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# Loop-Mediated Isothermal Amplification Method Targeting the TTS1 Gene Cluster for Detection of *Burkholderia pseudomallei* and Diagnosis of Melioidosis▽

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**Melioidosis is a severe infection caused by *Burkholderia pseudomallei*. The timely implementation of effective antimicrobial treatment requires rapid diagnosis. Loop-mediated isothermal amplification (LAMP) targeting the TTS1 gene cluster was developed for the detection of *B. pseudomallei*. LAMP was sensitive and specific for the laboratory detection of this organism. The lower limit of detection was 38 genomic copies per reaction, and LAMP was positive for 10 clinical *B. pseudomallei* isolates but negative for 5 *B. thailandensis* and 5 *B. mallei* isolates. A clinical evaluation was conducted in northeast Thailand to compare LAMP to an established real-time PCR assay targeting the same TTS1 gene cluster. A total of 846 samples were obtained from 383 patients with suspected melioidosis, 77 of whom were subsequently diagnosed with culture-confirmed melioidosis. Of these 77 patients, a positive result was obtained from one or more specimens by PCR in 26 cases (sensitivity, 34%; 95% confidence interval [CI], 23.4 to 45.4%) and by LAMP in 34 cases (sensitivity, 44%; 95% CI, 32.8 to 55.9%) ($P = 0.02$). All samples from 306 patients that were culture negative for *B. pseudomallei* were negative by PCR (specificity, 100%; 95% CI, 98.8 to 100%), but 5 of 306 patients (1.6%) were positive by LAMP (specificity, 98.4%; 95% CI, 96.2 to 99.5%) ($P = 0.03$). The diagnostic accuracies of PCR and LAMP were 86.7% (95% CI, 82.9 to 89.9%) and 87.5% (95% CI, 83.7 to 90.6%), respectively ($P = 0.47$). Both assays were very insensitive when applied to blood samples; PCR and LAMP were positive for 0 and 1 of 44 positive blood cultures, respectively. The PCR and LAMP assays evaluated here are not sufficiently sensitive to replace culture in our clinical setting.**

Melioidosis is a disease caused by the gram-negative bacterium *Burkholderia pseudomallei*. This environmental organism is distributed across much of Southeast Asia and northern Australia, where it is a cause of severe community-acquired sepsis (3, 19). Melioidosis is responsible for 20% of community-acquired septicemias and 40% of sepsis-related mortality in northeast Thailand (3, 19, 20). Manifestations of melioidosis are highly variable, and this infection cannot be differentiated with accuracy from other causes of sepsis on the basis of clinical features alone (3, 19). Diagnosis relies on the culture of the bacterium, but isolation and identification can take up to a week. Patients with melioidosis can deteriorate rapidly during this time, particularly since *B. pseudomallei* is resistant to many antibiotics used in the empirical treatment of sepsis (3, 19, 20). A rapid test is important for early diagnosis and treatment, and it has the potential to improve patient outcomes (3).

A range of serological tests have been developed previously, including the indirect hemagglutination assay, the enzyme-linked immunosorbent assay, and a rapid bedside immunochromatographic test (14). These tests have poor sensitivity and/or specificity due to high background seropositivity in areas in which the disease is endemic, combined with delayed or absent seroconversion of some patients with melioidosis (2, 4, 5, 14). Direct immunofluorescent microscopy (DIF) has been developed for use with fresh clinical specimens (24), and a monoclonal antibody-based latex agglutination test has been developed for rapid bacterial identification following laboratory culture (1, 21), but neither reagent is commercially available.

