIV co-infected Population. **Methods:** 323 specimens from approximately 400 patients were enrolled using probability sampling technique. Selection intensity (CD4 count < 100, critical symptoms of TB and HIV) with stringent conditions was set to reduce eligible population for TB-LAM test. Differences between two groups was tested using t-test in SPSS-24 statistical package. Sensitivity, specificity, NPV and PPV were computed using Diagnostic or Screening Test Evaluation 1.0 using OpenEpi (version 2.3). **Results:** There were significant differences ($P < 0.05$) among diagnostic accuracy for sensitivity, specificity, PPV, NPV and prevalence rate. The highest sensitivity was in GeneXpert (86.2%, 25/29) while TB-LAM the least sensitivity (57.5%, 46/80). EMS-AFB was significant ($P < 0.05$) and more sensitive than Spot-AFB and TB-LAM. Specificity was significant ($P = 0.04$) and slightly lower in the group tested with GeneXpert (90.8%) as compared to TB-LAM (100%), EMS-AFB (93.1%) and Spot-AFB (92.7%). The proportion of total study population with TB-LAM decreased linearly (23.2% - 7.4%) as the grades increases. **Conclusion:** This clearly suggest that TB-LAM might not be a robust testing for point of care diagnostics among TB-HIV co-infected Population using Zaria Metropolis of North Western, Nigeria.

Keywords: GeneXpert, Lipoarabinomannan, Tuberculosis, Human Immunodeficiency Virus, Sensitivity.

Acronyms

EMS	Early Morning Urine Sample
GeneXpert	Xpert
HIV	Human Immunodeficiency Virus
LAM	Lipoarabinomannan
LTBI	Latent Tuberculosis Infection
NPV	Negative Prevalence Value
PPV	Positive Prevalence Value
SPSS	Statistical Package for Social Sciences
TB	Tuberculosis
TN	True Negative
TP	True Positive
WHO	World Health Organization

Introduction

Tuberculosis (TB) ranks among the most fatal infectious diseases in the world. The major distinction between active tuberculosis and latent tuberculosis infection (LTBI) is changing at the moment, tuberculosis infection is described as a continuum with different *stages* of infection. Nigeria was rated the third (3rd) highest Tuberculosis (TB)-burden country in the world and number one (1st) in Africa, according to the World Health Organization (WHO, 2015). Given the current 16 per cent TB cases notification rate in the latest Global survey report, the country's TB treatment gap has also become the highest, accounting for 15 per cent of the global gap. Report from WHO (2015) at the lunch of 1st Nigeria, National Strategic Plan for TB Control opined that, at the current rate of transmission and development of Tuberculosis, over four million cases are forecasted to occur in Nigeria between 2015 and the end of 2020. According to the Global TB report, an approximated one million of the over four million cases would be co-infected with HIV, while 200,000 was expected to have multi-drug resistant TB.

In the majority of disease-endemic countries, sputum microscopy, a method introduced over a century ago, is still the first line diagnostic test for TB. In adult pulmonary TB without immune suppression it has reasonable sensitivity compared to culture (Dye *et al.*, 2013). However, for detection of TB in children, TB/HIV co-infected patients and extra-pulmonary TB (EPTB) sensitivity dropped to unacceptable levels (Dye and Williams, 2008). In HIV co-infection, reduced smear microscopy sensitivity is the result of a failed inflammatory response with consequent reduced caseating necrosis and lower numbers of acid-fast bacilli in the airway. Another diagnostic problem in severely ill TB/HIV co-infected patients is that they are often unable to self-expectorate sputum or have extra-pulmonary disease. Acquiring a specimen for TB diagnosis in these patients therefore requires assisted sputum sampling e.g. via hypertonic saline induction or alternative invasive sampling (Corbert *et al.*, 2006). In many TB/HIV endemic countries, capacity for assisted and invasive sampling is limited or unavailable. Furthermore, laboratory capacity for culture-based diagnosis is limited. Thus, especially in settings with dual epidemics of HIV and TB, the lack of accurate and rapid diagnostic TB testing is a major barrier to achieving global TB control.

The development of the GeneXpert MTB/RIF assay, a nucleic acid amplification test (NAAT) able to detect the presence of both *Mycobacterium tuberculosis* (*M.tb*) complex DNA and rifampicin drug resistance (associated strongly with MDR-TB) in less than 2 hours, provided a major advance in TB diagnosis. The WHO endorsed the use of the Xpert MTB/RIF assay for frontline TB diagnosis in HIV-infected and MDR-TB suspects in December 2010 (Theron *et al.*, 2014). When compared to smear microscopy the Xpert MTB/RIF assay provides clear clinical benefits for the early diagnosis of suspected pulmonary TB cases (Wells *et al.*, 2007). The Xpert MTB/RIF assay requires sputum for testing and has reduced performance in HIV-infected compared to uninfected patients and on extra-pulmonary compared to sputum. The use of inappropriate/ineffective/conventional diagnostic methods and delay in commencement of TB therapy has led to patient poor health seeking behavior, this brought about the need for this study in order to evaluate accuracy of rapid tuberculosis diagnostic tests that can be performed at point-of-care and as well identify factors responsible for the delay in treatment of (pulmonary) TB samples (Wells *et al.*, 2007). The Xpert MTB/RIF assay is not a true point-of care test because it is largely electricity dependent which is currently not readily available in most of Sub-Saharan African countries with highest TB burden. TB culture, takes up to 6 weeks to provide results, and requires considerable laboratory infrastructure (Davies *et al.*, 1999). The use of urine, rather than sputum, for TB diagnostic testing provides an appealing alternative. Collection is easy and less invasive, it is readily available, and its collection presents a very low infection risk to staff. In fact, urine antigen detection is the most common diagnostic technique employed for a number of infectious diseases. Lipoarabinomannan (LAM) is the most extensively evaluated, promising and is commercially available as a bedside test (Moodley *et al.*, 2011). The use of inappropriate or ineffective diagnostic methods has brought about the need for this study in order to evaluate accuracy of rapid tuberculosis diagnostic tests that can be performed at point-of-care. The general objective of this study is to Evaluate Diagnostic Accuracy of TB LAM Rapid Urine Antigen Screening Assay for TB Among HIV-Infected Population using Zaria Metropolis of North Western, Nigeria as a case study.

