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Evaluation of a loop-mediated amplification test for rapid diagnosis of tuberculosis in Ghana

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Abstract

Background: The unavailability of cheap but rapid, highly sensitive and specific diagnostic tools for tuberculosis (TB) remains a major setback for the global efforts to end TB by the year 2035. Nucleic acid-based TB diagnostic assays remain the most recommended and the Gene Xpert MTB/RIF (Cepheid Sunnyvale, United States) is the most widely used which has an added advantage of detecting rifampicin resistance. However, the machinery requirement of the GeneXpert MTB/RIF makes it unsuitable for use in rural and resource-limited settings eventually challenging the global efforts to end TB by the year 2035. Loop-mediated amplification (LAMP) of DNA technology presents a cheap alternative for the precision diagnosis of TB.

Objective: In this study, we evaluated the specificity and sensitivity of the TB-LAMP assay kit manufactured by Human Diagnostics Worldwide (Geneva, Switzerland) for the diagnosis of TB in Ghana**.**

Methods: We assessed the performance of the TB-LAMP assay against a panel of genotyped mycobacteria (including members of the *Mycobacterium tuberculosis* complex (MTBC) and a couple of nontuberculous mycobacteria) and sputum samples collected from presumptive TB patients using sputum culture as reference diagnostic assay.

Results: The TB-LAMP assay was found to be very specific in detecting members of the MTBC as positive samples whereas the nontuberculous mycobacteria were all negative. Using sputum culture as a reference, the TB-LAMP assay was found to have 99.2% sensitivity, 97.2% specificity, 98.5% positive predictive value (PPV), 98.6% negative predictive value (NPV) and 98.5% accuracy for detection of MTBC among sputum samples collected from presumptive TB patients in Ghana. The TB-LAMP assay additionally showed 100% accuracy in detecting members of the MTBC among a panel of mycobacteria.

Conclusion: The TB-LAMP is highly sensitive and specific for the diagnosis of TB. It is thence recommended for use as a primary screening tool before referral for culture and sensitivity assays for better management of TB.

Keywords: *Mycobacterium tuberculosis*, loop-mediated amplification, isothermal, diagnostics, Ghana

INTRODUCTION

Tuberculosis (TB) is caused by a closely related acid-fast bacillus called the *Mycobacterium tuberculosis* complex (MTBC). They are divided into human-adapted (hMTBC) namely *M. tuberculosis sensu stricto* (Mtbss) and *M. africanum* (Maf) as well as the animal adapted (aMTBC) that infects various animals [1]. There are 7 phylogenetic lineages (L) of the hMTBC including L1-4 and L7 classified under Mtbss and L5 and L6 under Maf [2]. The hMTBC exhibit unique global phylogeography with West Africa harbouring six out of the 7 lineages [2,3]. Tuberculosis is one of the top-ten causes of death and remains the leading cause of adult mortality by a single infectious disease other than the new global pandemic

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coronavirus disease of 2019 [4]. An estimated 10 million people fell ill with TB in 2019 among which 8.2% were people living with HIV. There were 56% men, 32% women and 12% children who fell ill with TB in 2019 [4]. An estimated 1.4 million (1.2 million HIV and about 2 hundred thousand non-HIV) people died from TB disease in 2019 [4]. The control of TB relies on the vaccination of neonates using the only WHO-approved Bacillus Calmette–Guérin (BCG) vaccine, case detection and treatment. However, case detection and treatment remain the most functional control strategy due to the inability of BCG to prevent TB disease other than disseminated disease among children under 5 years [5]. However, the lack of accurate and rapid TB diagnostics is a major reason why TB remains a very important global public health emergency.