Real-time PCR has been developed for the rapid detection of *B. pseudomallei* DNA, including assays that target genes encoding 16S rRNA, flagellin (*fliC*), ribosomal protein subunit S21 (*rpsU*) (18), type III secretion systems (TTS1 and TTS2) (11, 13, 17), and two sequences unique to *B. pseudomallei*, designated 8653 and 9438 (16). A prospective clinical evaluation conducted in Darwin, Australia, of a real-time PCR assay targeting the TTS1 type III secretion system gene cluster included 33 individuals with culture-confirmed melioidosis; the sensitivity and specificity for patient diagnosis were 91 and 95%, respectively (11). A retrospective study evaluated the real-time PCR targets 8653 and 9438, using samples collected in northeast Thailand from 28 patients with culture-confirmed

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A

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CGCGGCCGCTTCGATGCGGTCGCCGATCTGCATGCGGCCCCCGGTCAGGAAGGCGAGCGC
        490         500         510         520         530         540
GCCGACGCGATTGCCCGGGCCTGGGAGAGCGAGATGTTCGGACTCCAAGTATTTCCCCCC
        550         560         570         580         590         600
AATGCGCCACGCACGGCGGAGATTCTCGAATTGTCGTTGGACTTTCTTCTCATCCAGCGA
        610         620         630         640         650   F3    660
CGCATCGGGCCGATTCCCGTTAGTACGCTCGCCAGGCTGCGCGAGTCTCCTGCTCCCACA
        670   F2    680         690         700         710         720
CCGGTTGCTTGCGGCCGAGATCGCCTGCCTGGCGAGACGGCGCCGGACGACAGACGGCGC
        730   F1    740         750         760         770         780
TTCAATCTGCTCTTTCCGTTGCTGTGGTTGCAGGCGGGCATGCCGAGAACGGCCGTCCGC
 B1     790         800         810         820         830   B2    840
CTCGATTGCGCGATCGCCAAGCTGCTCGCGATCCGCAACGCGCTGCCTGACAATCCGCTA
        850   B3    860         870         880         890         900
GCAACAGATGACGCGCAAGCGGAACCGACACCGGCACCTCACGCCTCATCGAGCGAATGA
        910         920         930         940         950         960
```

B

| Primer | Primer sequence (5′-3′) |
|---|---|
| F3 | TCCTGCTCCCACACCG |
| B3 | GCTTGCGCGTCAT |
| FIP | CCACAGCAACGGAAAGAGCAGAGCTTGCGGCCGAGATC |
| BIP | GCCTCGATTGCGCGATCGCCTGTTGCTAGCGGATTGTCA |

FIG. 1. Names and locations of target sequences used as primers for *B. pseudomallei* LAMP. (A) The sequence of the second half of the *B. pseudomallei* K96243 gene BPSS 1406 is shown, together with the primer name (in boldface) and the location of each target sequence (in boldface and underlined). (B) Sequences of LAMP primers. Primer FIP consisted of the F1 complementary sequence (22 nucleotides [nt]) and the F2 direct sequence (16 nt). Primer BIP consisted of the B1 direct sequence (20 nt) and the B2 complementary sequence (19 nt). Primers B3 and F3 target sequences outside of the F2 and B2 regions.

melioidosis and 17 patients with bacteremia caused by other pathogens. The sensitivity was 71 and 54% for 8653 and 9438, respectively, and the specificity was 82 and 88%, respectively (16). These findings suggest that the detection of *B. pseudomallei* in clinical samples using molecular approaches has utility for the early diagnosis of melioidosis.

Loop-mediated isothermal amplification (LAMP) is an alternative method of rapid DNA amplification under isothermal conditions (12). This method employs a DNA polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA. The cycling reaction results in the accumulation of $10^9$ copies of target in less than an hour. The detection of product can be performed by a visual assessment of turbidity, by the use of a turbidometer, or by the addition of fluorescent reagents such as Sybr green I. The assay is quick and easy to perform, and all it requires is a laboratory water bath or heat block that maintains a constant temperature of 60 to 65°C. LAMP has been developed for the detection of a range of bacteria and viruses (7–10). The aim of this study was to develop a sensitive and specific LAMP-based DNA amplification method for the detection of *B. pseudomallei* that amplifies a region in the TTS1 gene cluster and to compare this assay to an established real-time PCR targeting the same gene region for the diagnosis of melioidosis.

## MATERIALS AND METHODS

**Laboratory strains.** LAMP was initially developed using *B. pseudomallei* K96243 and validated in the laboratory using a further 20 isolates representing three *Burkholderia* species. Ten of these were *B. pseudomallei*, including five clinical isolates from northeast Thailand (2686a, 2581a, 2692a, 2831a, and 3009a) and five clinical isolates from Australia (112c, 151, 543, 456a, and 571). Five were *B. thailandensis* (E205, E285, E305, E253, and E287), isolated from the environment in northeast Thailand, and five were laboratory strains of *B. mallei* (NCTC 10229, NCTC 10247, ATCC 23344, NCTC 3708, and EY2239). DNA was extracted from these isolates using a Wizard genomic DNA extraction kit (Promega).