Methods

Study area

The study was conducted in the National Tuberculosis and Leprosy Training Centre (NTBLTC), Zaria, Kaduna State, Nigeria to Diagnostic Accuracy in TB LAM Rapid Urine Antigen Screening Among HIV-Infected Patients in Zaria, Kaduna State.

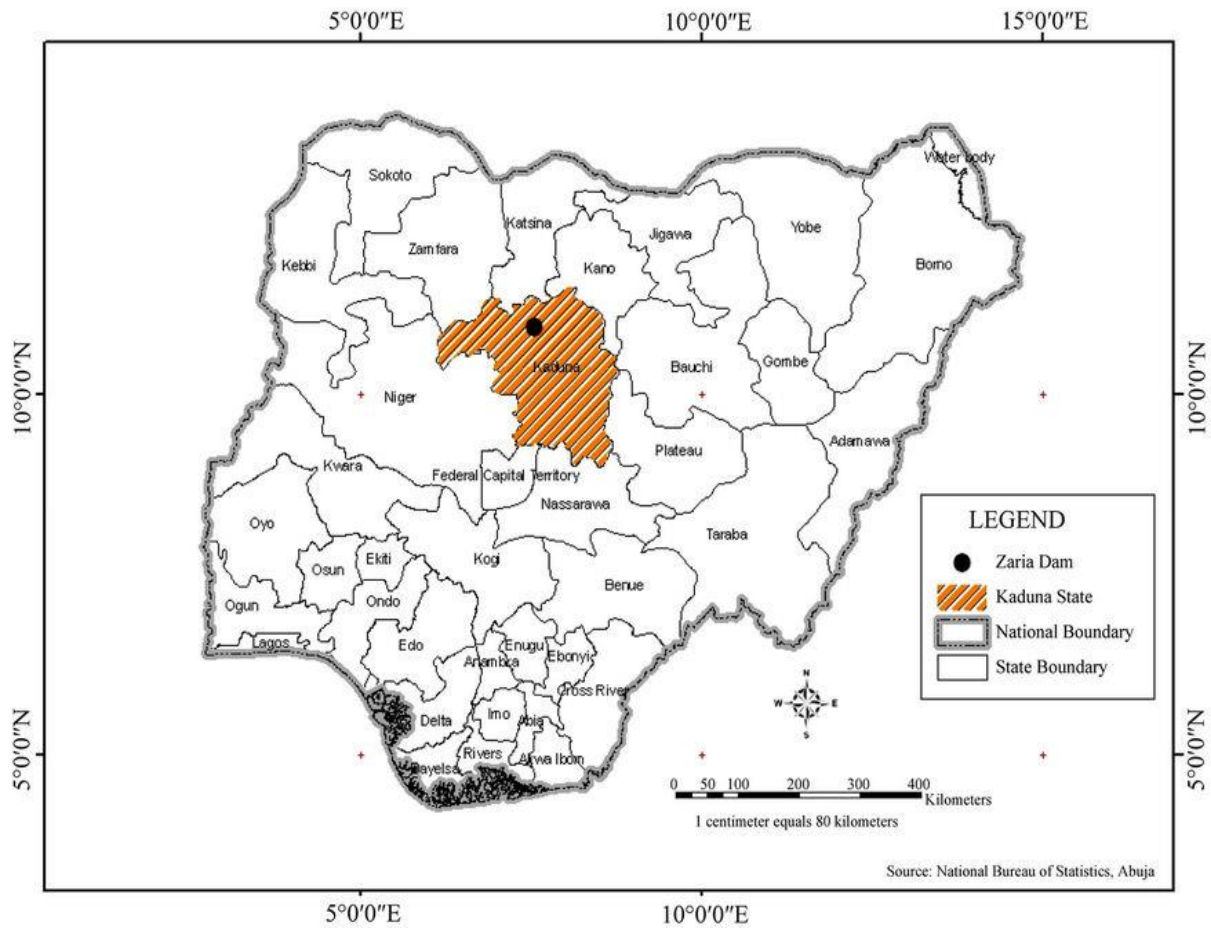


Figure 1. Map of nigeria showing kaduna state

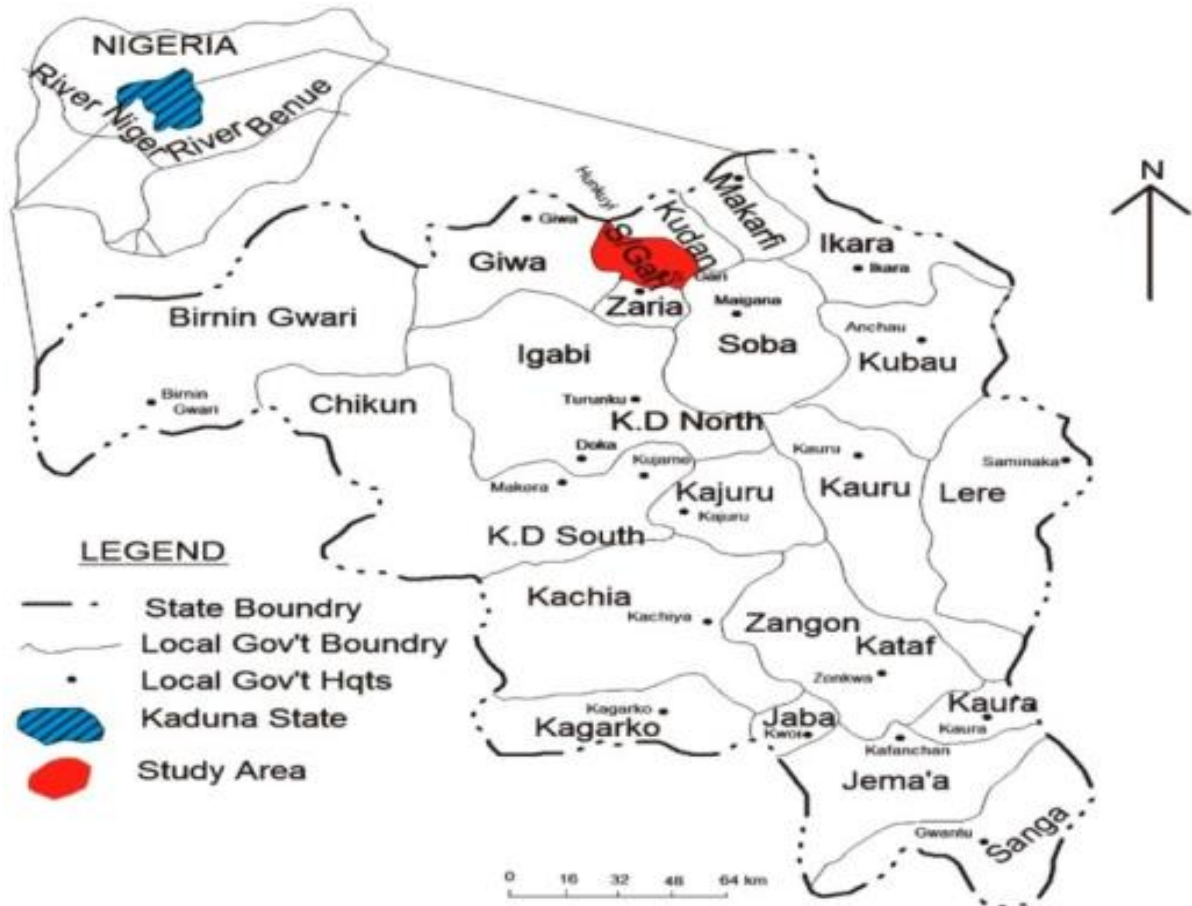


Figure 2. Map of kaduna state showing zaria, the study area

The NTBLTC Zaria, Kaduna State, Nigeria was established about twenty-five years ago, precisely in 1991. It has three mandates, patient care, training and research on TB and Leprosy. It is not a surprise that the mandate has been extended consciously to cover for HIV/AIDS. Structurally, the institution has recently been upgraded with enough facilities to handle international trainings on TB/HIV/AIDS. There are recently built clinics, laboratories, training classrooms, conference hall, library, hostel- they are designed to meet the standard of infection control in the facility. The centre also has a 24 hour wireless internet facility and a website to meet the challenges of international courses. The hospital wing of the centre has 140 beds, digitalized X-ray Unit, and a serology laboratory facility which complement the training unit of the centre in conducting courses on STOP TB Strategy, HIV care and support. Moreover, the installation of the BL3 containment laboratory for culture and DST couple with a PCR facility enhances the centre to conduct routine surveillance, researches and diagnosis of MDR/XDR TB. Furthermore, the center as the foremost National reference laboratory enable it to conduct training courses on drug resistant tuberculosis locally and internationally.

Sampling technique

HIV-infected patients with clinically suspected TB were selected and enroll for this study. Probability multistage sampling technique were employed to ensure that every element in a sample frame has an equal chance of being incorporated into the sample. A study of consecutive HIV-infected patients referred to the clinic in Zaria and its environs for ART. Patients were recruited prospectively and investigated at their (first) visit to the clinic. Patients were eligible for the study if they were at least 18 years of age (or above) and had no current tuberculosis diagnosis.