Until recently, sputum smear microscopy (with about 50% sensitivity) was the first line TB diagnostic tool [6,7]. Sputum culture assays (solid or liquid media cultures) give 80-95% sensitivity but require 2 to 8 weeks to produce results [8–10]. The long waiting window contributes to the loss of TB patients to follow-ups and monitoring hence negatively impacting proper management of TB. Nucleic acid amplification-based assays such as the line probe assays (LPA) MTBDR*plus* (Hain Lifesciences, UK) and GeneXpert MTB/RIF (Cepheid Sunnyvale, United States) which give relatively faster results are better options with over 98% sensitivity and specificity [11–13]. In addition to their high accuracy, GeneXpert MTB/RIF provides further information on susceptibility to rifampicin whereas the MTBDR*plus* detects susceptibility of the infecting pathogen to both rifampicin and isoniazid. The GeneXpert MTB/RIF and MTBDR*plus* take approximately 3 hours and 6 hours respectively to produce results. Even though MTBDR*plus* has an added advantage of diagnosing susceptibility to both isoniazid and rifampicin, GeneXpert MTB/RIF is the recommended molecular diagnostic tool in most TB diagnostic centres [14]. This is mainly due to the infrastructure required for the efficient running of MTBDR*plus* and the relatively longer turnaround time, hence, relegating it to reference laboratories and academic research institutions. The GeneXpert MTB/RIF also requires specialized instrumentation, long-term equipment maintenance and continuous electricity supply. Implementation of GeneXpert MTB/RIF in resource-limited countries is thus saddled with financial costs [15].

A potential DNA-based alternative for TB diagnostics under resource-limited settings is the emerging loop-mediated amplification tests for TB (TB-LAMP) [16–20]. It is based on isothermal amplification of the MTBC-specific insertion sequence 6110 (IS 6110) and DNA gyrase subunit B gene [21]. The method is very rapid and gives results within an hour at most. The test generates a fluorescent result that can be detected by naked eye visualization instead of expensive equipment, reducing operational costs. There are promising data on this assay's high sensitivity and specificity [16–20]. However, the geographical settings where these reports come from have limited diversity of the MTBC. There is therefore the need to evaluate this assay taking into consideration the existing diversity of the MTBC. Since Ghana is one of the few countries with the highest diversity of the hMTBC (6 out of the 7 main phylogenetic lineages) specifically L1-L6 causing TB, we sought to evaluate the applicability of the TB-LAMP assay in Ghana against sputum culture as the gold standard and evaluate its specificity against a spectrum of genotyped MTBC and nontuberculous mycobacterial isolates.

MATERIALS AND METHODS

Collection of sputum samples

Sputum samples used for this study came from the routine sputum transport system of the National Tuberculosis Control Program (NTP) to the Noguchi Memorial Institute for Medical Research (NMIMR) for microbiological diagnosis. We received sputum from presumptive TB patients reporting to TB diagnostic clinics in the Ashanti, Brong Ahafo, Central, Eastern, Greater Accra, Northern, Upper East, Volta, and Western regions of Ghana. The standard procedure for sputum sample collection as outlined by the NTP for routine diagnosis of TB in Ghana was followed. Sputum samples were transported in a cold chain to the NMIMR for further analysis.

LAMP assay for detection of MTBC

The TB-LAMP assay was carried out with the Loopamp™ MBTC kit following the manufacturer's protocol. Briefly, 60 µL of each raw sputum sample was pipetted into respectively labelled heating tube containing custom-made lysis buffer and closed tightly. The tubes were then inverted five times to ensure uniform mixing of the content before incubating in the heating unit of Humaloop provided by Human diagnostics at 90 ºC for 5 minutes. The heating tubes were then screwed into the absorbent tube and vigorously shaken until a milky solution was obtained. An injecting cap was screwed onto the adsorbent tube and the adsorbent tube was pressed to elute 60 µL of purified DNA into customized polymerase chain reaction (PCR) tubes containing lyophilized PCR master mix inside the cap. About 60 uL of purified DNA of the MTBC reference strain H37Rv and sterile nuclease-free water were added into respectively labelled tubes and used as positive and negative controls. The caps of the tubes were closed and inverted to allow the eluted DNA to settle inside the cap. The inverted tubes were incubated at room temperature for 2 minutes to ensure resuspension of the PCR master mix and quickly spun to collect the resulting mixture at the base of the PCR tubes. The reaction tubes were then incubated at 67 ºC for 45 minutes for isothermal amplification. The tubes were inserted into the detection unit with the UV light on for observation. Fluorescing and non-fluorescing tubes under ultraviolet illumination were respectively reported as positive and negative for the presence of MTBC.

