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Short Communication

Applicability of In-House Loop-Mediated Isothermal Amplification for Rapid Identification of *Mycobacterium tuberculosis* Complex Grown on Solid Media

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SUMMARY: A simple, rapid, and low-cost identification method is required in tuberculosis high-burden countries. We report the applicability of in-house loop-mediated isothermal amplification (LAMP) targeting 16S ribosomal RNA for the rapid identification of *Mycobacterium tuberculosis* complex grown on Lowenstein–Jensen media. Eighty acid-fast staining-positive clinical isolates were selected and used to evaluate the LAMP assay in comparison with polymerase chain reaction and conventional culture-based tests. The LAMP assay identified 60 *M. tuberculosis* isolates from 80 clinical isolates using simple heat-extracted DNA directly from the colony suspension. The results were in complete agreement with those obtained using the other methods, and the utility of the direct LAMP assay from a colony was demonstrated. The LAMP assay appears to be a practical and low-cost method that can be used for the rapid identification of *M. tuberculosis* isolates and suitable for endemic low-resource settings.

Tuberculosis (TB) is still the most common deadly infectious disease worldwide. In Thailand, almost 130,000 people suffer from active TB and approximately 11,000 deaths occur annually. The nation ranks 18th on the list of 22 ''TB high-burden countries'' in the world (1). Although most mycobacterial infections are still caused by *Mycobacterium tuberculosis* complex (MTC), nontuberculous mycobacteria (NTM) have been documented to cause a number of human pulmonary infections in developed and developing countries (2–3). The increasing incidence of TB and NTM infections caused by the acquired immunodeficiency syndrome (AIDS) epidemic has resulted in the need for rapid and accurate identification of isolates grown on media so that appropriate treatment can be prescribed.

The culture and identification of MTC is still the gold standard for diagnosing TB, although MTC can be directly identified from clinical specimens using genetic methods such as polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP) because these methods cannot differentiate between live and dead TB bacilli. However, conventional identification methods depending on biochemical assays and the phenotypic examination of colony formation on solid media are labor-intensive and time-consuming owing to the slow growth rate of MTC (4). In addition, the results of biochemical tests are sometimes inconclusive because of their low sensitivity and reproducibility. Thus, a rapid means of identifying MTC is essential for enhancing diagnostic services in mycobacteria laboratories and for improving the management of patients. PCR-based methods for the detection of MTC grown on Lowenstein–Jensen (L–J) media have been reported and proposed as an alternative method (5). Till date, the use of traditional nucleic acid amplification appears to be restricted to the laboratory setting, equipment, and technical expertise. The immunochromatography test (ICT) is commercially available and is widely used for the rapid confirmation of *M. tuberculosis* cultures (6–7). Although ICT is an easier method for the detection of MTC from culture samples, adequate growth with prolonged incubation is necessary to avoid false negative results (8). In recent years, several new molecular techniques, including LAMP, have been developed and used for *M. tuberculosis* detection (9–12). Compared with modern molecular methods, which are relatively complex and economically unsuitable for laboratories with limited resources, LAMP is a low-cost

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molecular assay that combines specificity and sensitivity. The advantages of the LAMP technique include its simplicity, i.e., isothermal amplification at a constant temperature of 60°C–65°C without any need for sophisticated equipments such as a thermal cycler, and the direct visual inspection of gene amplification in the reaction tube as opposed to analysis by gel electrophoresis (13–17). The positivity of the reaction can be easily detected by the naked eye as a color change or the observation of a white precipitate (15,17). Because of its simplicity and cost effectiveness, LAMP is a promising molecular technique that could be readily applied to the rapid detection and identification of *M. tuberculosis* in resource-limited settings.

This study aimed to assess the performance and applicability of in-house LAMP, known as TB-LAMP, to the rapid and accurate identification of MTC grown on L–J medium, which is used commonly in conventional mycobacterial culture. Sputum samples were collected, decontaminated by N-acetyl-L-cysteine-NaOH treatment, inoculated onto L–J slants, and examined for growth or contamination at 37°C (4). The bacteria that grew on L–J medium were examined to determine their colony morphology, growth rate, pigmentation, and Ziehl–Neelsen staining results. DNA was extracted from colonies recovered from L–J media that were positive for acid-fast bacilli (AFB) using a simple boiling method. In brief, putative small colonies were picked and suspended in a microcentrifuge tube containing 100 μl distilled water. DNA was extracted by boiling the cell suspension using a dry heat block at 80°C for 10 min. After brief spinning at 2,000 × g for 5 s, an aliquot of cell lysate was added directly to the LAMP reaction mixture as described in previous studies (12,18). In brief, LAMP for MTC was performed in a 20-μl reaction mixture, which contained 2.0 μl of 10 × LAMP buffer [200 mM Tris-HCl (pH 8.8), 100 mM KCl, 100 mM NH_4SO_4, and 1% Triton X-100], 14 mM dNTPs, 0.8 M betaine, 300 mM $MgSO_4$, 30 pmol FIP and BIP primers, 20 pmol of FL and BL primers, 5 pmol F3 and B3 primers, 8 U *Bst* DNA polymerase (New England Biolabs, Inc., Ipswich, Md., USA), 1 μl Fluorescent Detection Reagent (FDR; Eiken Chemical Co., LTD., Tokyo, Japan) and 7 μl cell lysate. The set of 6 primers used in this study was described in our previous studies (12,18). After incubation at 65°C for 1 h in a small heat block, DNA amplification was detected in LAMP reactions as a color change from orange to green using the naked eye (Fig. 1). The results were compared with those of multiplex PCR analysis, which could differentiate between MTC and NTM in a single tube, as well as classical biochemical tests, which comprised niacin accumulation, nitrate reduction, catalase production, and susceptibility to paranitrobenzoic acid (PNB) when heavy growth of each isolate was achieved (4).

