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TB-LAMP PILOT STUDY REPORT, NIGERIA

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EVALUATION OF TUBERCULOSIS LOOP -MEDIATED ISOTHERMAL AMPLIFICATION (TB -LAMP) ASSAY FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS IN NIGERIA

STUDY REPORT

May 2021

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LIST OF ACRONYMS

AFB	Acid-fast bacilli
DNA	Deoxyribonucleic acid
DST	Drug susceptibility testing
HIV	Human Immunodeficiency virus
IHVN	Institute of Human Virology Nigeria
LGA	Local government areas
MTB	Mycobacterium tuberculosis
NHREC	National Health Ethics Research Committee
NRL	National Reference laboratory
NTBLTC	National TB and Leprosy Training Centre
NTBLCP	National Tuberculosis, Leprosy and Buruli Ulcer Control Programme
PTB	Pulmonary Tuberculosis
PLHIV	People living with Human Immunodeficiency virus
RIF	Resistance to rifampicin
RR	Rifampicin resistant Tuberculosis
SDG-3	Sustainable Development Goal 3
-ve/+ve	Negative/Positive
SMC	Survey Management Committee
TB	Tuberculosis
TB-LAMP	Loop-mediated isothermal amplification
TB-LON	Tuberculosis Local Organizations Network (TB-LON)
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

Nigeria's estimated burden of TB ranks sixth in the world and first in Africa but, most of the people with the disease are undiagnosed. To narrow this detection gap is a top priority for NTBLCP and its partners. Smear microscopy with its attendant low sensitivity for PTB diagnosis is still used widely in Nigeria and other resource-limited countries because of the challenges associated with Xpert MTB/RIF assay. For these settings, therefore, the WHO has recommended TB-LAMP as a replacement for smear microscopy for PTB diagnosis in adults. Evidence supporting this recommendation showed a wide variation in quality and diagnostic accuracy of TB-LAMP thus, the need to validate the assay in Nigeria before its deployment to the field. As part of the USAID TB LON Regions 1 and 2 Project, KNCV TB Foundation Nigeria in a pilot study determined the accuracy of TB LAMP for PTB diagnosis and compared it with that of smear microscopy and Xpert MTB/RIF, in Nigeria.

It was a cross-sectional study of 2636 consenting eligible adult presumptive TB from five health facilities offering TB services in Nasarawa and Anambra States of Nigeria. Two "spot" sputum specimens from each participant were analysed for PTB using TB-LAMP, smear microscopy, Xpert MTB/RIF, and solid culture (reference standard). The sensitivity and specificity of TB-LAMP, Xpert, smear microscopy for PTB diagnosis were determined based on the standard test.

The MTB positivity rates of TB-LAMP, smear microscopy, Xpert MTB/RIF, and solid culture were 4.4%, 2.8%, 5.0%, and 4.9% respectively. The sensitivity and specificity of TB-LAMP for PTB diagnosis among all participants were 76.7% and 99.3% respectively. The TB-LAMP's diagnostic accuracy for PTB was higher compared to smear microscopy (sensitivity = 54.3%, specificity = 99.8%), but has a slightly lower sensitivity compared to Xpert MTB/RIF (sensitivity = 84.5%, specificity = 99.1%). For the HIV-seropositive participants, the diagnostic accuracy of TB-LAMP (sensitivity = 60.0%, specificity = 99.6%) did not differ significantly from that of smear

microscopy (sensitivity = 53.3%, specificity = 99.7%), and Xpert MTB/RIF (sensitivity = 66.7%, specificity = 99.6%), $p > 0.05$.

Based on the study results, TB-LAMP can be deployed and used in Nigeria as a reliable PTB diagnostic test in adults especially in areas where Xpert MTB/RIF is not accessible.

CHAPTER ONE

1.0: INTRODUCTION

Tuberculosis is one of the top ten causes of mortality globally as well as a leading cause of death from an infectious agent.[1] It is therefore a public health concern that explains the inclusion of the "end TB epidemic" by the year 2030 and 2035 as a target of the United Nations' SDG-3 and the WHO's End TB Strategy respectively.[2, 3] The disease is known to be associated with poverty thus it's high prevalence in developing countries including Nigeria. Because of TB's association with poverty, the current End TB Strategy employs a combination of multisectoral health and socio-economic interventions to achieve the target of reducing TB incidence by 90% (i.e., ≤10 cases per 100,000 population per year) and the number of TB deaths by 95%, compared with the year 2015 baseline, by the year 2035.[2]

In 2018, there were an estimated 10 million new infections, 1.2 million deaths among HIV-negative people, and 251 000 deaths among HIV-positive people. The new infection rate which varies widely between countries represents a 2% decline from the 2017 rate which though significant, falls short of the expected exaggerated pace necessary to end the TB epidemic globally.[4] In Nigeria, about 429,000 new cases were estimated for the year 2018, which translated to 4.3% of the global TB incident cases thus, Nigeria's burden of TB ranks sixth in the world and first in Africa.[1] On the other hand, out of the estimated global incident TB cases, 7 million cases were reported which though met the UN high-level meeting target for 2018, left a gap of about 3 million unreported or undiagnosed cases.[1] Unfortunately, with an estimated National TB missing case rate of 76% in 2018,[5] Nigeria accounted for about 12% of the global TB detection gaps, coming second to India that contributed about 25%.[1] It is

therefore clear that the country has missed the 2020 End TB Strategy target of a 20% reduction in TB incidence and a 35% reduction in TB deaths.[2]

For Nigeria to be re-channelled to the tracks of achieving the future global targets, more efforts should be directed towards closing the gaps between TB incidence and notification by improving access to early TB diagnosis and prompt treatment for everyone that needs it, which are the fundamental of Integrated patient-centred care and prevention of TB (Pillar 1) of End TB Strategy.[2] In line with this strategy, part of the Nigeria TB Programme's current key priority is to identify the missing TB cases and enrol them on treatment, by expanding the number of health facilities involved in TB care and the deployment of more GeneXpert instruments across the country.[5] In agreement with the WHO recommendation, the current guideline for TB diagnosis in Nigeria is the use of molecular diagnostics, such as Xpert MTB/RIF assay or the 'spot-morning' (two-day) sputum smear microscopy where the Xpert MTB/RIF is not accessible.[6] With the limited number of Xpert MTB/RIF serving the whole country, increasing break down of the instruments, and sub-optimal utilization, there has been recently a new shift to the use of AFB microscopy to fill the widening gap in diagnostic evaluation using the GeneXpert. In 2019, 22.2% of the presumptive PTB were tested with microscopy, and an AFB positivity rate of 12% was identified.[5]

Both Xpert MTB/RIF and sputum microscopy provide a rapid bacteriological diagnosis of TB and require similar laboratory infrastructure and biosafety precautions however, the former is recommended because it detects RR-TB (at least) and has higher diagnostic accuracy.[7] Beyond the limited number of the instruments in Nigeria, the use of Xpert MTB/RIF is associated with challenges including the need for constant power supply to maintain requisite ambient temperature for the instrument, which disrupts TB treatment services,[5] and often

leads to a shift to the use of microscopy for PTB diagnosis in settings where specimen transportation to a functional Xpert facility is not feasible. To expand the use of rapid molecular diagnostics for TB in resource-limited settings with these peculiar Xpert related challenges, WHO recommended the use of a simplified assay, the loop-mediated isothermal amplification (TB-LAMP) for detection of Mycobacterium tuberculosis as a replacement for sputum smear microscopy.[8] The assay, with a processing time of fewer than 2 hours for 14 samples, uses an ambient temperature-independent technique to rapidly amplify DNA and produces a result that can be manually read visually.[8]

Unidentified TB cases continue to sustain TB transmission in Nigeria because it is estimated that one untreated infectious TB case can infect up to 20 persons per annum. Therefore, identification of these missing cases (and treating them) is a top priority for the National Tuberculosis, Leprosy and Buruli Ulcer Control Programme (NTBLCP) of Nigeria.[5] To assist the NTBLCP in this vision, the USAID awarded the Tuberculosis Local Organizations Network (TB-LON) grant to KNCV and IHVN, targeting 18 states across three regions/projects. The KNCV Tuberculosis Foundation Nigeria is implementing the TB-LON Regions 1 and 2 Project in 14 States of Nigeria. To meet one of the project's objectives which is to improve access to high-quality, person-centred TB services; KNCV initiated the pilot implementation of the WHO recommended TB-LAMP to assess its usefulness as an alternative to sputum microscopy for TB diagnosis in Nigeria and support its countrywide scale-up.