Sputum decontamination and culture

Sputum samples were decontaminated by the 5% oxalic acid method [22] and inoculated on two pairs of Lowenstein-Jensen Media slants (supplemented with either

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glycerol or pyruvate), incubated at 37 ºC until macroscopic growth was observed. Colonies from positive cultures were sub-cultured on similar media and incubated as above until confluent growth was observed and confirmed as acid-fast bacilli by Ziehl-Neelsen staining and light microscopy.

Specificity of the TB-LAMP assay

We evaluated the specificity of the human Loopamp™ MTBC detection kit a TB-LAMP assay (Human Diagnostics Worldwide, Germany) for the detection of members of the MTBC. The DNA was extracted from a panel of mycobacteria spanning different lineages of the MTBC and some nontuberculous mycobacteria. The DNA was added into labelled Human Loopamp™ MTBC detection PCR tubes and processed similarly using the procedures described above for the sputum samples. Detection of positive and negative samples was also carried out using the same procedures described above with MTBC H37Rv and sterile nuclease-free water as positive and negative controls respectively.

Table 1: Distribution of samples from Eight Regions of Ghana

		Gender		
Region	Samples	Males	Females	NA
Ashanti	9	6	3	-
Brong Ahafo	4	3	1	-
Central	6	4	2	-
Eastern	29	21	8	-
Greater Accra	101	76	25	-
Northern	11	-	-	11
Upper East	3	2	1	-
Volta	40	33	7	-
Western	2	2	-	-
Total	205	147	47	11

*NA, not available

Table 2: Specificity of TB-LAMP for detecting diverse members of the MTBC

No	Genotype	TB-LAMP Results
1	MTBC-L4-Cameroun	Positive
2	MTBC-L4-Ghana	Positive
3	MTBC-L4-Harlem	Positive
4	MTBC-L4-Lam	Positive
5	MTBC-L4-Uganda 1	Positive
6	MTBC-L4-Uganda 2	Positive
7	MTBC-L2-Beijing	Positive
8	MTBC-L1	Positive
9	MTBC-L3	Positive
10	MTBC-L5.C1	Positive
11	MTBC-L5.C2	Positive
12	MTBC-L5.C3	Positive
13	MTBC-L5.C4	Positive
14	MTBC-L5.C5	Positive
15	MTBC-L6.C1	Positive
16	MTBC-L6.C2	Positive
17	MTBC-L6.C3	Positive
18	MTBC-Bovis	Positive
19	*M. aurum*	Negative
20	*M. smegmatis*	Negative

* MTBC, *Mycobacterium tuberculosis* complex; L1-lineage 1, L2-lineage 2, L3-lineage 3, L4-lineage 4, L5-lineage 5, L6-lineage 6; TB-LAMP, loop-mediated amplification tests for tuberculosis

Statistical analysis

Analysis was performed with STATA Statistical Software (Version 14, StataCorp LLC, College Station, TX). Sensitivity, specificity, accuracy (ACC), positive predictive value (PPV), and negative predictive value (NPV) of the Human TB-LAMP assay for detection of MTBC from direct sputum were estimated using the following formulae: Sensitivity = True positive (TP) / [TP + True negative (TN)]; specificity = TN / [False positive (FP) + TN]; ACC = (TP+TN) / [TP + TN + FP + False negative (FN)]; PPV = TP / (TP + FP) and NPV = TN / (FN + TN).

RESULTS

Demography

A total of 205 sputum samples from 205 presumptive TB patients were collected for this study. Among the 205 participants, data on gender was available for 194 participants among which 147 and 47 were males and females respectively (Table 1). Similarly, 149 of 205 participants provided information on age for which the youngest and oldest were 10 years and 79 years old respectively.

Detection of MTBC from direct sputum

After the TB-LAMP assay, 65.9% (135 out of the 205) of the sputum samples tested positive for MTBC (Figure 1). Of the 205 samples cultured for isolation of mycobacteria, 129 were positive, 71 were negative whereas the remaining five were contaminated. Out of the 129 mycobacteria positives cultures, 128 (99.2%) were positive for MTBC by the TB-LAMP assay with only one negative. Among the 71 culture-negative samples, 69 (97.1%) tested negative by the TB-LAMP assay with the remaining two being positive by the TB-LAMP assay. Using sputum culture as the standard for evaluation of the TB-LAMP, we found the TB-LAMP assay to have 99.2% sensitivity, 97.2% specificity, 98.5% positive predictive value (PPV), 98.6% negative predictive value (NPV) and 98.5% accuracy for detection of MTBC among sputum samples in Ghana.

