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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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Running Title: LAMP for rapid identification of MTC

Keywords: tuberculosis, identification, LAMP, immunochromatography
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 (AFB) were tested for MTC. DNA was extracted from a small portion of culture samples by heat lysis and subjected to LAMP analysis. Of these, 1445 were positive and 5 were negative by both tests. One culture that was ICT negative but that 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 0.83% (95%CI (0.59-1.00), respectively. The good correlation result suggested that LAMP assay was a reliable molecular test for rapid identification of MTC. It is practical for use in resource-limited, high burden settings.
Tuberculosis (TB) is a major public health problem and 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 high burden of TB, TB and HIV and multidrug-resistant TB (MDR-TB)(1). Although most mycobacterial infections are still caused by *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.

Culturing followed by identification of MTC is still the gold standard for TB diagnosis. Recently, numbers of genetic methods such as polymerase chain reaction (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 culture procedure, identification of MTC is needed for confirmation before reporting 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 the results and sometimes are not conclusive. Currently, the immunochromatographic test (ICT) which is commercially available has been used widely 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 the 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 (7). LAMP is a simple rapid and low-cost molecular technique with high specificity and sensitivity. The advantages of the LAMP technique include its simplicity due to isothermal profile
without the need for expensive and complicated equipment (8). The positivity of the reaction can be easily detected by the visualization of a color change or observation of white precipitation or turbidity due to 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 to the direct detection or rapid identification of MTC (11).

The aim of the study was to assess an in-house TB-LAMP for rapid identification of MTC grown in MGIT media by comparing with SD Bioline Ag MPT64 Rapid® (Standard Diagnostics, 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 Zheel-Neelsen staining results. DNA was then extracted from cell suspension positive for cord formation and AFB. Briefly, the initial 300 µl of MGIT cell suspension was collected in a microcentrifuge tube followed by brief centrifugation. After obtaining cell sediment, a volume of 100 µl distilled water was added. DNA was extracted by heating cell suspension using a dry heat block at 80°C for 10 min. After brief spinning, an aliquot of cell lysate was added to the LAMP mixture as described previously (12). After incubation at 65°C for 1 h in a small heat block, amplified DNA was detected for a color change (Fig. 1). LAMP results were compared with those of SD Bioline Ag MPT64 Rapid®. The ICT testing was performed according to manufacturer’s instruction. 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 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. The other discrepancy result was found with another one 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 the mutation of its coding gene (13). Sequencing of the \textit{mpt64} gene was performed using primers as described previously (14). Mutations are not frequent due to conserved sequences (13), but we found the 63-bp deletion in the open reading frame. The latter was further identified to MTB based on internal transcribed spacer sequencing as described previously (15). Non-tuberculous mycobacteria (NTM) were interpreted in five samples that were positive for AFB but negative by both tests (Fig 2). Sequencing confirmed negative results and identified NTM species to be \textit{Mycobacterium avium}, \textit{Mycobacterium intracellulare}, \textit{Mycobacterium abscessus} and \textit{Mycobacterium kansasii}. In overall, the TB-LAMP results showed nearly complete agreement with that 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 the excellent degree of agreement between the two methods. These concordant identification results confirmed the specificity and sensitivity of TB-LAMP for MTC. We observed the turnaround time. On average, identification results were available within 2 hours by TB-LAMP and 15 min by 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 the cost. It was found that heated MGIT content could be added directly to the LAMP mixture. Hence, LAMP is much safer than ICT. In addition, the lower cost of in-house LAMP is attractive. In conclusion, the study
confirmed that TB-LAMP was a reliable test for rapid identification of MTC. It is suitable for use in laboratories performing mycobacterial culture for rapid and inexpensive diagnostic services supporting patient management.

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**Conflict of Interests:** None to declare.

**Ethics approval:** Not applicable.
References


Figure legends

**Fig. 1.** 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.

**Fig. 2.** 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®.

<table>
<thead>
<tr>
<th>Identification method</th>
<th>ICT</th>
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<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>LAMP-TB</td>
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<td></td>
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<tr>
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<td>1</td>
</tr>
<tr>
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<tr>
<td>Kappa (95% CI)</td>
<td>0.83 (0.59-1.00)</td>
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</table>


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