Procedures for identification of participants

Potential participants were identified by health care workers at the TB diagnostic centre. All subjects who presented to any area of the clinic with symptoms suggestive of pulmonary TB (cough \pm fever/night

sweats/weight loss) were given basic information about the trial and referred to the study nurse, who was situated at the clinic and who worked alongside the clinic TB nurses. The study enrolled participants between the hours of 08.00am and 04.30pm on weekdays (Monday to Friday). The areas where subjects were identified was TB clinic. A cough triage system was in operation throughout the study period, whereby all PHC attendees were asked about the presence of cough on arrival at the clinic. Individuals reporting cough were directed towards the TB area within the clinic. Throughout the study period, all HIV-infected adults attending for treatment or care were also screened for TB symptoms at every clinic visit in accordance with national guidelines (WHO, 2015).

Sample size determination

The sample size was calculated by using the following formula: =

$$n = \frac{z^2 p (1-p)}{\epsilon^2}$$

Whereby z = level of confidence (1.96 for 95% confidence level)

p = expected proportion of patient with TB and HIV coinfection, in this case was unknown therefore p was taken as 100 -95=5%= 5/100=0.05 ϵ = margin of error equal 0.06

$$\frac{1.96 \times 1.96 \times 0.05 (1 - 0.05)}{0.06 \times 0.06} = 267$$

Therefore,

In order to minimize design effect in multistage random sampling the sample size was multiplied by 1.5 to get 400. Therefore, additional three random samples were added to minimize the effect of missing data. So, the total sample size considered in this study was 403.

Baseline analysis

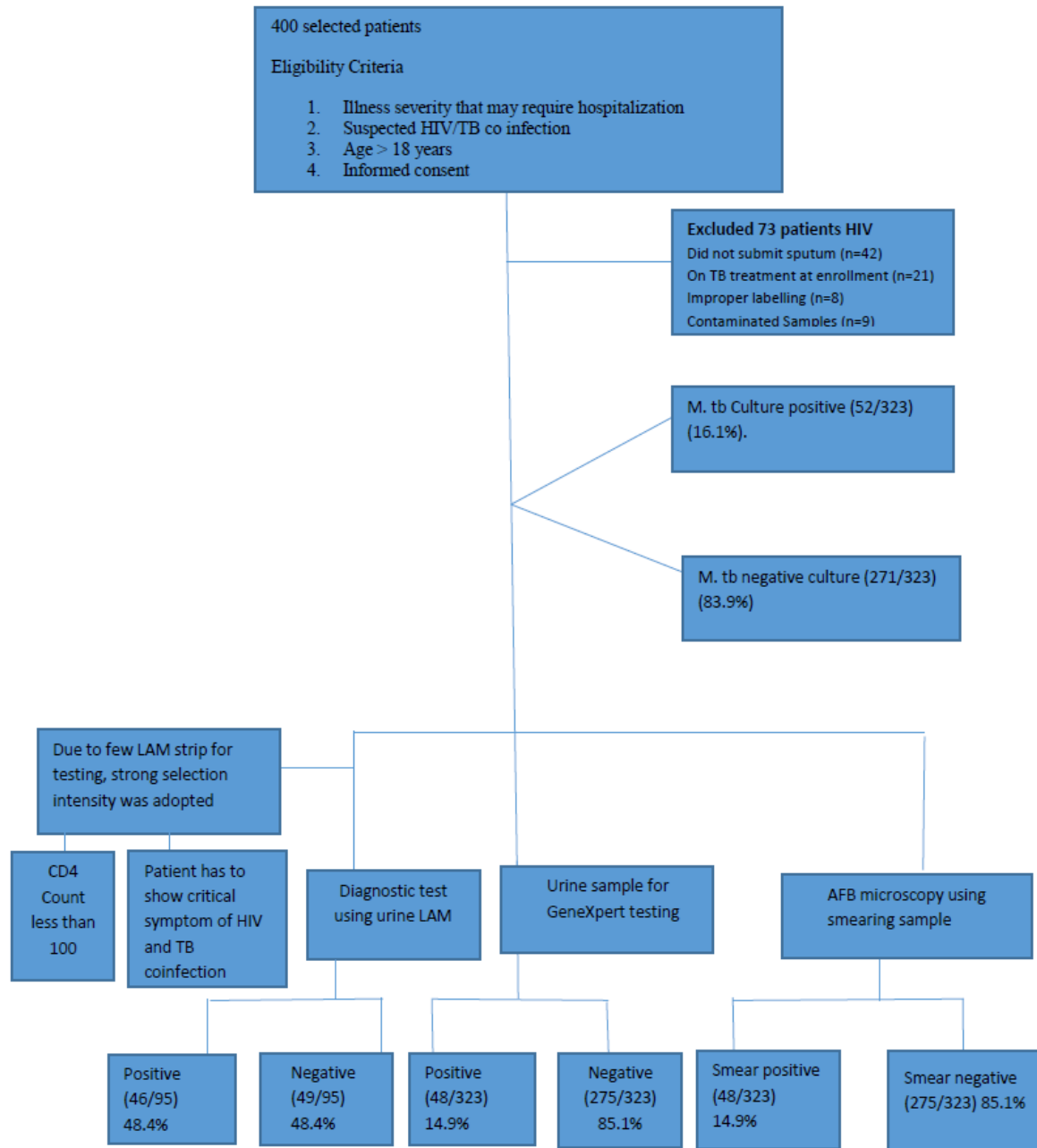
Comparison of baseline characteristics was performed to characterize the study population and to identify baseline imbalances occurring due to chance. No test of statistical significance was performed and confidence intervals and p values were not reported. The purpose of the comparison was to determine whether any baseline covariates needed to be adjusted for in the final analyses. The baseline analysis was performed for all participants and then separately for culture positive cases eligible for the primary outcome analysis.