**Primer design.** Four *B. pseudomallei*-specific LAMP primers were designed from the published sequence of strain K96243 (GenBank accession number AF074878) by using the Primer Explorer software (https://primerexplorer.jp/e/). The target was BPSS 1406 (encoding a hypothetical protein), situated within the gene cluster encoding TTS1. This region is not present in the closely related species *B. mallei* and *B. thailandensis*. The location and sequence of each primer in the target DNA are shown in Fig. 1. Primers were synthesized by Sigma-Proligo (Sigma-Genosys, TX).

**LAMP reaction.** The LAMP reaction was performed using the Loopamp DNA amplification kit (Eiken Chemical Co., Ltd., Tokyo, Japan). The LAMP master mix contained 12.5 μl of 2× reaction mix, 5 pmol of each of the outer primers, 40 pmol of each of the inner primers, and 1 μl of *Bst* DNA polymerase. The reaction volume was 25 μl total, including 2 μl of sample DNA extracted from laboratory isolates. For clinical samples, 7.5 μl of DNA was added and a 25-μl reaction volume was maintained by reducing the volume of sterile distilled water; this was based on the results of optimization experiments (see Results). Following optimization for time and temperature (see Results), the amplification was performed at 65°C for 90 min, followed by incubation at 95°C for 2 min to terminate the reaction. Sterile distilled water and *B. pseudomallei* K96243 DNA were included as negative and positive controls, respectively, in the assay.

**Analysis of LAMP products.** The LAMP reaction causes turbidity in the reaction mix that is proportional to the amount of amplified DNA. The presence of turbidity was determined by the naked eye. For further confirmation during assay development, amplified products were detected using 2.5% agarose gel electrophoresis followed by ethidium bromide staining.

**Real-time PCR assay.** The primer pair and probe targeting a region of *B. pseudomallei* TTS1 were described previously (11, 13). The forward primer locates to the intergenic region between *B. pseudomallei* K96243 BPSS 1406 and BPSS 1407 (encoding the type III secretion-associated protein SctD), and the reverse primer locates to BPSS 1407. The primers and probe were synthesized by using Sigma-Proligo (Sigma-Genosys, TX). The assay was performed as previ-

ously described (11), with the following modifications. Reactions were carried out in a total volume of 20 μl using a RotorGene 3000 real-time PCR machine (Corbett Robotics, Sydney, Australia). PCR mixtures contained primers and probes at final concentrations of 0.5 and 0.25 μM, respectively, 10 μl of quantiprobes (containing $MgCl_2$, *Taq* DNA polymerase, deoxynucleoside triphosphates, and reaction buffer [QuantiMix Easy Probes kit; Biotools B&M Labs, Madrid, Spain]), 5 μl of template DNA, and 3 μl of nuclease-free distilled water. Cycling conditions were 95°C for 15 min, followed by 50 cycles of 15 s at 94°C and 60 s at 60°C, and a final hold for 2 min at 45°C. The acquisition of signal was performed at 60°C at each cycle after the annealing step using the 6-carboxyfluorescein/Sybr channel. Negative (no template) and positive controls were included in each run. A standard curve was constructed by plotting the logarithmic values of a known number of bacterial copies versus the cycle threshold value. The assay was linear over 7 orders of magnitude (200 to 200 × $10^7$ target copies/reaction). The lower limit of the assay was calculated to be 20 target copies/reaction (5 μl template).

**Clinical validation.** Samples were taken from patients with suspected melioidosis who were admitted to Sappasithiprasong Hospital, Ubon Ratchathani, northeast Thailand, between 15 August and 30 September 2006. This was approved by the Human Research Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Patients with suspected melioidosis were identified during twice-daily ward rounds of the medical and intensive care wards. Multiple samples were taken from suspected cases, including blood, throat swab, sputum/tracheal aspirate, urine, and pus or surface swab from wounds and skin lesions. A 20-ml blood sample was divided between an EDTA tube for PCR (5 ml), a BacT/ALERT FA bottle (BioMérieux, NC) for standard culture (5 ml), and an Isolator 10 lysis centrifugation tube (Oxoid, Basingstoke, Hampshire, United Kingdom) for a quantitative count of *B. pseudomallei* cells (10 ml) (15). Urine, pus, and respiratory secretions were placed into plain sterile containers, and swabs were transported to the laboratory dry. Specimens were cultured, and *B. pseudomallei* was identified as previously described (6, 22, 23). DIF was performed on sputum/respiratory secretions, urine, pus, and other body fluids (24); blood and swabs are not suitable for DIF.