1.1: Justification and Literature Review

The diagnosis of TB using the GeneXpert MTB/RIF technology was introduced in Nigeria in 2011 and became the primary diagnostic method for TB in 2016.[5] As of 2019, there were 398 GeneXpert instruments distributed to 388 health facilities across 48% of the local government areas (LGAs) in Nigeria. The number of Xpert instruments per State of the Federation ranges from 5 to 38.[5] The limited number of instruments restricted access to testing making it a major challenge to the use of this molecular diagnostic method in Nigeria.[9] Also, the distribution of the limited number of instruments to public facilities is skewed to secondary health care while the utilization and outcomes of the test vary significantly across types and levels of healthcare facilities in the country.[10] Furthermore, the instrument is power dependent therefore the unstable power supply in the country especially in the rural areas, is another major challenge. Other challenges include the need for the annual instruments calibration check and other computer-related operation challenges to be performed by the laboratory staff, instrument breakdowns, etc.[9] Experience from a few sites in Nigeria suggests that a package approach of Xpert optimization programme made up of interventions categorized under demand creation, ensuring accessibility, and availability of testing, could help overcome some of the aforementioned challenges but, not eliminate them.[9] On the contrary, the demand creation intervention, when widespread, may likely overwhelm the limited Xpert services.

Due to the challenges associated with GeneXpert instruments, the low sensitivity of smear microscopy for PTB diagnosis among other limitations,[7] the unmet need for TB case detection in resource-limited settings still exists and possibly getting worse. Therefore, to improve the rapid and accurate diagnosis of PTB, the WHO recommended the use of an

ambient temperature-independent molecular assay, TB-LAMP (Eiken Chemical Company, Tokyo, Japan), as a replacement for smear microscopy for the diagnosis of pulmonary TB in adult presumptive TB; however, it was unclear whether the TB-LAMP technology was superior to smear microscopy in adult presumptive TB living with HIV.[8] This conditional recommendation by the WHO was based on very low-quality evidence obtained from a systematic review of 13 studies (conclude by 1st October 2015) from 11 countries, and involving 4760 participants.[8, 11] There were two eligible studies each from India and Vietnam; one eligible study each from Brazil, Peru, South Africa, Haiti Cote d'Ivoire, Madagascar, Malawi, Uganda, Tanzania. For all participants, the pooled results of the review showed that that the sensitivity of TB-LAMP (77.7 - 80.3%) was higher than that for smear microscopy (sensitivity differences: 7.1 - 13.2%). On the other hand, the pooled specificity of TB-LAMP ranged from 97.7% to 98.1% and was comparable to that of sputum-smear microscopy (specificity differences: -1.8% to -1.3%). Sub-analysis of the 385 participants with known HIV status showed lower ranges of sensitivity (63.8% - 73.4%) and specificity (95.0% - 98.8%) of TB-LAMP among HIV-positive adults when compared to all participants. Finally, for the smear-negative/culture-positive participants, the review showed low sensitivity for TB-LAMP of 40.3 - 42.2%, while the specificity was high (97.7 – 98.4%) and comparable to that observed for all participants. The very low evidence base for the TB-LAMP recommendation leaves a knowledge gap that calls for more validation studies in resource-limited settings.

In response to this call, a small sample (n = 78) single-centre study from Ethiopia showed that TB-LAMP had the same specificity with smear microscopy (98%) but, a lower sensitivity than smear microscopy (75% versus 78.5%).[12] Furthermore, a systematic review of the diagnostics accuracy of LAMP assay technology (TB-LAMP and in-house assay) for culture-proven TB,

analysed results of 9330 sputa reported in 26 studies from several countries including a few African countries (Kenya, Zambia, Uganda, Tanzania, and South Africa). [13] It found a pooled sensitivity of 89.6% and specificity of 94.0% for the LAMP technology for the diagnosis of PTB. The TB-LAMP based studies (9/26, 33.6%) yielded a sensitivity of 80.9% and specificity of 96.5% for all sputa. However, TB-LAMP had higher sensitivity (96.6% Vs 54.3%) and lower specificity (71.3% Vs 98.6%) for smear-positive sputa compared to smear-negative sputa for the diagnosis of PTB. Another study of 548 presumptive PTB cases in Madagascar, [14] found that the sensitivity of TB-LAMP (84.6%) and Xpert MTB/RIF (86.6%) were significantly higher than that of fluorescence smear microscopy (73.6%) for the diagnosis of PTB while their specificity, 98.4% Vs 97.4% Vs 99.0% respectively, were similar. However, for smear-ve/culture+ve cases, both TB-LAMP and Xpert MTB/RIF showed the same low specificity (54.7%) and comparable high sensitivity (98.4% Vs 97.4%) respectively.

A few other studies have also assessed the diagnostic ability of TB-LAMP for pulmonary TB using sputum culture as the gold standard.[15,16] In the Gambia, a study of 285 presumptive TB and 156 confirmed TB patients (on treatment for an average of two months), [15] found that for all participants, TB-LAMP had a sensitivity of 99% which was higher than sputum-smear microscopy; and a specificity of 94% which was similar to that of sputum-smear microscopy. The sensitivity of TB-LAMP was lower for smear -ve/culture +ve cases (90.0%) when compared to that of smear+ve/culture +ve (100%). Another study of 453 presumptive PTB patients in India showed that the sensitivity and specificity of TB-LAMP versus Xpert MTB/RIF for the diagnosis of PTB in culture-positive sputa were 100% and 99.2% versus 82.6% and 94.9% respectively. [16]

Though these studies generally support the use of LAMP assay as an alternative for smear microscopy, there is wide variability in their quality and results which justifies the need for more operational research on TB-LAMP in different epidemiological, and geographical settings as recommended by the WHO.[8] Given this, the need for such research was very critical in the most populous nation in Africa, ranked among the top 20 high TB burden countries, high TB/HIV burden countries, and high MDR-TB burden countries.[1] It was very important to be certain that TB-LAMP has a diagnostic accuracy for PTB that is at least equivalent to that of sputum-smear microscopy in Nigeria before its deployment to the field to mitigate the possibility of magnifying the current problem of low TB case notification. .

CHAPTER TWO

2.0 : STUDY GOAL AND OBJECTIVES

2.1 : Study Goal

To investigate the diagnostic accuracy of TB LAMP for PTB among presumptive TB at the State-level AFB microscopy/GeneXpert facilities in TB-LON Regions 1 and 2 states Project in Nigeria

2.2: Specific Objectives

- To determine the sensitivity and specificity of TB-LAMP for the diagnosis of PTB among adult presumptive TB patients, using solid culture as the reference standard
- To determine TB-LAMP accuracy for the diagnosis of PTB among adult presumptive TB patients, if used as an alternative test for smear microscopy
- To determine TB-LAMP accuracy for the diagnosis of PTB among adult presumptive TB patients living with HIV, if used as an alternative test for smear microscopy.
- Compare the diagnostic accuracy of TB-LAMP and GeneXpert for the diagnosis of PTB adult presumptive TB patients

2.3 : Study Design

The study is part of the five-year USAID funded TB LON Regions 1 and 2 Project being implemented by the KNCV Tuberculosis Foundation Nigeria. It was operational research and cross-sectional analytical in design. Participants were consenting adults with presumptive PTB from five purposively selected health facilities in the KNCV supported States in Nigeria.

