

Evaluation of TB-LAMP assay for the diagnosis of pulmonary tuberculosis in Lusaka, Zambia

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Abstract

Microscopy is still widely used for screening tuberculosis in Zambia. Although economical, this test has poor sensitivity. Assays based on Nucleic acid amplification technique (NAAT) detect *Mycobacterium tuberculosis* complex with a reliable sensitivity. However, the utilization of current NAAT based methods is limited by their cost, particularly in low income settings. We evaluated the performance of TB LAMP method (a low cost NAAT developed by Eiken Co., LTD) among presumptive TB patients in Lusaka. From January to July 2018 this study utilized leftover sputum samples (after routine fluorescent microscopy) to perform TB-LAMP assay at three health institutions. The positivity rate for TB-LAMP assay was compared to the routine fluorescent microscopy. Samples with discrepant results between the two methods were cultured in these instances, culture results were considered gold standard. Out of 1480 clinical samples analysed, TB-LAMP assay showed positivity rate of 22.4% versus 14.6% for fluorescent microscopy. Of the discrepant samples (i.e. TB-LAMP positive, microscopy negative) that were cultured, *Mycobacterium tuberculosis* complex was isolated in 70.1% of these. Therefore, TB-LAMP assay demonstrated superior performance to detect TB cases compared to fluorescent microscopy. The portion of smear negative – TB LAMP positive cases that yielded *Mycobacterium tuberculosis* complex upon culture highlights TB cases being missed by microscopy test during routine laboratory diagnosis. Adoption of TB-LAMP method for routine TB screening by the National TB control program in Zambia could increase case detection.

Introduction

Zambia is listed among the 30 high TB burden countries because of its high TB incidence rate, 361/100000 population [1]. Smear microscopy is widely employed for TB diagnosis in Zambia. Although, simple and economical, published sensitivities for smear microscopy are variably poor and low [2-5] particularly in paediatric and HIV cases [6]. A method with low sensitivity rate can be attributable to false negative laboratory results leading to missed TB cases.

The country is currently scaling up the use of Xpert MTB/ RIF method for screening presumptive TB cases. Xpert MTB/RIF is a nucleic acid amplification technique (NAAT) developed by Cepheid, USA and endorsed by WHO [7]. High sensitive rates have been reported for this method [8]. However, its full utilization in low income settings is constrained by high cost and equipment maintenance requirements.

In 2016, WHO endorsed another NAAT assay called TB –LAMP developed by Eiken Chemical Company (Tokyo, Japan) and uses loop mediated isothermal amplification technique [9]. Using Bst polymerase enzyme, amplification occurs at a fixed temperature (67 degrees Celsius). Test implementation and cost–effectiveness analysis have shown that TB-LAMP is potentially a cost–effective alternative to sputum smear microscopy in settings where the Xpert MTB/RIF assay cannot be implemented due to its infrastructure and equipment maintenance requirements [9]. Accuracy evaluation studies conducted in different parts of the world have reported sensitivities of TB LAMP as similar to other NAAT based assays [10-12].

Zambia, a third world country with high TB burden needed to understand the performance of this low-cost molecular tool (TB –

LAMP) in its setting for possible replacement of smear microscopy. This study therefore compared the performance of TB-LAMP assay with fluorescent microscopy at three laboratories in Lusaka.

Materials and methods

Study design and sample collection

This was a multicentre study conducted between January 2018 to July 2018 at three (03) hospital laboratories in Lusaka, namely; University Teaching Hospital, Chest diseases laboratory and Matero Level 1 Hospital laboratory. Sputum samples were clinically collected from presumptive TB patients aged 15 years and above seeking health care services at health centres in Lusaka (Chawama and Kamwala) where routine microscopy was performed. Left over sputum samples were then transported to the three study laboratories to perform TB-LAMP assay. Technicians performing TB-LAMP assay were blinded to microscopy results. Samples with discordant results between microscopy and TB-LAMP assay were cultured and in these instances culture results were considered as gold standard.

