

Short Communication

Loop-Mediated Isothermal Amplification for Rapid Identification of *Mycobacterium tuberculosis* in Comparison with Immunochromatographic SD Bioline MPT64 Rapid® in a High Burden Setting

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SUMMARY: Loop-mediated isothermal amplification (LAMP) was assessed for rapid identification of *Mycobacterium tuberculosis* complex (MTC) in comparison with an immunochromatographic test (ICT) using SD Bioline Ag MPT64 Rapid®. One hundred and fifty-one MGIT cultures positive for acid-fast bacilli were tested for MTC. DNA was extracted from a small portion of culture samples by heat lysis and subjected to LAMP analysis. Of these, 144 were positive and 5 were negative by both tests. One culture that was ICT negative but was LAMP positive was confirmed to have a mutation in the *mpt64* gene. The agreement was 98.68% (95% confidence interval [CI]: 94.80–99.77), and the kappa value was 0.83% (95% CI: 0.59–1.00). Good correlation results suggested that LAMP assay is a reliable molecular test for rapid identification of MTC and is practical for use in resource-limited, high burden settings.

Tuberculosis (TB) is a major public health problem and is still the most common deadly infectious disease worldwide. In Thailand, nearly 130,000 people suffer from active tuberculosis, and about 12,000 deaths occur annually. The nation is on the list of top 14 countries with a high burden of TB, TB combined with HIV, and multidrug-resistant TB (1). Although most mycobacterial infections are still caused by the *Mycobacterium tuberculosis* complex (MTC), a number of non-tuberculous mycobacteria (NTM) have been reported to cause a burden of pulmonary infections (2,3). Therefore, differentiation of MTC and NTM is important for prescribing appropriate treatment.

Culture followed by identification of MTC is still the gold standard for TB diagnosis. Recently, a number of genetic methods such as PCR or loop-mediated isothermal amplification (LAMP) have been applied to detect MTC directly from clinical specimens. However, these methods cannot differentiate live and dead TB bacilli. In the culture procedure, identification of MTC is needed for confirmation before reporting the culture results and proceeding to drug susceptibility testing. Previously, identification procedures depended on biochemical tests including susceptibility to para-nitrobenzoic acid (4).

These tests are labor-intensive, slow to yield results, and sometimes are not conclusive. Currently, the immunochromatographic test (ICT) that is commercially available, has been widely used for rapid culture confirmation of MTC both in liquid and solid media (5,6). Although ICT is a quick and easy-to-use test for differentiation between the MTC and NTM in acid-fast bacilli (AFB)-positive cultures, sufficient growth with prolonged incubation period is required to avoid false negative results (7). LAMP is a simple rapid and low-cost molecular technique with high specificity and sensitivity. The advantages of LAMP include its simplicity due to an isothermal profile without the need for expensive and complex equipment (8). The positivity of the reaction can be easily detected by visualization of a color change or observation of white precipitation or turbidity due to the pyrophosphate byproduct of the amplification reaction (9,10). Owing to its ease and cost-effectiveness, LAMP is a promising technique that could be easily applied for the direct detection or rapid identification of MTC (11).

This study aimed to assess an in-house TB-LAMP for rapid identification of MTC grown in MGIT media compared to the SD Bioline Ag MPT64 Rapid® (Standard Diagnostics, Gyeonggi-do, Republic of Korea), a commonly employed ICT in Thailand. Sputum samples were collected, decontaminated by N-acetyl-L-cysteine-NaOH treatment, inoculated in MGIT media, and examined for growth at 37°C (4). The bacteria grown on MGIT were observed for cord like morphology, growth rate, and Ziehl-Neelsen staining results. DNA was then extracted from cell suspensions positive for cord formation and AFB. Briefly, the initial 300 µl of MGIT cell suspension was collected in a microcentrifuge tube and

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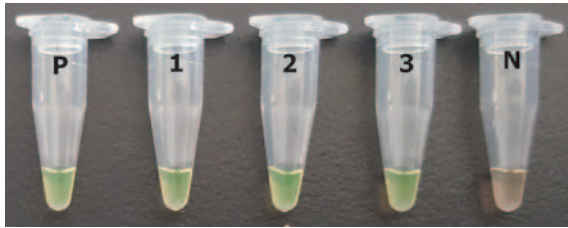


Fig. 1. (Color online) Identification of MTC by TB-LAMP. Coloric appearance of LAMP results, P, *M. tuberculosis* H37Ra positive control; N, MTC negative control; and 1–3, cultured samples.

briefly centrifuged. After obtaining the cell sediment, a volume of 100 μ l distilled water was added. DNA was then extracted by heating the cell suspension on a dry heat block at 80°C for 10 min. After brief centrifugation, an aliquot of the cell lysate was added to the LAMP mixture as described previously (12). After incubation at 65°C for 1 h on a small heat block, amplified DNA was examined for a color change (Fig. 1). The LAMP results were compared with those of the SD Bioline Ag MPT64 Rapid®. ICT testing was performed according to the manufacturer's instructions. The device could determine MTC rapidly based on the detection of a protein (MPT64) antigen secreted by MTC (7).