2.4 : Setting

The study was conducted in Nasarawa and Anambra States selected from TB LON Regions 1 and 2 respectively. As highlighted in figure 1, LON Regions 1 includes Bauchi, Kaduna, Katsina, Kano, Nasarawa, Plateau, and Taraba states, while Regions 2 comprises of A nambra, Akwa Ibom, Benue, Cross River, Delta, Imo, and Rivers. The selection of the two states was purposive based on the ease of the research operations and a representation of the Northern and Southern parts of the country

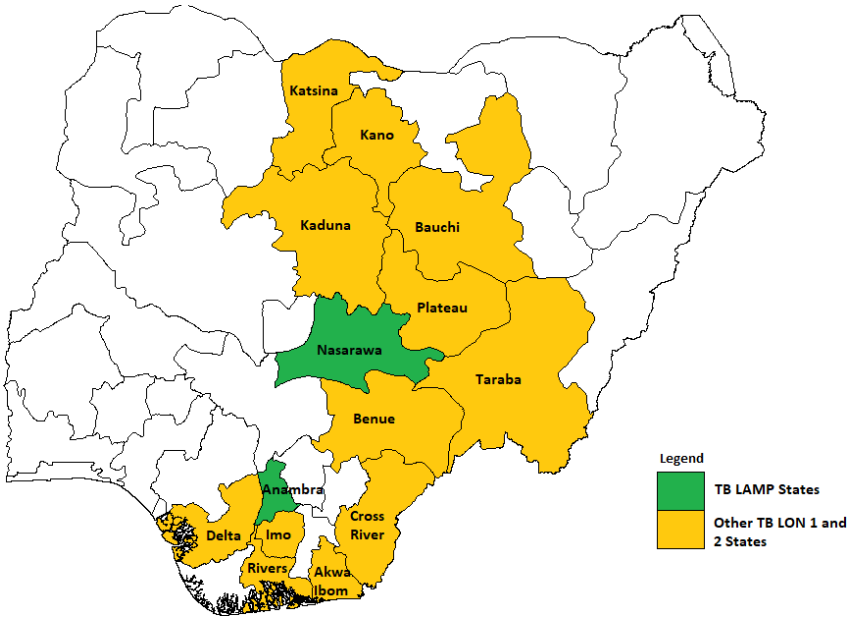

Figure 1: Map of Nigeria Indicating TB LON Regions 1 & 2 states and Selected TB-LAMP Study States

Nasarawa State is in the North-Central region of Nigeria. According to the 2016 estimate of the 2006 census, the State has a population of about 2,523,395 with a density of about 67 persons per square kilometre.[17, 18] The State is made up of 13 local government areas. With a case notification rate for all forms of TB of 100 per 100,000 population in 2019, the State ranks 3rd in Nigeria as regards TB burden.[5] It has about 19 Xpert MTB/RIF sites and about 80% of all TB cases reported in the State in 2019 were diagnosed by Xpert MTB/RIF. [5]

Anambra State is in the South-eastern region of Nigeria and consists of 21 local government areas. The 2016 population estimate of the State by the National Bureau of Statistics was 5,527,809.[17] It is the second-most densely populated state in Nigeria after Lagos State. [19] In 2019, the TB case notification rate was 41 per 100,000 population which ranked 26th out of all states (and FCT) in Nigeria; however, the HIV/TB rate and childhood TB Proportion among notified TB, ranked 9th and 5th in the country respectively.[5] There are about nine Xpert MTB/RIF testing sites in the State and they diagnosed about 60% of all TB cases in the State in 2019. [5]

Table 1: Basic Characteristics of Study Areas

Study area/State	Location/region	Population	No. of LGAs	Xpert sites	AFB sites	2019 TB detection rate (per 100,000)
Nasarawa	North-Central	2,523,395	13	19	56	18
Anambra	South-east	5,527,809	21	9	65	41

The validation of the performance of a diagnostic tool in a high-TB burden setting requires field studies in at least five sites.[7] Therefore, for this study, five healthcare facilities with AFB microscopy (Zhiel-Nelson) and GeneXpert capacity (or a nearby facility with Xpert capacity) were selected thus: two (one private and one public) and three (two public and one private)

health care facilities from Nasarawa and Anambra State respectively (table 2). The selection was based on their workload, location, supportive systems for testing optimization, and laboratory quality assurance records. TB-LAMP platform was installed by KNCV Nigeria at the five selected sites. All three government facilities had functional Xpert instruments while the two private facilities were supported by nearby government facilities with Xpert capacity. Research assistants, Microscopists, TB-LAMP operators, sputum movers, Xpert staff were recruited for each facility. On-site training was organized for laboratory staff for the TB-LAMP, Xpert and smear microscopy. Research Assistants, DOTS officers, sputum movers were also trained as appropriate. Participants were recruited from the selected health care facilities (hub) and their spoke sites.

Table 2: Sites and Available PTB Diagnostic Modalities

State	Name of facility	Status	TB-LAMP service	Smear microscopy service	Xpert service/Source
Anambra	General Hospital Enugu-ukwu	State Government facility	Yes	Yes	Yes
	General Hospital Umueri	State Government facility	Yes	Yes	Yes
	GEM Diagnostic Center Onitsha	Private facility	Yes	Yes	General hospital Onitsha
Nasarawa	Dalhatu Araf Specialist hospital (DASH), Lafia	State Government facility	Yes	Yes	Yes
	Karu Diagnostic center	Private facility	Yes	Yes	1) Federal Medical Center (FMC) Keffi 2) Maraba Gurku Medical Center (MGMC)

2.5 : Study Population

All adult patients (≥15 years) at the study site with cough of 2 weeks duration and above or persons living with HIV (PLHIV) with cough of any duration, with or without any other respiratory and constitutional symptoms that define presumptive TB in Nigeria,[5] were eligible for the study.

2.6 : Exclusion Criteria

These included adult presumptive TB at the study sites with a history of TB treatment including relapse cases, loss to follow-up, as well as those who could not produce an adequate volume of sputum.

2.7 : Sample Size Calculation

The sample size (n) was be determined using the formula:[21]

$n = 2 (Z_{\alpha/2} + z_{\beta})^2 x\ P\ (1 - P) / (p_1 - p_2)^2$

Where, n = sample size

$Z_{\alpha/2}$ = 1.96 i.e., Z score for α error of 5% (95% confidence level)

Z_{β} = 0.84 i.e., Z score for estimated study power of 80%

p_1 = TB Positivity rate of sputum microscopy among presumptive TB in Nigeria (0.12) [5]

p_2 = Assumed TB-LAMP detection rate (0.15) among presumptive TB patients. The study is designed to detect a 25% difference in TB positivity rate with respect to p_1.

P = pooled prevalence = ½ $(p_1 + p_2)$ = 0.135

$n = 2(1.96 + 0.84)^2 \times 0.135(1 - 0.135) / (0.12 - 0.15)^2$

= 2034.5 ≈ 2035

Assuming a loss to follow up rate of 20% = 407

n = 2035 + (0.2 × 2035) = 2442

Finally, a sample of 500 eligible presumptive TB was projected for each of the five study sites which gave a total of 2500 participants. However, by the end of the study, a total of 2,872 participants were recruited (Fig. 3)

2.8 : Sampling Approach

After ethical and other approvals, all consecutive consenting eligible presumptive TB at each study site and spoke facilities were recruited into the study. Participants' recruitment lasted for six months (August 2020 – February 2021).