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Ethical approval

This study was approved by The University of Zambia Biomedical Research Ethics Committee (UNZABREC reference no. 009-11-17). All the patients were informed of the potential utilization of their clinical specimens for research after clinical usage and written informed consents were obtained.

Fluorescent microscopy

Direct sputum smear microscopy was performed following procedures already reported by others [13]. Briefly, heat fixed smears were flooded with 0.1% auramine O solution and allowed to stain for 15 minutes. The smears were then rinsed with distilled water and decolorized with 0.5% acid alcohol for 2 minutes. Smears were rinsed again with distilled water and flooded with 0.5% potassium permanganate. Lastly, smears were air dried following a final rinse and examined under fluorescent microscopes.

TB -LAMP assay

TB-LAMP assay was performed as described in the manufacturer kit insert (Eiken Chemical CO., LTD). Operators transferred a small volume of sputum (60µL) to heating tubes already containing lysis buffer. The tubes were mixed and placed in a heating block (LoopampLF-160) at 90°C for 5 minutes. After cooling, the heating tubes were joined to tubes containing absorbent material which remove inhibitors. The extracted solution was then expressed directly from absorbent tubes into the reaction tubes, containing dried form reagents. After reconstituting the sample with the reagent, tubes were thoroughly mixed and placed into the reaction block at 67°C for 40 minutes and the results were examined visually by checking for fluorescence in positive samples.

TB Culture

Sputum samples were cultured using BACTEC TM 960 MGIT (Mycobacterium Growth Indicator Tube) system as described by Becton, Dickson and Company, Franklin Lakes, 106 NJ, USA. Briefly, in biosafety level 3 laboratories, sputum samples were decontaminated using 4% sodium hydroxide and 0.5mL of homogenised sample was added to MGIT tubes containing PANTA/Growth supplement and loaded into the BACTEC 960 instrument. Positive tubes were differentiated using Tauns Capilia identification kit (CapiliaTB-Neo,

Tauns Laboratories, Inc. 761-1, Kamishima, Izunokuni, Shizuoka, Japan) after Zielh neelsen identification of acid-fast bacilli and ruling out contamination on blood agar.

Statistical analysis

We compared the positivity rates between fluorescent microscopy and TB-LAMP assay for the detection of *Mycobacterium tuberculosis* Complex in sputum samples. Chi-square (McNemar Test) at 95% confidence interval (CI) was employed to test the observed difference and the *p*-value of 0.05 was set for statistical significance.

Results

In total, 1480 patients participated in the study and males aged between 30 – 39 years were the majority (35.5%) (Table 1).

Positivity rates for TB-LAMP and Fluorescent microscopy

At all the three study laboratories, the positivity rate for TB-LAMP was higher than that of fluorescent microscopy with the biggest margin difference observed at Chest diseases laboratory, 23.5% vs 11.5%, respectively. Overall, the positivity rate for TB-LAMP assay was 22.4% (95% CI 20.24% ~ 24.49%) while for fluorescent microscopy was 14.6% (95% CI 12.79% ~ 16.40%), *p* < 0.000001 (Table 2).

Discrepant results between TB-LAMP and fluorescent microscopy

A total of 129 specimens had discrepant results between smear microscopy and TB-LAMP assay. Out of this number, 122 cases were negative on smear microscopy but positive with TB-LAMP assay and 7 were positive with smear microscopy but negative with TB-LAMP assay (Table 3).

Culture results

Sputum samples with discrepant results (129) were cultured. Out of the 122-smear negative but TB-LAMP positive cases that underwent culture, 15 cultures were contaminated and therefore excluded from the analysis. Of the remaining 107 tests, *Mycobacterium tuberculosis* complex (MTBC) was isolated from 70.1% (75/107) cases. Five (05) specimens grew *Mycobacterium other than tuberculosis* (MOTT) and 27 were culture negative (Table 4).