DNA was extracted from clinical specimens within ~2 h of collection. EDTA-blood was spun at 1,500 × *g* for 10 min, and the buffy coat was removed using a Pasteur pipette, 200 μl of which was used for DNA extraction. Ten milliliters of urine was centrifuged at 1,500 × *g* for 5 min, and 9 ml of supernatant was removed to obtain a 10× concentrated urine sample; 200 μl of this was used for DNA extraction. Respiratory secretions were used neat unless they were highly viscous, in which case an equal volume of sterile distilled water was added. DNA was extracted directly from pus and other body fluids. Swabs were placed into 500 μl of sterile distilled water for 10 min and then vortexed for 1 min. Using these preparations, 200 μl of each sample was transferred into a 1.5-ml reaction tube containing 200 μl lysis buffer and 20 μl of 20-mg/ml proteinase K. The mixture was vortexed and incubated in a water bath at 56°C with continuous shaking for 10 min. DNA was extracted using an automated DNA extractor (KingFisher ml; Labsystems) and the InviMag blood DNA mini kit/KingFisher ml, as recommended by the manufacturer. DNA from blood was eluted in a volume of 100 μl, and DNA from other specimens was eluted in a volume of 200 μl. DNA was stored at 20°C until use.

The LAMP and PCR assays were performed in the Wellcome Unit (Thailand) laboratory in Bangkok upon the completion of the clinical study and sample collection. The technician performing the assays was blinded to the culture result.

**Statistical analyses.** All statistical analyses were performed using STATA/SE version 9.0 (College Station, TX). Standard bacterial culture results were used to calculate the sensitivity and specificity of PCR and LAMP assays. The McNemar test was used to compare the sensitivities, specificities, and accuracies of the two groups.

## RESULTS

**Optimization of LAMP.** The LAMP assay was initially developed using *B. pseudomallei* K96243. Genomic DNA was quantified using the Quanti-iT high-sensitivity DNA assay kit (Invitrogen, CA) and a RotorGene 3000 machine in the DNA concentration measurement mode. Two microliters of DNA at a concentration of 14.7 ng/ml was used in the LAMP reaction mixture for the optimization experiments. To determine the optimal temperature, the reaction was incubated at 61, 63, or 65°C for 60 min and stopped at 95°C for 2 min. Incubation at 65°C gave the greatest observable turbidity by eye; this was confirmed by the visualization of amplification products using 2.5% gel electrophoresis and ethidium bromide staining (data not shown). The assay was performed at 65°C for 60, 90, or 120 min. Reactions incubated for 90 and 120 min gave equivalent products and were superior to that of 60 min, based on the degree of visual turbidity and gel electrophoresis results (data not shown). The assay was standardized throughout the remainder of the study by using an incubation temperature of 65°C and time of 90 min.

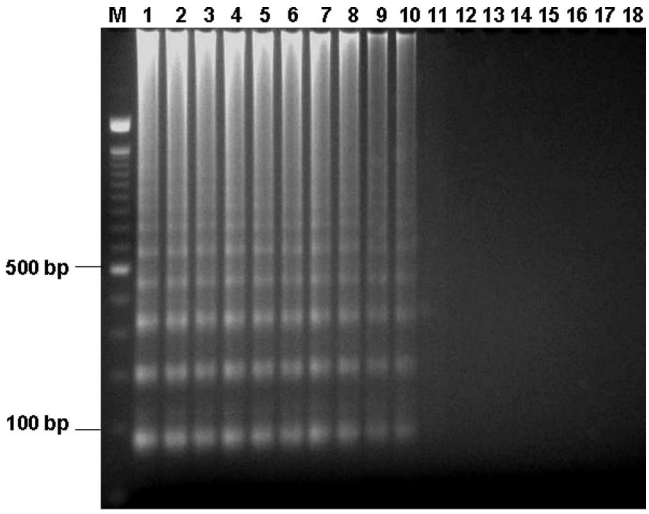


FIG. 2. Sensitivity of the LAMP assay for the detection of *B. pseudomallei*. A 10-fold dilution series of *B. pseudomallei* K96243 DNA was performed in duplicate from a calculated 380,000 copies (lanes 1 and 2) to 0.038 copies (lanes 15 and 16). Lane M, 100-bp ladder; lanes 17 and 18, distilled water. The lower limit of detection was 38 copies per reaction (lanes 9 and 10).