2.9 : Methods and Data Collection

After obtaining written informed consent, each participant submitted in appropriate containers, two 'spot' sputa (≥ 1.5 ml each) produced by direct coughing. One sputum specimen (in wide mouthed sputum cup) was used for TB-LAMP, smear microscopy, and Xpert test at the study site's laboratory while the other specimen (collected in falcon tube) was used for solid culture at a designated TB reference laboratory. The sputum specimen for culture were maintained in a cold chain and transported to either of these three reference laboratories within 48 hours - Abia Specialist Hospital and Diagnostic Center Amachara, Umuahia and University of Port-Harcourt Teaching Hospital Port-Harcourt for the specimens from Anambra State; Zankli Research Center in Bingham University Karu, Nasarawa state for the specimens from Nasarawa State. In line with the WHO's recommended same day "spot-spot diagnosis" strategy,[22] a part of every sputum for culture, was used for smear microscopy at reference laboratory before being processed for solid culture.

At the study sites and spoke facilities, all specimens were registered and coded appropriately with a sticky unique identifier by a trained healthcare worker before transportation to the laboratory. Also, the same participant's code was stuck on his/her corresponding informed consent forms and data sheets. The three diagnostic modalities in each study site were manned by three different teams of trained laboratory staff working independently and blinded to the patient's characteristics. Laboratory procedures for the TB-LAMP (figure 2) and other diagnostic methods were carried out according to the respective standard operating protocols and the manuals of the diagnostic instruments. [6, 23]

For this study, the result from each diagnostic method was recorded in the appropriate registers at the study sites as per protocol, and in the study's datasheet (annexe 1). The result of Xpert for each participant was used for his/her prompt treatment where necessary, in line with the NTBLCP guidelines.[6]

For this study, non-response included participants whose sputum specimens were rejected at the culture laboratory, culture results that showed non-tuberculous mycobacteria (NTM) only or culture-contaminated, and situations where smear microscopy, culture, or speciation were not done.

2.10 : Description of the TB-LAMP Instrument

The HumaLoop T brand of the TB-LAMP system was used for the study. It is an integrated platform for sample preparation, amplification, and visual result reading, manufactured by Eiken Chemical Company Ltd. (Tokyo, Japan) and distributed by HUMAN Diagnostics. [24] For the diagnosis of PTB, the instrument employs three major steps [8,23] as shown in figure 2: heating of sputum at 90°C in an extraction solution to inactivate and lyse bacteria as well as extract the DNA; amplification of the extracted DNA at 67°C using the polymerase enzyme;

and naked eye visualization of a positive test in a fluorescence detector under ultraviolet light using double-stranded DNA binding dyes. The HumaLoop T has a rapid throughput of up to 14 sputum specimens per cycles and up to 5 cycles per day. The instrument is supplied with an independent power system (solar panel and portable battery) to ensure steady power supply during its operation.[24]

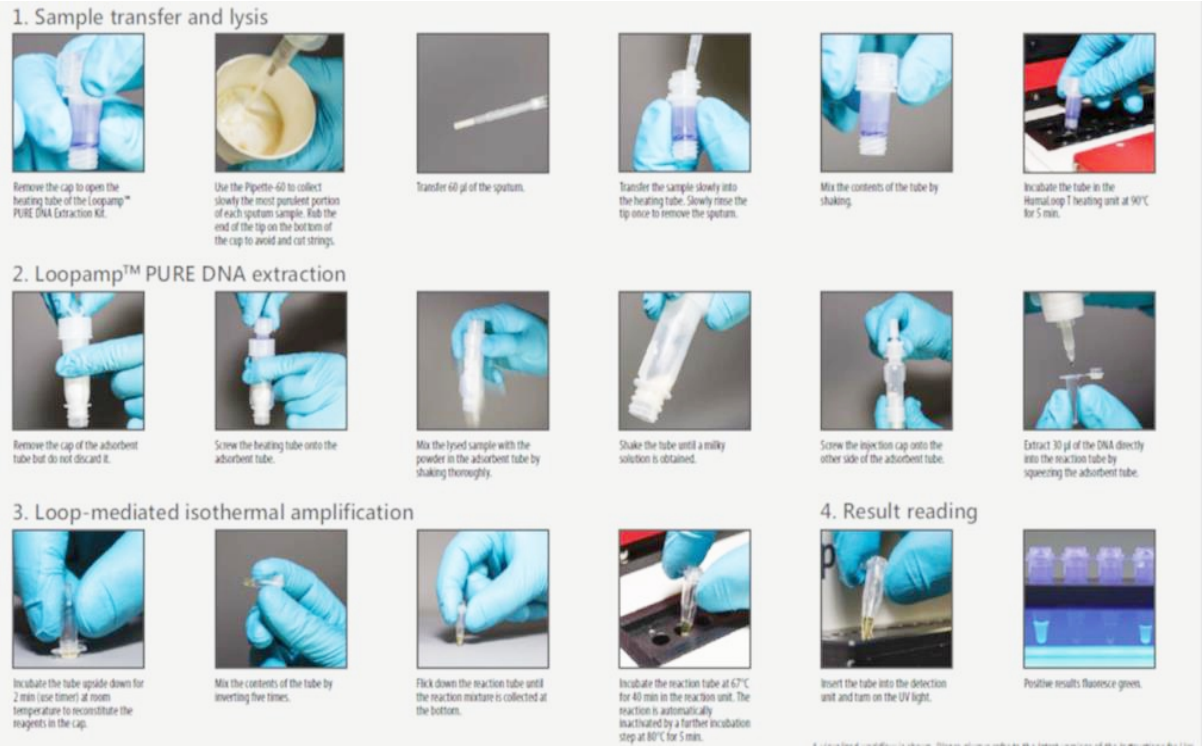

Figure 2: Schematic description of the workflow for TB-LAMP[23]

2.11 : Quality Assurance

On-site user training and proficiency testing of laboratory staff on the use of the new diagnostic instrument (TB-LAMP) as well as retraining on sputum smear microscopy and Xpert instrument were conducted before the commencement of the study. The sequence of spot sputa production into the two specimen containers (sputum cup and falcon tube) was alternated across participants; for instance, while the first participant produced the first

sputum in the sputum cup, the second consecutive participant was encouraged to produce the second specimen in the falcon tube. The alternation of sputum presentation to the two lines of TB testing was to minimize selection bias. All sputum specimens were transported to the study site laboratory in a cold chain. Also, all sputum specimens for culture were stored in a cold chain for not more than 48hrs before transport to the designated TB reference laboratory.

There was uniformity of smear microscopy method (i.e., ZN) and type of GeneXpert machine (i.e., Xpert MTB/RIF Ultra assay) across the study sites. Calibration and maintenance of the TB-LAMP and GeneXpert Instruments were carried out routinely according to the manufacturer's specifications. Laboratory staff manning a testing modality were blinded to the results from other testing modalities to minimize bias.

Also, there was a regular study management's weekly virtual meeting throughout the study duration for the study activities' monitoring and evaluation. Regular supervisory and monitoring visits were made by study management team members to the implementing laboratories and the culture laboratories, to ensure compliance with the study protocol.

2.12 : Study Tools and Variables

The study datasheet (annexe 1) was adapted from the *Request for examination of biological specimen for TB (Form 03)*[20] and the National TB guideline.[6] Study variables used for data analyses include participant's age, sex, HIV status, smear microscopy results, culture and speciation results, TB-LAMP results, and Xpert test results.

2.13 : Outcome Measures

The primary outcome measures were:

- the AFB/MTB positivity rates of TB-LAMP, sputum microscopy, Xpert MTB/RIF, sputum culture among presumptive TB cases at the study sites
- The sensitivity and specificity of TB-LAMP, sputum microscopy, and Xpert MTB/RIF for the diagnosis of PTB among all participants in the study centres, using sputum culture as the reference standard.

The secondary outcome measures were:

- The sensitivity and specificity of TB-LAMP, and Xpert MTB/RIF for the diagnosis of PTB among smear +ve culture +ve participants, using sputum culture as the reference standard.
- The sensitivity and specificity of TB-LAMP, and Xpert MTB/RIF for the diagnosis of PTB among smear -ve/culture +ve participants, using sputum culture as the reference standard.
- The sensitivity and specificity of TB-LAMP for the diagnosis of PTB among participants living with HIV at the study centres, using sputum culture as the reference standard.