The specificity and sensitivity of TB-LAMP were intensively examined in our previous study of direct detection from clinical specimens (12) and liquid culture (18). TB-LAMP was specific to MTC and could directly detect as few as 9 tubercle bacilli in sputum samples. However, the direct detection of MTC from clinical specimen cannot differentiate between living and dead TB bacilli, while liquid culture examination is not sustainable in many developing countries because of its

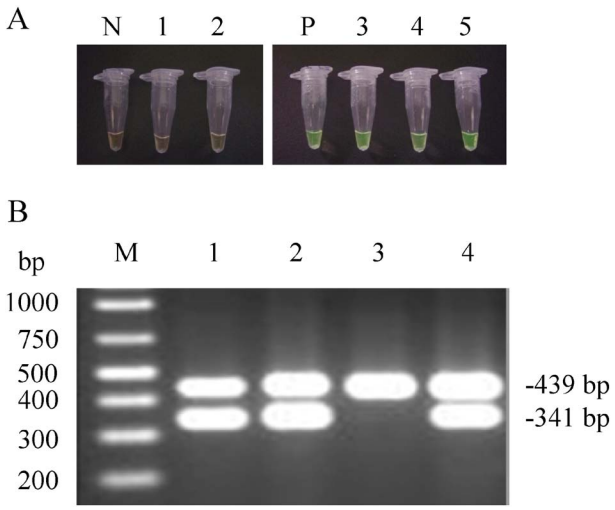

Fig. 1. Identification of *M. tuberculosis* complex isolates by TB-LAMP and multiplex PCR.

DNA extracted by heat lysis from mycobacterial clinical isolates positive for acid-fast bacilli was used for TB-LAMP and multiplex PCR reactions. *M. tuberculosis* complex and non-tuberculous mycobacteria were identified by visual observation of the color change in LAMP and by agarose gel electrophoresis in multiplex PCR. A. Visual appearance of LAMP results showing N: negative control, 1–2: non-tuberculous mycobacterial isolates, P: positive control, and 3–5: *M. tuberculosis* complex isolates. B. Multiplex PCR results analyzed by agarose gel electrophoresis. A 439-bp DNA band specific for *Mycobacterium spp.* reported to contain NTM in the samples. PCR positive for 439 bp specific for *Mycobacterium spp.* and 341 bp specific for MTC reported as containing MTC in the samples. Lane M: DNA marker; Lane, 1, 2 and 4: PCR product from *M. tuberculosis* complex isolates and Lane 3: PCR product from non-tuberculous mycobacterial isolates.

cost, although it is recommended in many settings. Solid culture examination is still considered essential, and it has been used in many developing countries. Thus, we examined the feasibility of TB-LAMP for the detection of MTC in early culture-positive samples. Using TB-LAMP, 60 clinical isolates, which were presumed to be MTC on the basis of visual observations of the colonies, were rapidly identified as MTC by TB-LAMP. As expected, the remaining 20 isolates, which were presumed to be NTM, yielded negative results with TB-LAMP. The TB-LAMP results were in complete agreement with the multiplex PCR results (Fig. 1). In addition, all the isolates with the biochemical characteristics of MTC and susceptibility to PNB exhibited positive results by TB-LAMP (Table 1). There were no false-positive identification results using TB-LAMP among all 20 NTM isolates (Table 1). These concordant identification results confirmed the specificity of TB-LAMP for MTC, and the simple DNA extraction from the tiny colony on L–J medium at the beginning of culture growth made identification by this method much quicker than that by the combination of biochemical tests and susceptibility to PNB. In addition, the overall procedure of the TB-LAMP assay allowed *M. tuberculosis* identification to be completed in less than 2 h without any requirements of expensive or complex instruments. The LAMP system uses a simple aluminum heat block that can be powered by a handy battery, therefore, it can be used in difficult settings that experience power interrup-

Table 1. Concordance of identification results by TB-LAMP with conventional methods

	M. tuberculosis complex*	Non-tuberculous mycobacteria
TB-LAMP		
Positive	60 (100%)	0 (0%)
Negative	0 (0%)	20 (100%)

*Isolates were identified by conventional methods, multiplex PCR, biochemical tests, and PNB susceptibility.

tions. This study clearly demonstrated the utility of TB-LAMP for the rapid identification of MTC grown on L–J medium. Conventional identification has a long delay because of the need for heavy growth and labor-intensive procedures; however, it could be substituted with this alternative nucleic acid isothermal amplification method. The preparatory steps required to extract DNA for the LAMP reaction in a simple heating method also reduced the infection risk and the cost of the test. Conventional nucleic acid amplification tests are not widely used in developing countries because of contamination by carry-over products. An advantage of LAMP is that the results can be observed without any further analysis unlike conventional nucleic acid amplification tests. Naked-eye observation of the color change in the reaction mixture without opening the lid of the reaction tube reduces the risk of cross-contamination.

TB is the most common mycobacterial infection in developing countries; therefore, this simple and economic identification method based on TB-LAMP could be suitable for use in any laboratories that perform mycobacterial culture.

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Conflict of interest None to declare.

Ethics approval Not applicable as this study used clinical isolates.

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