Selection method

The participants were selected by using two levels of sampling (multistage sampling). Under this technique an exclusion criterion to remove outliers, for example improper labelling, patients that do not submit sputum and those on TB treatments which reduced the population of the study to three hundred and twenty three (323). The sample was further selected into *M.tb* culture (52) positive and negative (271). At the last stage, the sample was further spread to AFB microscopy (n=323) and GeneXpert (n=323) but due to few strips available for testing LAM antigen, a strong selection method was adopted (patients with CD4 count less than 100 which gave us 95 samples). Therefore the total number of 400 participants was involved in the study. Selection method ensured that all participants had an equal chance of being included in the research.

Flow chart for multistage random sampling

323 specimens from approximately 403 patients were enrolled in this study.



Study population

The study population comprised of adult TB suspects (n=400) of eighteen years and above. Different medical examinations were done by the hospital physicians including observation of complex symptoms suggestive of TB such as fever, cough of more than two weeks and not responding to antibiotic treatment, night sweats and weight loss. The diagnostic facility in TB centre in Zaria year was selected because it house the National TB reference laboratory and management care centre. Subjects were recruited from newly diagnosed TB suspects, voluntary counseling and testing (VCT) clinics (Normal subjects controls) and patients not on treatment. New TB patients are those patients who have not taken any anti-TB drugs before. Different Medical examinations were performed by the hospital clinicians including observation of complex symptoms suggestive of tuberculosis. Only individuals that were HIV-infected and/or had a high risk of drug-resistant TB were included, due to their high risk of mortality and prioritization for Xpert MTB/RIF testing, according to WHO recommendations (WHO, 2015).

Selection criteria

Inclusion criteria

- Adult (≥ 18 years old)
- Current pulmonary TB symptoms (defined as cough of any duration)
- Confirmed HIV infection and/or high risk for drug-resistant TB (adapted from WHO guidelines for programmatic management of MDR-TB)
- Smear non-conversion at month 2 or 3 of standard HIV treatment regimen or retreatment regimen
- Household exposure to known MDR/XDR-TB case
- Health care workers
- Prison inmate in previous 12 months
- Severely unwell requiring immediate admission to hospital
- Willing to give inform consent
- **Exclusion criteria:**
- Previous MDR/XDR-TB diagnosis or treatment (documented or self-reported)
- Suspicion of extra-pulmonary TB only (absence of cough)
- Unable to give informed consent
- Failure of standard treatment or retreatment regimen
- Relapse or return after default
- Any other previous TB treatment (At any time point)
- HIV negative patient

Informed consent

The nurse/research assistant used in this study checked the eligibility criteria and completed the form entitled 'Eligibility criteria. Subjects that met the eligibility criteria were provided information (verbal and written) about the study in English and those that were willing to participate were taken through the informed consent process and were asked to sign the consent form. Agreement of participants who were illiterate was indicated by thumbprint on the consent form and a literate witness signed on their behalf.

Sputum specimen collection

Spontaneously expectorated sputum specimens were collected at the study clinic (two spot specimens per patient). Sputum collection occurred outside. Each sputum specimen was collected into a sterile, wide-mouthed specimen container with a tightly fitting screw top. Simple instructions on how to submit a good quality sputum specimen were given to each participant with the aid of a pictorial card with instructions in English, based on instructions shown previously to increase case detection. Every effort was taken to prevent contamination of the exterior of the specimen container. Each specimen container was placed into an individual disposable watertight sealed plastic bag. The nurse/research assistant instructed participants to wait for 50 minute between producing the first and the second sputum specimen. The first sputum specimen was used for Xpert MTB/RIF testing and the second specimen for culture, line probe assay (LPA) \pm 100 drug susceptibility testing (DST).

Sputum specimen testing

GeneXpert

A four-module GeneXpert machine with desktop computer was installed for each two-week time period in the TB diagnostic laboratory according to the randomization schedule. Xpert MTB/RIF testing followed the manufacturer's instructions. Xpert version G3 cartridges were used. The change in cartridges was based on what was supplied by the distributor at the time of cartridge restocking. Xpert MTB/RIF testing was performed by a laboratory technician. Under the laboratory strategy, participants were requested to return to the clinic for results after three working days, based on the typical turnaround time for receipt of smear microscopy results at the clinic prior to the study. For point-of-care clusters, Xpert MTB/RIF was performed on site by the trained study nurse in a dedicated room. N95 respirator

masks were used and there was a biosafety cabinet. Under the point-of-care strategy, participants were advised to wait for their result the same day. If they were unable or unwilling to wait at the clinic, they were advised to return the following day or any subsequent day.