2.14 : Ethical Considerations

The study objectives, procedure, and benefits to the individual/society were discussed with every eligible client before the informed consent form (annexe 2) was administered. The participation of subjects was voluntary. Every participant was assured of his/her confidentiality and assured that his/her sputum specimens would be used for TB diagnosis and the study only.

The approval for the study was obtained from the National Health Research Ethics Committee (NHREC) of Nigeria (Approval number: NHREC/01/01/2007-03/09/2020).

2.15 : Data Management

Data for each participant from all five study sites and three culture laboratories were collected by trained staff using the appropriate study's datasheets. Data from each State were entered into a separate secure excel data sheet template then, merged into a single data set. Data cleaning and participants' de-identification were done as appropriate.

Data analyses was conducted with IBM SPSS (version 20) and supplemented with OpenEpi (Version 3.01).[25] Data analyses involved descriptive and inferential statistics at 95% confidence level. Results were presented as appropriate using mean ± SD, frequencies, percentages, tables, and figures. Using sputum culture results as the reference standard, true positives (TP), true negatives (TN), false positive (FP), and false-negative (FN) rates of TB-LAMP, smear microscopy, and Xpert MTB/RIF for diagnosing PTB were determined for all participants and the sub-category of people living with HIV. These rates were used to determine each diagnostic test's PTB diagnostic accuracy (sensitivity, and specificity) for the appropriate participants' category using the formulae:

Sensitivity = [True-Positive / (True--ositive + False Negative)] ×100

Specificity = [True-Negative / (True Negative +False Positive)] ×100

The confidence intervals for MTB Positivity, sensitivity, and specificity rates were determined by the Wilson score interval method. McNemar test for Binary matched-pair data was used to compare the diagnostic performance of TB-LAMP with smear microscopy and Xpert MTB/RIF for all participants, and for PLHIV. Where the diagnostic performance of the tests for

the sensitivity and specificity did not agree, Youden's J statistic was used to determine superiority.

CHAPTER THREE

3.0: RESULTS

3.1: Study Participants

As shown in Figure 3, a total of 2872 consenting presumptive TB were recruited from the two study States, Anambra (n = 1533, 53.4%) and Nasarawa (n = 1339, 46.6%), over the six months study period. However, data from 2636 (91.8%) participants were analyzed, Anambra State (n = 1410, 53.5%) and Nasarawa State (n = 1226, 46.5%). Data from 236 participants were excluded from analyses for reasons including sputum rejection at culture lab (n = 45), culture contamination (n = 147), culture result of non-tuberculous mycobacteria (NTM) only (n = 38), etc.

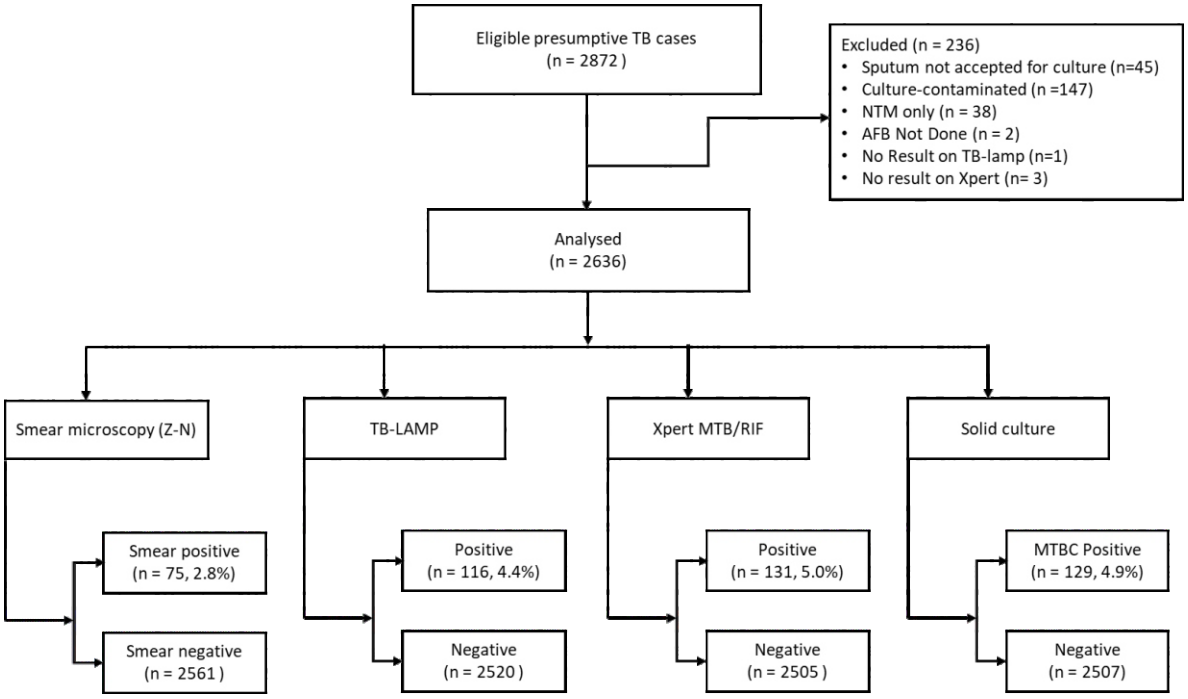

Figure 3: TB-LAMP Study Flow Chart

3.2 : Characteristics of the Participants

Table 3 shows the relevant basic characteristics of the study participants. There were 955 (36.2%) males and 1681 (63.8%) females. The mean age of all participants was 39.8 ± 15.52 (15 – 93) years. The modal age group was 25 – 34 years (n = 717, 27.2%). Most of the participants were married (n = 1924, 73.0%), traders (n = 1032, 39.2%), and HIV sero-negative (n = 1280, 48.6%).

Table 3: Basic Characteristics of Study Participants

Characteristic	Sub-group	Frequency (n = 2636)	Percentage (%)
State of Residence	Anambra	1410	53.5
	Nasarawa	1226	46.5
Sex	Male	955	36.2
	Female	1681	63.8
Age group (years)	15-24	391	14.8
	25-34	717	27.2
	35-44	627	23.8
	45-54	396	15.0
	55-64	272	10.3
	>=65	233	8.8
Marital Status	Married	1924	73.0
	Single	704	26.7
	Widowed	6	0.2
	Others	2	0.1
Occupation	Trading	1032	39.2
	Civil servant/Public servant	296	11.2
	Skilled worker	264	10.0
	Student/Corper	362	13.7
	Housewife	228	8.6
	Farming	295	11.2
	Others	105	4.0
	Unemployed	25	0.9
	No response	29	1.1
HIV Status	Sero-negative	1280	48.6
	Sero-positive	715	27.1
	Unknown	641	24.3

3.3 : PTB positivity rates of TB-LAMP and Other Testing Modalities

As shown in table 4, for all participants, TB-LAMP was positive for MTB in 116 participants giving a positivity rate of 4.4% [95%CI (3.68 – 5.25)]. The MTB positive rates of other diagnostic modalities were smear microscopy (2.8%), Xpert MTB/RIF (5.0%), sputum Culture (4.9%). For the HIV sero-positive population, the MTB positivity rates range from the lowest (1.4%) for smear microscopy to the highest (2.1%) for sputum culture.