**Sensitivity and specificity of LAMP.** The specificity of LAMP was determined by testing isolates representative of three closely related *Burkholderia* spp. All 10 clinical isolates of *B. pseudomallei* were positive by LAMP. Five *B. thailandensis* isolates and five *B. mallei* isolates were negative by LAMP. The sensitivity was determined by defining the lower limit of detection using a 10-fold serial dilution of genomic DNA from *B. pseudomallei* K96243. The starting DNA concentration was determined using a Quanti-iT high-sensitivity DNA assay kit (Invitrogen, CA). The number of copies per reaction was calculated based on the G+C content, with 1 ng *B. pseudomallei* DNA being equivalent to 1.34 × $10^8$ genome equivalents (18). LAMP was performed in duplicate for each dilution. The turbidity was determined by eye, and the amplification product was visualized by 2.5% gel electrophoresis. The lower limit of amplification detectable by both eye and gel electrophoresis was calculated to be 38 copies/reaction (Fig. 2).

**Optimization of sample volume extracted from clinical specimens.** Clinical samples from patients with melioidosis may have a very low *B. pseudomallei* count. LAMP was further optimized for use with DNA extracted from clinical specimens to maximize the amount of template added. This was initially done using 10 specimens from patients with culture-confirmed melioidosis; samples were of EDTA-blood ($n = 5$), urine ($n =$

TABLE 1. Sensitivity and specificity of PCR and LAMP assay compared to those of culture for *B. pseudomallei* using sample type as the denominator[a]

| Sample type | Sensitivity | | | Specificity | | |
|---|---|---|---|---|---|---|
| | PCR | LAMP | *P* | PCR | LAMP | *P* |
| Blood | 0/44 (0) | 1/44 (2.3) | 0.32 | 189/189 (100) | 187/189 (98.9) | 0.16 |
| Sputum | 13/24 (54.2) | 11/24 (45.8) | 0.32 | 168/168 (100) | 166/168 (98.8) | 0.16 |
| Urine | 6/8 (75.0) | 5/8 (62.5) | 0.32 | 188/189 (99.5) | 185/189 (97.9) | 0.08 |
| Pus, fluid collection | 8/14 (57.1) | 8/14 (57.1) | | 18/18 (100) | 18/18 (100) | |
| Throat swab | 0/17 (0) | 4/17 (23.5) | 0.05 | 159/159 (100) | 156/159 (98.1) | 0.08 |
| Other swab | 3/9 (33.3) | 6/9 (66.7) | 0.08 | 7/7 (100) | 7/7 (100) | |
| Overall | 30/116 (25.9) | 35/116 (30.2) | 0.23 | 729/730 (99.9) | 719/730 (98.5) | 0.002 |

[a] Values for sensitivity and specificity are given as the number of samples positive and negative, respectively, by the indicated test out of the total number of samples tested. Values in parentheses indicate the percent positive for sensitivity and percent negative for specificity.

2), pus ($n = 1$), sputum ($n = 1$), and throat swab ($n = 1$). A further nine specimens were examined from patients who were culture negative for *B. pseudomallei*; sample types were of EDTA-blood ($n = 4$), urine ($n = 2$), pus ($n = 1$), sputum ($n = 1$), and throat swab ($n = 1$). DNA was extracted from these samples as described above. The sample volume in the LAMP reaction was varied between 2.5, 5.0, and 7.5 μl per reaction. The total volume was adjusted by altering the amount of sterile distilled water added to the reaction mix, such that a total volume of 25 μl was maintained. The reaction mixture was incubated at 65°C for 90 min and stopped at 95°C for 2 min. All non-melioidosis samples were negative at each of the template volumes tested. Of the 10 melioidosis samples, 4, 3, and 5 were positive (using visual turbidity) for sample volumes of 2.5, 5.0, and 7.5 μl, respectively. This result was confirmed by gel electrophoresis (data not shown). One of two urine samples, the pus sample, and the sputum sample were positive for all three volumes used. The second urine sample was positive only for 2.5 and 7.5 μl. The throat swab was positive only for 7.5 μl. None of the blood samples was positive at any volume. The sample volume was standardized to 7.5 μl for LAMP using DNA extracted from clinical specimens.