The Xpert MTB/RIF assay can generate three types of results other than positive and negative: error, invalid and no result. These results are associated with different problems with the sample and with the assay. In the event of any of these results, and if sufficient sample/buffer mix remained, a repeat Xpert MTB/RIF test was performed. If insufficient sample remained or if there was still no definitive result, a second sputum specimen was collected at the earliest convenience (usually when the patient collected the initial result). Second sputum specimens were tested under whichever strategy was operating at the time that the second specimen was collected. A repeat Xpert test was also recommended in the protocol in the event of a result demonstrating the presence of rifampicin-resistant *M. tuberculosis*. The repeat test could be performed with remaining sample/buffer mix from the original specimen or with a fresh specimen collected when the participant collected their result. This was included because of the concern about suboptimal specificity and the potential for false-positive rifampicin resistance results at the time of study design, so as to gather data on the reliability of repeat Xpert tests in differentiating true positive and false positive results.

Mycobacterial growth indicator tubes (MGIT) were inoculated and incubated for up to 6 weeks. Identification of *M. tuberculosis* was confirmed from all positive cultures using niacin and nitrate and/or Rapid MPT64 antigen assay (Manufactured by Standard Diagnostics, Inc. (SD), Yongin, Korea). The Genotype MTBDRplus assay (Hain Lifescience, Nehren, Germany) was performed on culture positive isolates to identify mutations associated with rifampicin and isoniazid resistance. Phenotypic DST for key first-line and second-line drugs (rifampicin, isoniazid, ofloxacin, and kanamycin) was performed using the 1% proportion method on Middlebrook 7H10 agar plates, only for isolates with rifampicin and/or 103 isoniazid resistance on LPA. Standard drug concentrations were used: 1 µg/ml rifampicin, 0.2 µg/ml isoniazid, 5 µg/ml kanamycin and 2 µg/ml Ofloxacin.

HIV Testing

All participants with unknown or negative HIV status were counselled and offered rapid HIV testing prior to enrolment. This mirrored routine clinical practice at the clinic, where all TB suspects are offered HIV counselling and testing before sputum collection. All HIV-infected participants were referred for CD4+ T-cell count and HIV care and treatment as per routine clinic procedures.

Urine sampling and LF-LAM methodology

All patients were required to give a random urine sample (30ml) collected in a sterile container at enrolment. Urine collected the following day, first void urine of the day between 05:00 am and 07:00 am. The LF-LAM was done on matched frozen-thawed spot and urine specimens. Sputum-based reference testing was performed in parallel. All samples were tested using the Alere Determine®-TB LAM Ag lateral flow assay (Alere, USA). Briefly, 60µl of urine pipetted onto the lateral flow strip-loading bay (pipettes provided with the strips). After 25-35 minutes, two independent readers blinded to the reference test results read the LF-LAM.

This was done via the following procedure: test validity was confirmed by identifying the presence of a band in the positive control window; the intensity of the colour band (if any) in the patient window was read by comparison with the pre-January 2014 manufacturer provided visual reference scale card (graded 0–5 depending on band intensity). Using the manufacturer-recommended grade 2 cut-point, a band of visual intensity \geq grade 2 in the patient window was classified as a ‘positive’ test while the complete absence of a band (grade 0) and faint band (grade 1) in the patient window was classified as a ‘negative’ test. The test reported as invalid if no control, either band identified in the patient window or if a broken / incomplete band seen in the patient window. Invalid tests repeated once, but thereafter LF-LAM was considered to have failed. Discrepancies found between two readers in binary readout of positive or negative were resolved by the third reader.

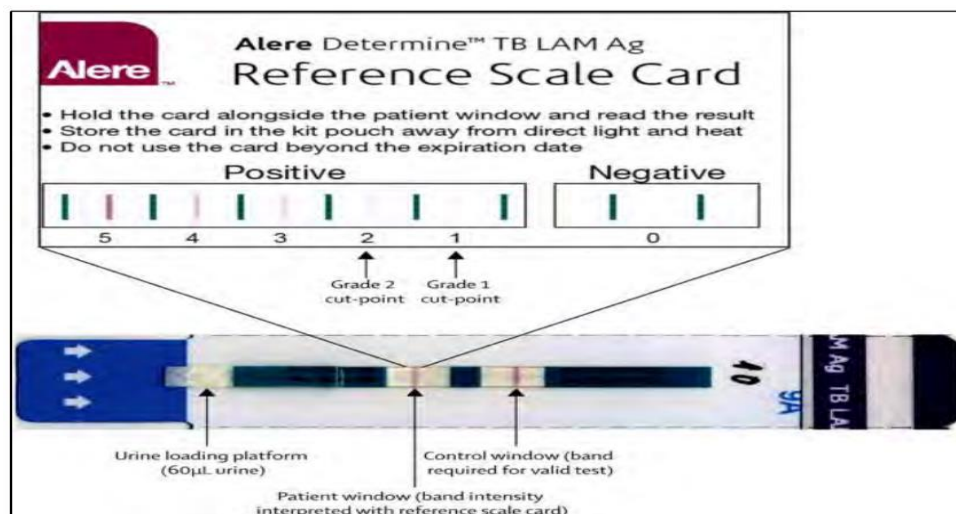
Data collection

The quality of data was modified starting from the time of questionnaires preparations. The questionnaires were developed by reviewing relevant literatures on the subject to ensure reliability. Every day the questionnaires was viewed and checked for completeness and its relevance by principal investigator. The necessary feedback was given every morning before the actual procedure and analysis was started by using proper method for variables under study. An extensive quality control procedure was implemented in each collection site and laboratory to reduce the possibility of false-positive results, including from cross-contamination.