Table 4: MTB Positivity Rates of TB-LAMP and other Diagnostic Modalities

A)	All Participants (n = 2636)		
Diagnostic modality	MTB Detected/Positive		MTB Positivity rate (95%CI)
	Yes	No	
	Frequency (%)	Frequency (%)	
TB-LAMP	116 (4.4)	2520 (95.6)	4.4 (3.68 – 5.25)
Smear microscopy	75 (2.8)	2561 (97.2)	2.8 (2.28 – 3.55)
XPert MTB/RIF	131 (5.0)	2505 (95.0)	5.0 (4.20 – 5.87)
Sputum culture	129 (4.9)	2507 (95.1)	4.9 (4.13 – 5.79)
B)	HIV Sero-positive participants (n = 715)		
Diagnostic modality	MTB Detected/Positive		MTB Positivity rate (95%CI)
	Yes	No	
	Frequency (%)	Frequency (%)	
TB-LAMP	12 (1.7)	703 (98.3)	1.7 (0.96 – 2.91)
Sputum microscopy	10 (1.4)	705 (98.6)	1.4 (0.76 – 2.56)
XPert MTB/RIF	13 (1.8)	702 (98.2)	1.8 (1.03 – 3.12)
Sputum culture	15 (2.1)	700 (97.9)	2.1 (1.28 – 3.43)

3.4: Sensitivity and specificity of TB-LAMP Versus Other Modalities for PTB Diagnosis among all Participants

Table 5 shows the sensitivity and specificity of TB-LAMP, smear microscopy, and Xpert MTB/RIF for the diagnosis of PTB in all participants, using solid culture as the standard test. All diagnostic modalities had a specificity of above 99%. TB-LAMP has a sensitivity of 76.7% (95%CI: 68.75-83.20) while that of smear microscopy and Xpert MTB/RIF were 54.3% (95%CI: 45.67-62.61 and 84.5% (95%CI: 77.26-89.73) respectively. The Kappa score of TB-LAMP and Xpert MTB/RIF were within the 0.8 - 1.0 range.

Table 5: Sensitivity and Specificity TB-LAMP and other Modalities Compared to Sputum Culture for all participants

Modality	Test outcome Vs Standard		Sensitivity (95%CI) %	Specificity (95%CI) %	Kappa score (κ) (95%CI)	p-value
TB-LAMP (n = 2636)	True Positive	99	76.7 (68.75-83.20)	99.3 (98.92-99.58)	0.80 (0.76-0.84)	<0.001
	False Negative	30				
	True Negative	2490				
	False Positive	17				
Sputum microscopy (n = 2636)	True Positive	70	54.3 (45.67-62.61)	99.8 (99.52-99.91)	0.67 (0.64-0.71)	<0.001
	False Negative	59				
	True Negative	2502				
	False Positive	5				
Xpert MTB/RIF (n = 2636)	True Positive	109	84.5 (77.26-89.73)	99.1% (98.67-99.42)	0.83 (0.79-0.87)	<0.001
	False Negative	20				
	True Negative	2485				
	False Positive	22				

Table 6 shows the results of comparing the PTB diagnostic performance of TB-LAMP with Smear microscopy and Xpert MTB/RIF for all participants. The sensitivity of TB-LAMP was significantly higher than smear microscopy ($p < 0.001$) but the reverse was the case for the Specificity ($p = 0.003$). Further analysis showed J-Index scores of 0.76 and 0.54 for TB-LAMP and smear microscopy respectively. The sensitivity of TB-LAMP was significantly lower than Xpert MTB/RIF ($p = 0.004$) while their specificities were similar ($p = 0.297$).

Table 6: Comparison of Diagnostic Performance of TB-LAMP with Smear microscopy and Xpert MTB/RIF for all participants

A) TB-LAMP Versus Smear microscopy						
Diagnostic accuracy		TB-LAMP (Sensitivity = 76.7%)				
Smear microscopy (Sensitivity = 54.3%)		TP	FN	Total	X^2	p-value
	TP	69	1	70	27.13	<0.001
	FN	30	29	59		
	Total	99	30	129		
Diagnostic accuracy		TB-LAMP (Specificity = 99.3%)				
Smear microscopy (Specificity = 99.8%)		TN	FP	Total	X^2	p-value
	TN	2488	14	2502	9.0	0.003
	FP	2	3	5		
	Total	2490	17	2507		
B) TB-LAMP Versus Xpert MTB/RIF						
Diagnostic accuracy		TB-LAMP (Sensitivity = 76.7%)				
Xpert MTB/RIF (Sensitivity = 84.5)		TP	FN	Total	X^2	p-value
	TP	99	11	110	8.3	0.004
	FN	1	19	20		
	Total	100	30	130		
Diagnostic accuracy		TB-LAMP (Specificity = 99.3%)				
Xpert MTB/RIF (Specificity = 99.1%)		TN	FP	Total	X^2	p-value
	TN	2476	9	2485	1.09	0.297
	FP	14	8	22		
	Total	2490	17	2507		

3.5: Sensitivity and specificity of TB-LAMP and Xpert: Smear +ve/culture +ve participants

For participants that were smear +ve/culture +ve (n = 70), the sensitivity of TB-LAMP (98.6%) did not differ significantly from that of Xpert MTB/RIF (97.1%), p = 0.480. Details are shown in tables 7 and 8.

Table 7: Sensitivity and Specificity of TB-LAMP and Xpert Compared to Sputum Culture for Smear +ve/culture +ve participants

Modality	Test out come Vs Standard		Sensitivity (95%CI) %	Specificity (95%CI) %	Kappa score (95%CI)	p-value
TB-LAMP (n = 70)	True Positive	69	98.6 (92.34-99.75)	-	-	-
	False Negative	1				
	True Negative	0				
	False Positive	0				
Xpert MTB/RIF (n = 70)	True Positive	68	97.1 (90.17 – 99.21)	-	-	-
	False Negative	2				
	True Negative	0				
	False Positive	0				

Table 8: Comparison of Sensitivity of TB-LAMP with Xpert MTB/RIF for Smear +ve/Culture +ve participants

TB-LAMP Versus Xpert MTB/RIF						
Diagnostic accuracy		TB-LAMP (Sensitivity = 98.6 %)				
Xpert MTB/RIF (Sensitivity = 97.1%)		TP	FN	Total	X^2	p-value
	TP	68	0	68	0.5	0.480
	FN	1	1	2		
	Total	69	1	70		

3.6 : Sensitivity and specificity of TB-LAMP and Xpert: Smear -ve/culture +ve participants

Tables 9 and 10 compared the sensitivity of TB-LAMP and Xpert MTB/RIF among participants that were smear -ve/culture +ve (n = 59). TB-LAMP had a sensitivity of 50.8% (95%CI: 38.35-63.16) while Xpert MTB/RIF had a sensitivity of 69.5% (95%CI: 56.85-79.75), p = 0.001.

Table 9: Sensitivity and Specificity TB-LAMP and Xpert Compared to Sputum Culture for Smear -ve/culture +ve participants

Modality	Test outcome Vs Standard		Sensitivity (95%CI) %	Specificity (95%CI) %	Kappa score (95%CI)	p-value
TB-LAMP (n = 59)	True Positive	30	50.8 (38.35-63.16)	-	-	-
	False Negative	29				
	True Negative	0				
	False Positive	0				
Xpert MTB/RIF (n = 59)	True Positive	41	69.5(56.85-79.75)	-	-	-
	False Negative	18				
	True Negative	0				
	False Positive	0				

Table 10: Comparison of Sensitivity of TB-LAMP with Xpert MTB/RIF for Smear -ve / Culture +ve participants

TB-LAMP Versus Xpert MTB/RIF						
Diagnostic accuracy		TB-LAMP (Sensitivity = 50.8%)				
		TP	FN	Total	X^2	p-value
Xpert MTB/RIF (Sensitivity = 69.5%)	TP	30	11	41	10.08	0.001
	FN	0	18	18		
	Total	30	29	59		

3.7 : Sensitivity and specificity of TB-LAMP and Xpert: HIV Seropositive participants

Table 11 shows the sensitivity and specificity of TB-LAMP and other diagnostic modalities among HIV seropositive participants (n = 715), using solid culture as the standard test. The specificity values of all diagnostic modalities were above 99%. The sensitivity of TB-LAMP was 60.0% (95%CI: 35.75-80.18). The sensitivity of smear microscopy and Xpert MTB/RIF were 53.3% (95%CI: 30.12-75.19) and 66.7% (95%CI: 41.71-84.82) respectively. Their kappa scores were within the 0.60 – 0.79 range.