Table 1. Tabulating participation of patients by gender and age group

	Males	Females	Total (%)
<i>Age group</i>			
< 20 years	22 (2.6%)	23 (3.6%)	45 (3.0%)
20 – 29 years	170 (20.0%)	192 (30.5%)	362 (24.5%)
30 – 39 years	302 (35.5%)	179 (28.4%)	481 (32.5%)
40 – 49 years	198 (23.3%)	125 (19.8%)	323 (21.8%)
50 - 59 years	81 (9.5%)	52 (8.2%)	133 (9.0%)
60 years and above	75 (8.8%)	57 (9.0%)	132 (8.9%)
Unknown	2 (0.2%)	2 (0.3%)	4 (0.3%)
Total	850	630	1480

Table 2. Positive and negative rates for TB-LAMP and fluorescent microscopy at each study laboratory

STUDY LABORATORY	POSITIVES % (no.)		NEGATIVES % (no.)	
	TB - LAMP	Fluorescent Microscopy	TB-LAMP	Fluorescent Microscopy
University Teaching Hospital	19.5% (100/512)	13.3% (68/512)	80.5% (412/512)	86.7% (444/512)
Chest Diseases Laboratory	23.5% (110/468)	11.5% (54/468)	76.5% (358/468)	85.5% (414/468)
Matero Level 1 Hospital	24.2% (121/500)	18.8% (94/500)	75.8% (379/500)	81.2% (406/500)
Overall	22.4% (331/1480)	14.6% (216/1480)	77.6% (1149/1480)	85.4% (1264/1480)

Table 3. Comparison of smear microscopy and TB-LAMP results

		Smear microscopy		Total
		+	-	
TB-LAMP	+	209	122	331
	-	7	1142	1149
Total		216	1264	1480

Table 4. Culture results for samples with discrepant results between TB-LAMP assay and fluorescent microscopy

PURE-TB-LAMP (1480)	Smear microscopy (1480)		MGIT-CULTURE (129*)			
	Positive	Negative	MTBC isolated	NTM isolated	No growth	Contaminated
Positive	209	122*	75	5	27	15
Negative	7*	1142	3	1	3	0

*Discrepant results between TB-LAMP and smear microscopy

Discussions

In the current study conducted in Lusaka Zambia, TB-LAMP assay has shown higher positivity rate than fluorescent microscopy. We utilised clinical sputum samples of which most of the samples were obtained from male presumptive TB patients. The age group 30–39 years showed the highest frequency of participation (32.5%). This finding is consistent with what was observed in the Zambia national TB prevalence survey 2013–2014 which showed that the prevalence of laboratory confirmed TB was high in males and in the HIV positive individuals in the age group 35–44 years [14].

Positivity of tests

Overall, the positivity rate for TB-LAMP assay was 22.4% and that of fluorescent microscopy was 14.6%, $p=0.00000005$. Our results are in agreement with previous studies on TB-LAMP evaluation which have reported higher diagnostic performance of this assay than smear microscopy. For instance, in a South African study, the sensitivity for TB-LAMP test was almost double that of smear microscopy (74% versus 45%) [15]. A much higher sensitivity for TB-LAMP (97.2%) was reported in a multi-center study conducted in India, Uganda and Peru [16]. Overall, a meta-analysis compiled by WHO reported 78% sensitivity for TB-LAMP versus 63% that of smear microscopy [9]. Based on these evidences, WHO has endorsed TB-LAMP (Eiken Chemical.Co.) as a second NAAT (other than Xpert MTB/RIF) for TB diagnosis among adult patients with signs and symptoms of TB. On the other hand, low sensitivity of TB-LAMP was reported from Malawi where TB-LAMP sensitivity was lower than fluorescence microscopy (65% versus 87.5%) [17]. In such settings further studies are needed before TB LAMP assay is adopted for routine application. Worth noting about the design for the Malawian study is that concentrated fluorescence smear microscopy with routine double reading were employed. Such procedures are unusual in routine practice particularly in peripheral laboratories for low income countries.