Most data on TB history, TB contact history, HIV status and presenting clinical features were collected prospectively on a standard Case Report Form (CRF). The integrity of this whole study dependent immensely on the way in which data was collected. A lot of effort and quality control were placed into every stage of data collection right from the clinics to the laboratory where most of the diagnostic work was done. The study involved urine samples as specimens for research. Patient recruitment and clinical data collection were done by consultant-led groups of attending clinicians, with no association to the study team. This helped in the reduction of errors emanating from biases. Research nurses who helped in the collection of data were well trained. All the data collected at the recruitment sites were carefully recorded in a spreadsheet. Also, well-designed forms were used to immediately record results and all the data from the laboratory diagnosis and was recorded and stored in a Microsoft Excel spreadsheet. Two senior scientists were involved in the analysis of the results especially the LAM strip test procedures. All this was done to ensure that the quality control of data was maintained and followed.

Grading of LAM strip results

The colour intensity of a bar in the patient window was graded 0-5 using a scale reference card. The manufacturer recommends a grade-1 cut-point, which means that the appearance of any complete band (grade 1-5) is interpreted as “test positive” while the complete absence of a band is interpreted as “test negative”. A broken or incomplete bar in the patient window, irrespective of band intensity, is interpreted as a “test indefinite” result. The use of the alternative grade-2 cut-point suggested by Peter *et al.*, (2012) means that the appearance of only a grade 2-5 band is interpreted as “test positive”, while both the complete absence of a band and a grade-1 band is interpreted as “test negative” (Peter *et al.*, 2012).



Statistical analysis

A complete case analysis was conducted (i.e. only including cases with post-baseline follow-up). Incomplete cases were described and their baseline characteristics were compared with those of the complete cases to determine the extent of any difference. Frequency tables were produced for all categorical baseline characteristics. Accuracy of diagnostic tests (sensitivity, specificity, positive and negative predictive values) was compared using a binary marginal generalized linear regression model

(GLM) for sample analysis. The GLM is very flexible, allows for the inclusion of covariates and accounts for the fact that results of multiple samples from the same patient or test results of different tests on the same sample may be dependent. The odds ratio was reported with 95% confidence intervals and a p value from the Wald test. Where a major imbalance existed in any of the baseline individual-level covariates and the covariate could plausibly influence the outcome, a supplementary analysis was performed for the effects of the individual-level covariates. This was considered supportive to the primary analysis. The starting point for the calculation of the sensitivity and specificity was the construction of a 2x2 table with the index test results on one side of the table and the reference standard (culture)

This was calculated using the 2x2 table Sensitivity = TP/ (TP+FN) Specificity = TN/ (TN+FP) Positive Predictive Value (PPV) = TP/ (TP+FP) Negative Predictive Value (NPV) = TN/ (TN+FN)

Key; **TP**-True positive, **FN**-False Negative, **TN**-True Negative, **FP**-False Positive, all calculations were done using a statistical software called Diagnostic or Screening Test Evaluation 1.0 using OpenEpi (Open Source Epidemiologic Statistics for Public Health, Version 2.3).

Ethics approval and trial registration

The trial was approved by the Ethics Committee of the National Tuberculosis and Leprosy Training Centre (NTBLCTC) located at *Saye* village, Zaria and Research Ethical Committee of Ministry of Health and Human Services, Kaduna State. Written informed consent was obtained from all patients at enrollment. Before collecting sample the study participants were informed about the purpose, merit and demerit of the study in local languages, written informed consent was obtained from all participants. In addition the confidentiality was kept. Any participants who are not volunteers were not enforced to be included as study subject.

Results

Table 1. Diagnostic accuracy of Spot AFB, EMS AFB, Gene expert MTB and TBLAM among TB patients

Diagnostic tools	Sensitivity	Specificity	PPV	NPV	Prevalence
Spot AFB	79.4% (223/249)	92.7% (268/289)	56.3% (27/48)	95.5% (268/275)	10.5% (34/323)
EMS AFB	84.8% (28/33)	93.1% (270/290)	58.3% (28/48)	98.2% (270/275)	10.2% (33/323)
GeneXpert MTB	86.2% (25/29)	90.8% (267/294)	48.1% (25/52)	98.5% (267/271)	9.0% (29/323)
TBLAM	57.5% (46/80)	100% (15/15)	100% (15/15)	30.6% (15/49)	15.8% (15/95)
P value	0.002**	0.04*	0.05*	0.01**	0.001**

**p<0.01-Highly significant; *p<0.05- Significant

Table 1: showed the diagnostic accuracy of Spot AFB, EMS AFB, Gene expert MTB and TBLAM among TB patients. There were significant differences (P<0.05) among the diagnostic accuracy for sensitivity, specificity, PPV, NPV and prevalence rate. The highest sensitivity was recorded in

GeneXpert (86.2%, 25/29) while TBLAM recorded the least sensitivity (57.5, 46/80). EMS AFB was significant ($P < 0.05$) and more sensitive than Spot AFB and TB LAM. Specificity was significantly ($P = 0.04$) and slightly lower in the group tested with Gene expert (90.8%) as compared to TB LAM (100%), EMS AFB (93.1%) and spot AFB (92.7%). TBLAM had the highest accuracy (100%) for PPV while GeneXpert had the least accuracy (48.1%). The PPV for spot AFB and EMS AFB were comparable (56.3% and 58.3%). The NPV had high accuracy for Spot AFB, EMS AFB, Gene expert MTB with the exception of TBLAM (30.6%). The prevalence ranged from 9.0% in GeneXpert through 15.8% in TBLAM. Therefore, the null hypotheses was rejected while the alternate hypothesis was accepted.