Table 11: Sensitivity and Specificity TB-LAMP and other Modalities Compared to Sputum Culture for HIV Seropositive participants

Modality	Test outcome Vs Standard		Sensitivity (95%CI) %	Specificity (95%CI) %	Kappa score (95%CI)	p-value
TB-LAMP (n = 715)	True Positive	9	60.0 (35.75-80.18)	99.6 (98.75-99.85)	0.66 (0.59-0.73)	<0.001
	False Negative	6				
	True Negative	697				
	False Positive	3				
Smear microscopy (n = 715)	True Positive	8	53.3 (30.12-75.19)	99.7 (98.96-99.92)	0.63 (0.56-0.71)	<0.001
	False Negative	7				
	True Negative	698				
	False Positive	2				
Xpert MTB/RIF (n = 715)	True Positive	10	66.7 (41.71-84.82)	99.6 (98.75-99.85)	0.71 (0.64-0.78)	<0.001
	False Negative	5				
	True Negative	697				
	False Positive	3				

Table 12 shows that the sensitivity and specificity of TB-LAMP for PTB diagnosis among HIV sero-positive participants did not differ from smear microscopy, and Xpert MTB/RIF ($p > 0.05$).

Table 12: Comparison of Diagnostic Performance of TB-LAMP with Smear microscopy and Xpert MTB/RIF for HIV Sero-positive participants

A) TB-LAMP Versus Smear microscopy						
Diagnostic accuracy		TB-LAMP (Sensitivity = 60.0%)				
Smear microscopy (Sensitivity = 53.3%)		TP	FN	Total	X^2	p-value
	TP	7	1	8	0.33	0.564
	FN	2	5	7		
	Total	9	6	15		
Diagnostic accuracy		TB-LAMP (Specificity = 99.6%)				
Smear microscopy (Specificity = 99.7 %)		TN	FP	Total	X^2	p-value
	TN	696	2	698	0.33	0.564
	FP	1	1	2		
	Total	697	3	700		
B) TB-LAMP Versus Xpert MTB/RIF						
Diagnostic accuracy		TB-LAMP (Sensitivity = 60.0%)				
Xpert MTB/RIF (Sensitivity = 66.7%)		TP	FN	Total	X^2	p-value
	TP	9	1	10	0.5	0.480
	FN	0	5	5		
	Total	9	6	15		
Diagnostic accuracy		TB-LAMP (Specificity = 99.6%)				
Xpert MTB/RIF (Specificity = 99.6%)		TN	FP	Total	X^2	p-value
	TN	694	3	697	0	1.0
	FP	3	0	3		
	Total	697	3	700		

CHAPTER FOUR

4.0: DISCUSSION

The study's goal was to investigate the diagnostic accuracy of TB-LAMP for PTB among presumptive TB at the State-level AFB microscopy/GeneXpert centres in Nigeria within the TB-LON Regions 1 and 2 Project in Nigeria. However, it was primarily designed to determine whether TB-LAMP is adequately accurate to be used as an alternative to smear microscopy for PTB diagnosis among adult presumptive TB in Nigeria. This enquiry was necessary because of the variations in quality and diagnostic accuracy of TB-LAMP recorded in a few countries thus, the need for more operational studies in different epidemiological and geographical settings.[8] Therefore, there was a strong justification for the study in Nigeria - the most populous nation in Africa and one of the top 20 high TB burden countries in the world.[1]

The participants' age groups and occupations represent the current demographics of Nigeria.[26] The proportion of females recruited for the study was almost twice that of the males which may suggest that women access TB treatment services more than males in the study areas. The reason for this disproportionate access across gender is not clear. However, further data analysis showed that the odds of being diagnosed PTB based on culture results was three times higher in males when compared to females [OR = 3.3 (95%CI: 2.27 – 4.75)]. This male preponderance of PTB in this study agrees with the known epidemiology of PTB in Nigeria.[5] Trading was the most common occupation among study participants which agrees with the 2018 National Demographic Survey that found 'sales and services as the predominant occupation in Nigeria.[26] Tuberculosis is associated with HIV infection which explains the higher HIV prevalence among PTB patients and vice versa. The 2019 NTBLCP report,[5] showed a national TB/HIV prevalence of 10.4% however; at the State levels, a TB/HIV

prevalence of up to 36.2% has been reported.[27] Therefore, the high HIV seroprevalence rate of 27% among participants in this study was expected because of the deliberate referrals from HIV diagnostic or treatment centres in the study areas.

In Nigeria, smear microscopy is still used for PTB diagnosis in areas where Xpert MTB/RIF assay is not accessible.[6] In 2019, 22% of the presumptive TB examined for diagnosis in the country were tested with microscopy. Assuming high diagnostic performance of all test modalities used in this study, the low comparative MTB positivity rate of smear microscopy in this study may suggest that many PTB cases are missed, with the obvious public health implication. On the other hand, the slightly less PTB positivity rate of culture compared to Xpert may be due to the limitations inherent with the use of solid media including errors associated with the manual reading of results.[6]

For all adult participants in this study, both the diagnosis of PTB with TB-LAMP and Xpert MTB/RIF showed a kappa statics score of 0.8 and above which indicates a strong degree of agreement with the results of the standard diagnostic test (sputum culture).[28] Thus, the diagnostic performance of TB-LAMP in this study may be considered to be superior to smear microscopy which showed a moderate degree of agreement with the results of the standard test (κ = 0.67). The PTB diagnostic superiority of TB-LAMP over smear microscopy is also supported by its higher J-Index score assuming the tests' false negative and false positive rates are given equal consideration.[29] The findings of this study as regards superiority of TB-LAMP over smear microscopy agrees with the findings of two systematic reviews, [8,11,13] and other related studies,[14, 15] but, differed from a small sample, a single-centre study from Ethiopia.[12] On the other hand, this study result supports the established superiority of Xpert MTB/RIF over smear microscopy for PTB diagnosis which is the reason it is used as the primary

diagnostic method in Nigeria.[6] It also suggests that Xpert PTB/RIF is superior to TB-LAMP for PTB diagnosis in the general population because the sensitivity was significantly higher than TB-LAMP while their specificities were similar (table 5).[29]

The sensitivity of TB-LAMP in this study was markedly higher than smear microscopy ($p < 0.001$) which means that TB-LAMP has a significantly lower FN rate when compared to smear microscopy. This observed lower FN rate of TB-LAMP to smear microscopy is critical in the current campaign by NTBLCP to reduce missing TB which serves as a pool of reservoir for the continued transmission of TB in the community.[5] Because one untreated infectious TB case can infect about 15 persons per year, therefore the TB control in Nigeria has set the finding of the missing TB cases as its most important priority. It is believed that TB-LAMP, an automated molecular-based diagnostic test, with FN rate that is lower than smear microscopy as confirmed by this local study, will help achieve that objective if deployed to areas currently served by smear microscopy.

The study also shows that TB-LAMP and Xpert MTB/RIF had very high and comparable sensitivities for the diagnoses of smear +ve/culture +ve PTB cases however, for the smear -ve/culture +ve PTB cases, the sensitivity of TB-LAMP was significantly lower with a difference of 19% ($p = 0.001$). This observed sensitivity difference may explain the significant sensitivity difference between TB-LAMP and Xpert for all participants in this study. The superiority of Xpert MTB/RIF over TB-LAMP for smear -ve/culture +ve PTB cases in this study supports its endorsement as the most sensitive rapid assay for PTB diagnosis in smear -ve respiratory samples.[30] As the case with Xpert MTB/RIF, the addition of a second or third TB-LAMP test for paucibacillary sputum sample may increase its sensitivity.[30] However, the operational advantage and ease of additional TB-LAMP testing in resource-poor settings like Nigeria, need

further studies. The sensitivity of TB-LAMP for smear -ve/culture +ve PTB participants, in this study, seems closer to the pooled values of 40 – 42% in a systematic review[8,11] when compared to the sensitivity of 90% reported by the Gambian study.[15]

For the HIV sero-positive participants, the TB-LAMP sensitivity of 60% and specificity of 99.6% may be comparable to the sensitivity and specificity results (63.8% - 73.4% versus 95.0% - 98.8%) of the systematic review that guided the WHO's TB-LAMP recommendation.[8,11] Furthermore, this study shows that PLHIV, TB-LAMP, smear microscopy, and Xpert MT B/RIF had a moderate degree of agreement with the standard test results (κ = 0.60 – 0.79) which suggests the comparable diagnostic performance of the three tests.[28] Likewise, the specificity and sensitivity of TB-LAMP for PTB diagnosis in PLHIV when compared independently with smear microscopy and Xpert MTB/RIF did not differ significantly thus suggesting that TB-LAMP is equivalent to smear microscopy and Xpert for PTB diagnosis among adults PLHIV.[29] Nevertheless, considering the low culture-based PTB rate in the HIV sero-positive cohort of this study (table 4), larger sampled studies among PLHIV may be required to confirm this study's findings.