The specificity and sensitivity of TB-LAMP used in this study were examined previously (12). This TB-LAMP targeting the 16S rRNA gene was specific to MTC and could directly detect as low as 9 tubercle bacilli in sputum samples. In this study, 151 MGIT growth samples positive for AFB were selected. Of these, 145 were positive by LAMP. One sample that was positive by ICT was negative by LAMP. Another discrepant result was found with a sample that was negative by ICT but was positive by LAMP. A false negative result by ICT was reported to be possibly caused by the absence of MPT64 antigen due to mutation of its coding gene (13). Sequencing of the *mpt64* gene was performed using primers as described previously (14). Mutations are not frequent due to conserved sequences (13); however, we found a 63-bp deletion in the open reading frame of this particular sample. The latter was further identified as MTB based on the internal transcribed spacer sequence as previously described (15). NTM were interpreted in 5 samples that were positive for AFB but were negative by both tests (Fig 2). Sequencing confirmed negative results and identified the NTM species as *M. avium*, *M. intracellulare*, *M. abscessus*, and *M. kansasii*. Overall, the TB-LAMP results showed nearly complete agreement with those of the immunochromatographic test (Table 1). The agreement was 98.68% (95% CI 94.80–99.77) and the kappa coefficient value was 0.83 (95% CI 0.59–1.00) suggesting an excellent degree of agreement between the 2 methods. These concordant identification results confirmed the specificity and sensitivity of TB-LAMP for MTC. We observed the turnaround time and found that on average, identification results were available within 2 h by TB-LAMP and within 15 min using ICT. LAMP generated small amounts of contaminated residues without the bulk of cassettes, and simple heating for extracting DNA could reduce both the infection risk and cost. It was found that the heated

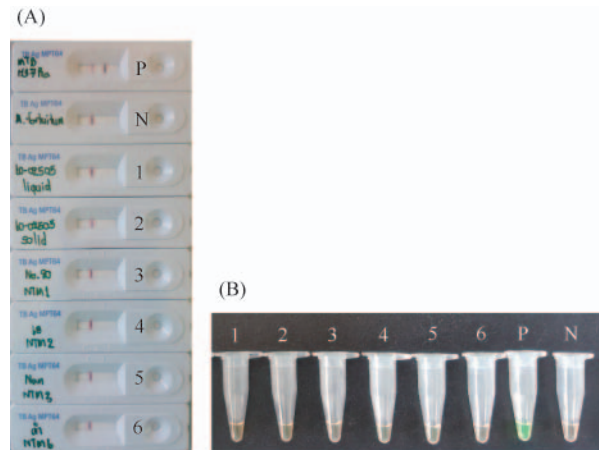


Fig. 2. (Color online) Identification of NTM by TB Ag MPT64 (A) and LAMP-TB (B). Results of 5 NTM (1 and 2 were the same isolate), P, *M. tuberculosis* H37Ra positive control and N, *M. fortuitum* negative control. NTM 1–2, *M. avium*; 3, *M. intracellulare*; 4–5, *M. abscessus*; 6, *M. kansasii*.

Table 1. Comparison of identification of MTC by LAMP-TB with ICT using SD Bioline Ag MPT64 Rapid®

Identification method		ICT		Total
		Positive	Negative	
LAMP-TB	Positive	144	1	145
	Negative	1	5	6
	Total	145	6	151
Agreement (%; 95% CI)		98.68 (94.80–99.77)		
Kappa (95% CI)		0.83 (0.59–1.00)		

MGIT content could be directly added to the LAMP mixture. Thus, LAMP is much safer than ICT. In addition, the reduced cost of the in-house LAMP makes it attractive. In conclusion, the study confirmed that TB-LAMP is a reliable test for the rapid identification of MTC. It is suitable for use in laboratories that perform mycobacterial culture for rapid and inexpensive diagnostic services supporting patient management.

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Conflict of interest None to declare.

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