Table 2. Accuracy of the Determine TB LAM Antigen Assay by band Intensity threshold TB-LAM Lateral Flow Band Intensity Threshold Considered 'Positive'

	≥ grade 1	≥ grade 2	≥ grade 3	≥ grade 4	≥ grade 5	P value
Proportion of total study population with TB LAM result (%)	23.2	20.0	18.9	17.9	7.4	
Sensitivity %	77.8 (21/27)	63 (17/27)	59.3 (16/27)	55.6 (15/27)	14.8 (4/27)	0.001**
Specificity %	98.5 (67/68)	97.1 (66/68)	97.1 (66/68)	97.1 (66/68)	95.6 (65/68)	0.07
PPV %	91.8 (67/73)	86.8 (66/76)	85.7 (66/77)	84.6 (66/78)	73.9 (65/88)	0.03*
NPV %	95.5 (21/22)	89.5 (17/19)	88.9 (16/18)	88.2 (15/17)	57.1 (4/7)	0.01**

** $p < 0.01$ -Highly significant; * $p < 0.05$ - Significant; Abbreviations: PPV, predictive value of a positive test; NPV, predictive value of a negative test

Table 2: shows the accuracy of determine TB LAM Antigen Assay by band Intensity threshold. There were significant differences ($P < 0.05$) across different grades for sensitivity, PPV and NPV with the exception of specificity ($P = 0.07$). The proportion of total study population with TB LAM decreased linearly (23.2% - 7.4%) as the grades increases. Grade 1 recorded the highest sensitivity (77.8%, 21/27), specificity (63%, 17/27), PPV (91.8%, 67/68) and NPV (95.5, 21/22) while ≥ grade 5 had the least sensitivity (14.8%, 4/27), specificity (95.6%, 65/68), PPV (73.9%, 65/88) and NPV (57.1, 4/7).

Discussion

The highest sensitivity for GeneXpert (86.4%) in this study was similar the reports of some authors (Bowles *et al.*, 2011). In 27 prospective studies utilizing fresh collected sputum samples and where full results were presented, the pooled sensitivity of a single Xpert compared to a reference standard of *M. tuberculosis* culture (calculated as a random effect weighted proportion) was 87% (95% CI 86- 88) and specificity was 98% (95% CI 98-98) (Bowles *et al.*, 2011). There was substantial heterogeneity in sensitivity between studies, partly reflecting the different study populations. This implies that the diagnostic system can deliver acceptable performance in rural setting not only under normal laboratory conditions but also within a primary health care clinic when operated by a nurse. The sensitivity of spot and EMS AFB of 79.4 and 84.8% in this study was lower than the sensitivity recorded in GeneXpert could linked to the fact that sputum smear microscopy are reduced in HIV-infected individuals. There is some evidence from these studies of reduced sensitivity for detection of *M. tuberculosis* in HIV infection (Theron *et al.*, 2011), although two studies showed no difference in sensitivity and one study amongst hospital inpatients in Zambia demonstrated a modest increase in sensitivity (Ograndy *et al.*, 2012) amongst HIV-infected participants (Ograndy *et al.*, 2012). In a study by Davis *et al.* (2013), two sputum specimens collected on the first day had similar sensitivity to the standard 'spot-morning-spot' approach (56.4% of culture-positives vs. 57.7%, $p > 0.5$) which was closer to the trend observed in this study. The sensitivity using standard light microscopy (with culture as the reference standard) was 55%

from the single specimen and 56% from the two specimens was lower as compared to the estimates recorded in this study. Accuracy of TBLAM in this study was 57.5% which was lower as compared to the research of some authors. The low accuracy in this study could be connected to the low positive samples detected by TBLAM due to low sample size because of scarcity of TBLAM kits. Lawn *et al.* (2012) reported low sensitivity for TBLAM using urine sample in TB patient that are not coinfecting with HIV. Specificity, NPV and PPV was high and similar with the value of above 90 % reported in the literatures (Ograndy *et al.*, 2012). This suggests high repeatability of the diagnostic methods used in this study. Increase in the grading system of TBLAM resulted to a corresponding decrease in sensitivity, specificity, PPV and NPV, respectively. An unresolved issue is that of the optimal band positivity threshold, and our findings differ from those of Lawn *et al.* (2012) who reported high specificity using a grade 1 positivity threshold. These could be linked to batch-to-batch variation in TB LAM test strips, differences in visual interpretation of faint bands and/ high bacterial contamination of urine samples causing weak false-positive bands.

Conclusions

TB LAM has the propensity for diagnostic Accuracy (57.5, 46/80) of screening Assay for TB among HIV-Infected Population though GeneXpert was the best (86.2%, 25/29) for point of care diagnostic and on the spot diagnosis of tuberculosis.

Acknowledgement

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