Specificity of TB-LAMP assay

We tested the specificity of the TB-LAMP assay for the detection of MTBC irrespective of phylogenetic lineages or genotype. We found that among a panel of 20 mycobacteria, the TB-LAMP accurately detected every member of the MTBC but the two nontuberculous mycobacteria samples were not detected (Table 2).

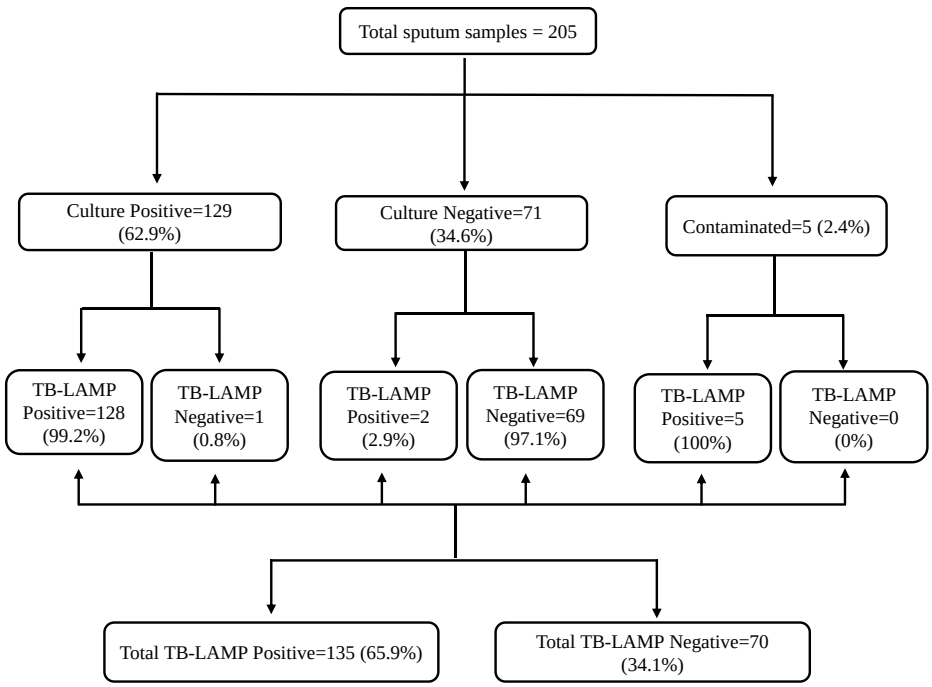

Figure 1: Detection of MTBC by sputum culture and the TB-LAMP assay.
*MTBC, *Mycobacterium tuberculosis* complex; LAMP, loop-mediated amplification

DISCUSSION

The promulgation of nucleic acid-based assays for diagnosis of TB has opened a new chapter for high precision diagnosis of the world's leading killer of mankind by a single infectious disease over the decades until the emergence of SARS-CoV-2 [4]. The WHO recommended GeneXpert MTB/RIF which in addition to diagnosing TB can also detect resistance to rifampicin (the backbone of the directly observed treatment short-course) has been a game-changer. Nevertheless, these two nucleic acid-based applications which are common in most well-equipped laboratory settings are unavailable in resource-poor landscapes where the TB burden is mostly the highest. Thus, there is a need for a cheap, fast, and accurate diagnostic tool that can be used in resource-limited settings to bolster the diagnosis of TB. The LAMP technology which is nucleic acid-based is fast, accurate, easy to use and requires less infrastructure and hence comes as the best option for peripheral or second-tier laboratories for diagnosis of TB. Whilst TB-LAMP assays have been evaluated in other parts of the world, it is yet to be evaluated in a geographical setting where TB pathogens of the highest diversity are found [16,21,23,24]. Using the Human Loopamp^{TM} MTBC detection kit, we tested the specificity of the TB-LAMP assay with a panel of genotyped clinical mycobacteria including the six phylogenetic lineages of the hMTBC, *M. bovis* (an animal strain) and a couple of nontuberculous mycobacteria in Ghana (one of the two countries with the highest diversity of the MTBC in the world). In addition, we evaluated the sensitivity and specificity of the assay using sputum culture as the reference. The observation that all eighteen genotyped MTBC strains were detected as positive MTBC whereas the two genotyped nontuberculous bacteria (*M. aurum* and *M. smegmatis*) gave negative results by the TB-LAMP assay validates the discriminatory power of the assay. Non-tuberculous mycobacteria are a major cause of TB-like symptoms especially among immunocompromised persons [25] and are mostly resistant to the standard anti-TB drugs [26,27]. Therefore, affected patients if misdiagnosed do not respond to the regular anti-TB therapy and are mostly mismanaged as drug-resistant TB patients with its accompanying clinical implications. Our finding is therefore essential because accurate separation of MTBC caused by TB from other TB-like symptoms caused by nontuberculous mycobacteria helps to promulgate proper management for specific patients. This finding also confirms the stability and universality of the target-biomarker of the TB-LAMP assay among members of the MTBC irrespective of the genotype and hence supports the use of this assay, especially under geographical settings with a high diversity of MTBC genotypes such as West Africa. When we evaluated the TB-LAMP using sputum from presumptive TB patients with culture as a reference,