**Clinical evaluation of LAMP and PCR.** A total of 846 samples were obtained from 383 patients with suspected melioidosis. Sample types were blood ($n = 233$), sputum ($n = 192$), urine ($n = 197$), throat swab ($n = 176$), surface swab from wounds or skin lesions ($n = 16$), and pus or other body fluids from sterile sites ($n = 32$). Melioidosis was diagnosed for 77 patients, who were culture positive for *B. pseudomallei* in 116 samples.

A positive PCR result and LAMP result were obtained for one or more specimens from 26 (sensitivity, 34%; 95% confidence interval [CI], 23.4 to 45.4%) and 34 (sensitivity, 44%; 95% CI, 32.8 to 55.9%) of 77 patients with culture-confirmed melioidosis, respectively ($P = 0.02$). All samples from 306 patients that were culture negative for *B. pseudomallei* were negative by PCR (specificity, 100%; 95% CI, 98.8 to 100%), but samples from 5 of 306 patients (1.6%) were positive by LAMP (specificity, 98.4%; 95% CI, 96.2 to 99.5%) ($P = 0.03$). The diagnostic accuracies of PCR and LAMP were 86.7% (95% CI, 82.9 to 89.9%) and 87.5% (95% CI, 83.7 to 90.6%), respectively ($P = 0.47$). The final diagnoses for the five patients who were culture and PCR negative for *B. pseudomallei* but LAMP positive were the following: leptospirosis (1), necrotising fasciitis with a pus culture positive for *Staphylococcus aureus* (1), gastroenteritis responsive to ciprofloxacin (1), and respiratory infection of unknown cause (2). Melioidosis commonly presents as pneumonia, but we consider it unlikely that these two cases were caused by *B. pseudomallei*. This is based on the fact that treatment with antibiotics with activity against *B. pseudomallei* was given to these patients for only 3 and 10 days, respectively, and both patients were well 10 months later. The recommended duration of antimicrobial treatment for melioidosis is a minimum of 12 weeks, and less than this is highly associated with failure to cure and relapse. However, we are not able to exclude the possibility of melioidosis in either case.

A second comparison between PCR and LAMP was performed using the specimen type as the denominator (Table 1). Of 116 specimens that were culture positive for *B. pseudomallei*, PCR and LAMP were positive for 30 (25.9%) and 35 (30.2%) specimens, respectively ($P = 0.23$). Of 730 specimens that were culture negative for *B. pseudomallei*, PCR and LAMP were positive for 1 (0.1%) and 11 (1.5%) specimens, respectively ($P = 0.002$). The single culture-negative sample that was positive by PCR was from a patient with culture-proven melioidosis (a culture of samples taken from another body site was positive for *B. pseudomallei*) and likely represents a false-negative culture result. Six samples positive by LAMP but negative by culture were also from patients with melioidosis, but the remaining samples were from five patients with alternative diagnoses; the sample types were blood ($n = 2$) and throat swab ($n = 3$). The clinical features of these patients were discussed above.

Both assays were extremely poor at detecting the presence of *B. pseudomallei* in blood. PCR and LAMP were positive for 0 and 1 of 44 blood samples that were culture positive for *B. pseudomallei*, respectively. The median *B. pseudomallei* count in blood for the 44 culture-positive samples was 2.4 CFU/ml (interquartile range [IQR], 0.2 to 13.5 CFU/ml).

Forty-eight patients with culture-confirmed melioidosis were receiving effective parenteral antimicrobials at the time sampling was performed (45 patients, ceftazidime treatment; 2 patients, amoxicillin-clavulanic acid treatment; and 1 patient, cefoperazone plus sulbactam treatment). This group had a lower bacterial count in blood (median, 0.5 CFU/ml; IQR, 0.1 to 7.7 CFU/ml) than patients not receiving antibiotics at the time of sampling (median, 8.4 CFU/ml; IQR, 0.8 to 61 CFU/ml) ($P = 0.03$).

