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 is still the most common deadly infectious disease. Treatment of TB is complex (MTC), a number of non-tuberculous mycobacteria (NTM) have been reported to sometimes be resistant to drug susceptibility (11). Although genetic methods such as PCR or loop-mediated isothermal amplification (LAMP) have been applied to detect TB, these methods cannot differentiate live and dead TB bacilli. In addition, 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, Gyunggi-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 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.

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.

Received April 3, 2018. Accepted September 27, 2018.

DOI: 10.7883/yoken.JJID.2018.128

*Corresponding author: Mailing address: National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand. Tel: 66-2-5801567, Fax: 66-2-9659700, E-mail: benjapsk@health.moph.go.th
b Briefly centrifuged. After obtaining the cell sediment, a volume of 100 μl 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 turn-around 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 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.

**Acknowledgments** This work was financially supported by the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand. The work is a part of the program of the Japan-Thailand Research Center Collaboration on Emerging and Re-emerging Infections (RCC-ERI)-Osaka University. The study was supported partially by Japan Agency for Medical Research and Development (AMED) and a grant for the Joint Research Program of the Research Center for Zoonosis Control, Hokkaido University from Ministry of Education, Culture, Sports, Science and Technology (MEXT).

**Conflict of interest** None to declare.

**REFERENCES**