the sensitivity and specificity were respectively 99.2% and 97.2%. Whereas the recorded sensitivity is comparable to the 99% reported in the previous evaluation in the Gambia [21], it is higher than the 86% reported from another evaluation in Haiti [28] and 88.8% reported in China [16]. Similarly, the specificity recorded in this study is higher than the 94% recorded in the Gambia but comparable to the 96.9% recorded in China and lower than the 98.4% recorded in Haiti and a multicenter evaluation report of 98.7% [16,21,24,28].

The recorded 100% negative predictive value and the overall 98.5% accuracy of the TB-LAMP assay by Human Diagnostics Worldwide (Geneva, Switzerland) suggest that the problem of false-positive diagnosis and its concomitant mismanagement of such cases can be avoided in clinical settings. This is very important to ensure that people who do not need to be put on anti-TB therapy are spared at least six months of extensive exposure to drugs meant for people infected with members of the MTBC. Such patients may be efficiently managed with common antibiotics if properly diagnosed and will not have to be confronted with potential side effects of anti-TB drugs. The performance of the TB-LAMP in this study was very similar to that of the WHO-recommended GeneXpert MTB/RIF [11–13,29]. However, the GeneXpert MTB/RIF can detect resistance to rifampicin in addition to TB diagnosis which is an added advantage. Nevertheless, the equipment requirement and cost of running GeneXpert MTB/RIF make it unsuitable for use under rural and or resource-limited settings whereas the 60 ^{o}C isothermal amplification of TB-LAMP can be easily adapted for use under such special settings using pocket palm warmer packs (temperature ranges from 40 ^{o}C to 70 ^{o}C depending on the manufacturer) that have been evaluated for use in LAMP assays targeting *M. ulcerans* [30].

Conclusion

The sensitivity and specificity of the TB-LAMP assay recorded in this study and the previous evaluations are highly comparable to the performance of GeneXpert MTB/RIF. Even though this assay is not meant to detect drug resistance and/or bacterial load, it has the potential to be used as a primary screening tool before referral for culture and sensitivity assays, especially in rural and/or resource-limited settings for better management of TB.

DECLARATIONS

Ethical considerations

Sputum samples used for this study came from the routine sputum transport system of the National Tuberculosis Control Program. The system coordinates the shipment of sputum from peripheral laboratories to centralized sophisticated laboratories for specialized diagnosis. Samples were used without reference to patients' data. This study did not warrant ethical review board approval because it was part of the routine activities of the National Tuberculosis control program under the Ghana Health Service

Consent to publish

All authors agreed to the content of the final paper.

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Competing Interests

No potential conflict of interest was reported by the authors.

Author contributions

IDO, DY conceptualized the study and were involved in the research investigations, methodology design, provision of resources, data curation, formal analysis, and writing and reviewing of the manuscript. TA, PM, SYA, PA, ABM helped to conduct the research investigations and data analysis, and were involved in the writing and reviewing of the manuscript. SO, SOY, GTO, GA, CL, AF, AP provided study resources and helped to write and review the final manuscript.

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Availability of data

Data is available upon request to the corresponding author.

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