**Comparison of LAMP and PCR to DIF.** A total of 421 samples were suitable for DIF, of which 415 samples were examined (sputum, $n = 189$; urine $n = 196$; and pus, $n = 30$). Forty-three of the 415 samples were culture positive for *B. pseudomallei*. DIF was positive for 19 of the 43 samples (sensitivity, 44.2%; 95% CI, 29.1 to 60.3%) and was negative for all samples that were culture negative for *B. pseudomallei* (specificity, 100%; 95% CI, 99.0 to 100%). PCR was positive for 25 of these 43 samples (sensitivity, 58.1%; 95% CI, 42.1 to 73.0%) ($P = 0.08$, McNemar test), and LAMP was positive for 22 of these 43 samples (sensitivity, 51.2%; 95% CI, 35.5 to 67.0%) ($P < 0.44$).

## DISCUSSION

LAMP represents an innovative technology that has the potential to detect bacterial DNA in clinical samples from patients with a range of infectious diseases. The LAMP assay developed here for the detection of *B. pseudomallei* was significantly more sensitive than real-time PCR targeting the same gene cluster for the clinical diagnosis of melioidosis. However, both assays had a low level of diagnostic sensitivity compared to the findings of a previous study conducted in Darwin that reported the sensitivity of the real-time PCR assay to be 91% (11). The Darwin study did not indicate how many patients had received effective antibiotics by the time samples were taken. In our study, 48 of the 77 patients (62%) who were culture positive for *B. pseudomallei* were receiving effective parenteral antimicrobials at the time sampling was performed. We function as a melioidosis research team, and patients are first seen, investigated, and treated by the attending physicians. The administration of antibiotics would be predicted to have less effect on molecular-based tests than on culture-based tests, but it is possible that dead bacteria are cleared more rapidly than live ones. The time of sampling in relation to antibiotic administration is likely to be critical, and samples should ideally be taken at presentation and prior to antimicrobial treatment, provided this does not impose a delay in their administration. Further evaluation of LAMP using earlier sampling strategies is important and warranted.

The LAMP assay was shown to be specific for *B. pseudomallei* in the laboratory setting, in that other closely related *Burkholderia* species were negative. However, five patients with samples that were culture negative and PCR negative for *B. pseudomallei* were positive by LAMP. Follow-up of these patients did not indicate that a diagnosis of melioidosis had been missed. An alternative possibility is that other bacterial pathogens give a false-positive result. Further evaluation of the specificity of our primers is required in both laboratory and clinical settings.

Although rapid molecular techniques have become established for a range of infectious diseases in high-technology settings, PCR is not readily transferable to low-technology settings. PCR is time-consuming and requires a thermal cycler that is expensive to purchase and relatively complex to use. LAMP has clear advantages over PCR, in that it does not need a thermal cycler and produces a simple end point that can be interpreted by eye. These features have been much heralded and represent an important step forward for the development of technology that may be suitable for lower-technology settings. However, DNA extraction from clinical samples was still required during this study. The need for kit-based DNA extraction requires technical expertise and increases the cost of the test. The use of boiled samples has been assessed for LAMP assays designed to detect other pathogens, and sample preparation that does not depend on complex DNA extraction processes requires further evaluation for the detection of *B. pseudomallei* in clinical samples. Additional factors for low-technology settings are that LAMP reagents require storage in a −20°C freezer, which increases the cost and requires a reliable electricity source and/or backup. Furthermore, the technical care required to prevent contamination applies as much to LAMP as to PCR.

The diagnostic sensitivities of PCR (58%) and LAMP (51%) were higher than that of DIF (44%) for 43 *B. pseudomallei* culture-confirmed specimens suitable for DIF. The sensitivity of DIF for this set of specimens was lower than that described in a previous evaluation of this test (66%) (24). However, DIF is cheap and quick to perform, and it remains useful for the presumptive identification of *B. pseudomallei* in sputum, urine, and pus samples.

In summary, LAMP represents a viable alternative to PCR for the rapid diagnosis of melioidosis. However, the diagnostic sensitivity of both assays was low in this evaluation. The timing of sampling is likely to prove critical; further studies are required to fully evaluate the utility of LAMP in clinical practice.

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