4.1 : Study strength and limitations

This study is a novel effort at testing the diagnostic performance of TB-LAMP technology for PTB diagnosis in Nigeria before its deployment to the field. The detailed planning, and diligent execution based on the SOP, as well as strict monitoring and evalu ation by the relevant project teams, strengthened the validity of the study's outcome.

Furthermore, the study was limited by the use of solid sputum culture techniques with the attendant limitations however, the culture processing period of up to eight weeks and proper application of the laboratory SOPs ensured optimal mycobacterial growth and reduced

contamination rate. The first and second waves of COVID-19 pandemic which affected hospital attendance by all patients prolonged the study period.

4.2 : Conclusion

In the general population in Nigeria, the TB-LAMP's diagnostic accuracy for pulmonary tuberculosis among adult presumptive TB was superior to smear microscopy but, inferior to Xpert MTB/RIF. However, for the HIV sero-positive population, the PTB diagnostic accuracy of TB-LAMP among adult presumptive TB was equivalent to Xpert MTB/RIF and smear microscopy.

4.3 : Recommendation

Therefore, it is recommended that TB-LAMP be deployed and used as an alternative diagnostic test for PTB among adults especially in areas of Nigeria where Xpert MTB/RIF is not accessible.

ANNEXES

Annexe 1: Study Datasheet

Facility ID /__/ State ID /__/__/ Patient ID No. /_/_/_/_/_/

Date of recruitment: …… Date of sputum submission: ……

1) Age as at last birthday (years) ……

2) Sex [1]Male ☐ [2]Female ☐

3) Marital status [1]Married ☐ [2]Single ☐ Others (specify) ……

4) Weight (kg) ……

5) Height (meters) ……

6) Occupation ……

7) State / LGA of residence …… / ……

8) HIV status of participant [1] Negative ☐ [2] Positive ☐

9) History of use of antibiotics in the last 2 weeks [1]Yes ☐ [2] No ☐

If the answer to '9' above is "No", continue from item 12 below,

10) If yes to '9', state the name of antibiotics …… [1]Do not know ☐

11) How many days ago did s/he stop the antibiotics? ……days [1]Still on it ☐

12) Smear microscopy results:

Smear no.	Results (tick one)				
	Negative *(0 AFB/100 HPF)*	1 - 9/100 HPF *(Scanty; report actual number)*	+ *(10-99 AFB /100 HPF)*	++ *(1-10 AFB/HPF)*	+++ *(>10 AFB/HPF)*
Specimen 1 (Spot)					
Specimen 2 (Spot)					

13) TB-LAMP results:

Date sputum received	Final reaction volume *(30-35µl)*	Laboratory serial no.	Results (tick one)		
			Positive	Negative	Invalid

14) Xpert MTB/RIF test result:

Laboratory serial no.	Results (tick one)				
	Detected	Trace	Not Detected	Invalid	No result/Error
	Rifampicin resistance (tick one)				
	Detected		Not detected	Indeterminate	

15) Culture & speciation results:

Date Sputum received	Media used *(solid)*	Laboratory serial no.	Results (tick one)					
			Negative *(0 colony)*	1 – 9 *(<10 colonies)*	+ *(10-100 colonies)*	++ *(>100 colonies)*	NTM*	Contaminated

**Non-tuberculous mycobacteria*

Annexe 2: Informed Consent Form

Title of the research: EVALUATION OF TB-LAMP FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS IN NIGERIA

Name and affiliation of the researcher of applicant: Dr Bethrand ODUME, Chief of Party USAID TB LON Regions 1 & 2 Project; on behalf of KNCV Tuberculosis Foundation Nigeria located at Plot 564, Block 'B' Fourth Floor, AUJ Complex, 565 Independence Ave, Central Business District, Abuja

Sponsor of research: This study is sponsored by KNCV Tuberculosis Foundation Nigeria / USAID Tuberculosis Local Organizations Network (TB-LON) Regions 1 and 2 Project

Purpose of the research: The purpose of this research is to find out whether the New machine test known as HumaLoop T (TB-LAMP) manufactured by Eiken Chemical Company, Tokyo, Japan, is better than the use of the usual microscope or as good as the old machine in current use (Xpert MTB/RIF) for the diagnosis of pulmonary tuberculosis in Nigeria.

The procedure of the research: You will submit 3 sputum specimens today – one will be examined with the new machine (TB-LAMP), microscope, and the old machine in current use (Xpert MTB/RIF) while the 2nd sputum will be sent to a bigger laboratory for culture to see whether the organism causing tuberculosis will grow from the sputum. The 3rd sputum will also be examined with the microscope. You may be asked to submit another sputum if the ones you have submitted were not adequate. In total, we expect to recruit 2500 participants in this study throughout the country.

The research will last until we get enough participants but, you will only be involved for the period it takes you to produce 3 sputum specimens which is expected not to last for more than 1 hour.

Risks: The study poses no risk to you and shall not delay your treatment if the electric machine (Xpert MTB/RIF) shows that your sputum is positive for tuberculosis causing germs. The research procedure is not expected to expose you to any physical or psychological harm. Your participation in this research will not cost you anything.

Benefits: The sputum culture result may help us know whether you need a special type of drug for treatment in case your result is positive. If the new machine is found to be as good

as the standard machine in current use, it will improve TB case detection in Nigeria because power failure and other challenges associated with the electric machine will be minimized.

Confidentiality: All information collected in this study will be given code numbers and no name will be recorded. This cannot be linked to you in any way and your name or any identifier will not be used in any publication or reports from this study. As part of our responsibility to conduct this research properly, authorized officials from KNCV Tuberculosis Foundation Nigeria may have access to these records.

Voluntariness: Your participation in this research is entirely voluntary**.** The services you will receive from this hospital will not be affected in any way in case you choose not to participate in the research. You are free to withdraw from the research at any time after signing this form but, note that some of the information and data from the sputum you have already submitted cannot be removed anymore and will be used in reports and publications without your details mentioned.

Due inducement(s): You will not be paid any fees for participating in the research but, you shall be compensated for the cost of your transport to this hospital.

What happens to research participants and communities when the research is over: Outcomes and recommendations from the study results will be circulated widely as appropriate and posted on the hospital's notice board for your information.

Statement of the person obtaining informed consent:

I have fully explained this research to_____and have given sufficient information, including about risks and benefits, to make an informed decision.

DATE:_____SIGNATURE: _____

NAME: _____

Statement of the person giving consent:

I have read the description of the research or have had it translated into a language I understand. I have also talked it over with the health staff to my satisfaction.

DATE:_____SIGNATURE/THUMB PRINT: _____

NAME: _____

Contact information of Research/Project Supervisor:

Dr Nkiru NWOKOYE
KNCV Tuberculosis Foundation Nigeria
Plot 564, Block 'B' Fourth Floor, AUJ Complex, 565 Independence Ave, Central Business District, Abuja
Telephone: 0813 650 1810

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