Outcomes for cultured discrepant samples

Detection of *Mycobacterium tuberculosis*, particularly in smear-negative tuberculosis cases, is a priority for TB control. In the current study, among the TB-LAMP-positive and smear-negative cases (discordant samples) that were cultured, 70% grew *Mycobacterium tuberculosis complex*. This figure is within the estimated performance for molecular assays of 50-80% sensitivity among smear negative-culture positive specimens [18]. In a similar study from Cameroon, TB-LAMP test was observed to be positive in 62 patients from the

total of 96 who were smear negative but culture positive [19]. Another study in China reported 53.81% sensitivity for TB-LAMP assay among smear negative-culture positive cases [20]. These findings indicate the volume of TB cases misclassified as negative by laboratories performing smear microscopy and therefore pose a high likelihood of clinical mismanagement of TB patients. As already hypothesized, undetected TB cases can lead to development of a severe disease in individual patients and continued spread of TB in the communities [21]. Fortunately, all the smear negative –TB LAMP positive patients who enrolled in our study and were confirmed having *Mycobacterium tuberculosis* by culture were recalled to health centres and started TB treatment as it was an ethical requirement.

On the other hand, 27 samples which were positive with TB-LAMP assay came out negative on MGIT culture. These results are usually interpreted as false positive results. However, other studies have shown that despite the culture method being considered as gold standard, it can misclassify positive TB samples as negative under certain conditions. For example, a study by Kim et al, (2018) investigated a 14.9% proportion of results that were positive with TB-LAMP assay but negative on culture and observed that all those samples were ultimately positive by real time PCR (polymerase chain reaction) [22]. Factors such as delay in culturing samples and previous TB treatment were speculated to be the cause of negative cultures. Other factors associated with missed detection of *Mycobacterium tuberculosis* by BACTEC MGIT 960 system include low bacterial load, low growth rate of the bacteria and harsh decontamination with 4% sodium hydroxide [23,24].

Another five (05) samples that were TB-LAMP positive and smear negative, grew Non tuberculosis mycobacteria (NTM). NTMs are known to be fast growers therefore have a potential to mask the growth of *Mycobacterium tuberculosis complex* (slow grower) in co-infected patients. Data from Zambia national TB prevalence survey reported 15.1% as the prevalence of NTM and 0.2% of these were co-infected (*Mycobacterium tuberculosis* and NTM) [25]. A similar proportion (0.2%) was reported as mixed infection of NTM and *Mycobacterium tuberculosis* by a study in Nigeria [26]. We however could not establish whether the five samples which were positive on TB-LAMP assay and grew NTM were mixed infection.

The impressive positivity rate of TB-LAMP assay in the current study and the isolation of *Mycobacterium tuberculosis complex* from smear-negative discrepant samples confirm the superiority of TB-LAMP assay over fluorescent microscopy. In line with WHO (2016) policy guidance, we speculate that replacement of smear microscopy with TB-LAMP assay by National TB control program (NTP) in Zambia can help increase TB detection in the country. Since the country is already rolling out another NAAT method (Xpert MTB/RIF), finding a synergistic and complementary application of the two molecular tools would be valuable to the NTP in Zambia. For instance, TB-LAMP could be placed in peripheral laboratories exploiting the attribute of instrument (Loopamp LF- 160) robustness to harsh conditions while GeneXpert machines can be utilised at relatively high level of health care facilities with improved laboratory infrastructure [27].

Study limitations

In the current study, we could not culture all the samples to enable computation of sensitivities and specificities for TB-LAMP and fluorescent microscopy. Alternatively, while working within available resources, this study cultured all discrepant samples to verify the positives and negatives.

Conclusions

TB-LAMP assay has shown high positivity rate (22.4%) compared to fluorescent microscopy (14.6%), demonstrating its potential to increase TB case detection if adopted as a testing method for presumptive TB patients. The significant proportion (70.1%) of smear-negative but TB-LAMP positive cases that grew *Mycobacterium tuberculosis* complex upon culture, supports an urgent need by the national TB control program (NTP) in Zambia to replace smear microscopy with a profound method for screening presumptive TB patients.

Authorship and contributions

Authors E.S.S, G.M and D.N conceptualised the study while A.L.S, P.S.L and F.M.Z reviewed the study outcome. Authors M.M, C.M and J.M participated in data acquisition. The final manuscript was written by E.S.S

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Competing interest

None of the authors declared any conflict of interest regarding the publication of this manuscript.

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