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ORIGINAL PAPER

LAMP-CRISPR-Cas12-based diagnostic platform for detection of *Mycobacterium tuberculosis* complex using real-time fluorescence or lateral flow test

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Abstract

A CRISPR-based nucleic acid detection platform, termed LACD (loop-mediated isothermal amplification coupled with CRISPR-Cas12a-mediated diagnostic) has been developed. In the LACD system, the core primer used in conventional LAMP (forward inner primer or backward inner primer) was engineered to contain a PAM (protospacer adjacent motif) site (TTTT) at the linker region. As a result, the LAMP amplicons contained a specific PAM site for CRISPR-Cas12a recognition. At the CRISPR-mediated detection stage, the resulting LAMP products can activate the corresponding CRISPR-Cas12a effector upon the formation of the CRISPR-Cas12a/gRNA/target DNA complex. The single-strand DNA (ssDNA) reporter molecules are then rapidly cleaved due to the CRISPR-Cas12a's *trans*-enzyme activity. The ssDNA degradation can then be visualized on a lateral flow biosensor or measured by a real-time fluorescence instrument. Our LACD assay allows any target sequence to be detected (even targets which do not contain any PAM sites) as long as they met the design requirement for LAMP. The feasibility of the LACD methodology for nucleic acid detection was validated on the *Mycobacterium tuberculosis* complex (MTC). This proof-of-concept assay can be reconfigured to detect a variety of target sequences by redesigning the engineered LAMP primers.

Keywords Fluorescence detection; Lateral flow test; CRISPR · Cas12a · LAMP · LACD · Nucleic acid detection

Introduction

Nucleic acid detection is important for many applications including medical diagnosis and molecular biology research [1]. Although polymerase chain reaction (PCR)-based technologies (e.g., conventional PCR and real-time PCR) are the most widely used methods for nucleic acid detection [2], they require expensive laboratory equipment [3]. The requirement for thermal cyclers has thus precluded their deployment in "on-site" field and point-of-care settings.

Isothermal amplification-based detection technologies have successfully overcome the limitations associated with PCR-based assays [4]. The development of isothermal amplification approaches (e.g., loop-mediated isothermal amplification (LAMP), multiple cross displacement amplification (MCDA), and recombinase polymerase amplification

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(RPA)) has successfully eliminated the requirement for thermocycling instruments and can be used for real-time detection, supporting field and point-of-care testing [2, 5, 6]. However, these technologies cannot typically discriminate between inputs that differ by a single nucleotide, require optimization, and may lead to false-positive detection due to nonspecific amplification, cross-contamination, or primer dimerization [7, 8]. Therefore, there is still a need to develop novel diagnostic platforms for rapid, ultrasensitive, and highly specific detection of nucleic acids.

The CRISPR-Cas systems (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated system) are involved in archaeal and bacterial adaptive immunity against foreign nucleic acid [9]. They have been adapted for use as prominent molecular tools for genome editing and transcription regulation. CRISPR-Cas systems use a guide RNA (gRNA) that localizes Cas effector proteins (e.g., Cas9, Cas12, and Cas13) to target sites which then cleaves the target sequence [9]. Thus, these systems allow manipulation and investigation of the target gene function. Beyond genome editing applications, some Cas effector proteins such as Cas12a, Cas12b, Cas13a, and Cas14 have been successfully validated for their potential to develop the next generation of nucleic acid detection technologies due to their collateral cleavage ability of target sequences and nonspecific ssDNA/ssRNA (single-stranded nucleic acids) and using signal readout (e.g., lateral flow or fluorescence signal readout) [10, 11].

Recently, CRISPR-Cas-based diagnostic methodologies have been successfully developed and demonstrated [12]. For example, CRISPR-Cas12a- and 14-based DETECTR (DNA Endonuclease Targeted CRISPR *Tans* Reporter) assays have been developed for the diagnosis of HPV (human papillomavirus) and identification of SNPs, respectively [13, 14]. Similarly, CRISPR-Cas12a-based HOLMES (One-Hour-Low cost Multipurpose highly Efficient System) assays have also been developed for the detection of SNPs [15]. A CRISPR-Cas13a-based SHERLOCK (Specific High-Sensitivity Enzymatic Reporter UnLOCKing) assay was developed for HPV, ZIKV (Zika virus), and DENV (Dengue virus) with attomolar sensitivity and single-based mismatch specificity. These CRISPR-Cas-based diagnostic platforms require several important reaction components including a Cas effector, gRNA, nucleic acid amplification mixture, CRISPR-Cas buffer, a reverse transcription system (for SHERLOCK), and reporter molecules (single-stand nucleic acid) [16]. Although ultrasensitive and highly specific, these existing CRISPR-Cas-based technologies depend on target nucleic acid amplification through PCR or RPA pre-amplification, which require sophisticated instruments or special reagents, respectively, limiting their convenience. In addition, to activate the CRISPR-Cas/gRNA complex, the target sequence must contain a PAM (protospacer adjacent motif) site at an appropriate location. Thus, these existing CRISPR-Cas-based detection assays are restricted and cannot target sequences that do not contain any PAM sites.

Here, we developed a novel CRISPR-Cas12a-based diagnostic platform called LACD (LAMP coupled with CRISPR-Cas-mediated diagnostic) for ultrasensitive and highly specific detection of nucleic acids within 1 h. For demonstration purposes, *Mycobacterium tuberculosis* complex (MTC) which causes human tuberculosis (TB) was detected using our proof-of-concept technique (LACD) to validate its feasibility as a diagnostic assay. Here, we explain the basic LACD mechanism and provide further details about our LACD diagnostic test.

Materials and methods

Primer and gRNA design

Specific LAMP primer sets, which targeted the MTC *IS6110* gene, were designed using PrimerExplorer v5 (http://primerexplorer.jp/lampv5e/index.html) based on the principle of LAMP and LACD (Fig. 1 and Fig. S1) [17]. The specificity of the LAMP primer set was determined using the National Center for Biotechnology Information BLAST. OligoAnalyzer online software version 3.1 (Integrated DNA Technologies, Coralville, IA) was used to assess secondary structure and primer dimer. The guide RNA (gRNA) was designed according to the LACD principle. Further details about primer design, locations, sequences, and the gRNA are shown in Fig. S2 and Table S1. All of the oligomers were synthesized and purified by TianYi-Huiyan Biotech. Co., Ltd. (Beijing, China) at HPLC purification grade.

LAMP and CRISPR-Cas12a trans-cleavage reaction

The LAMP assay was performed using a commercial isothermal amplification kit (HuiDeXing Biotech. Co., Ltd. Tianjing, China). Briefly, LAMP was carried out in a 25 μl mixture containing 12.5 μl 2× isothermal reaction buffer, 1 μl enzyme (8 U of Bst 2.0 DNA polymerase), 1.6 μM each of FIP and BIP, 0.8 μM each of LF and LB, 0.4 μM each of F3 and B3, and template (1 μl for pure templates and 5 μl for samples). Real-time turbidity was monitored using LA-320C to optimize the LAMP temperature. CRISPR-Cas12a trans-cleavage reactions were performed as previously described (refer to Supplementary) [13].

Lateral flow biosensor (LFB) assay

LFB (4 mm × 60 mm), displayed in Fig. 2A, was made by HuiDeXing Biotech. Co., Ltd. (Tianjing, China) according to our design (refer to Supplementary). For lateral flow detection, a 9-μl aliquot of products from the CRISPR-Cas12a

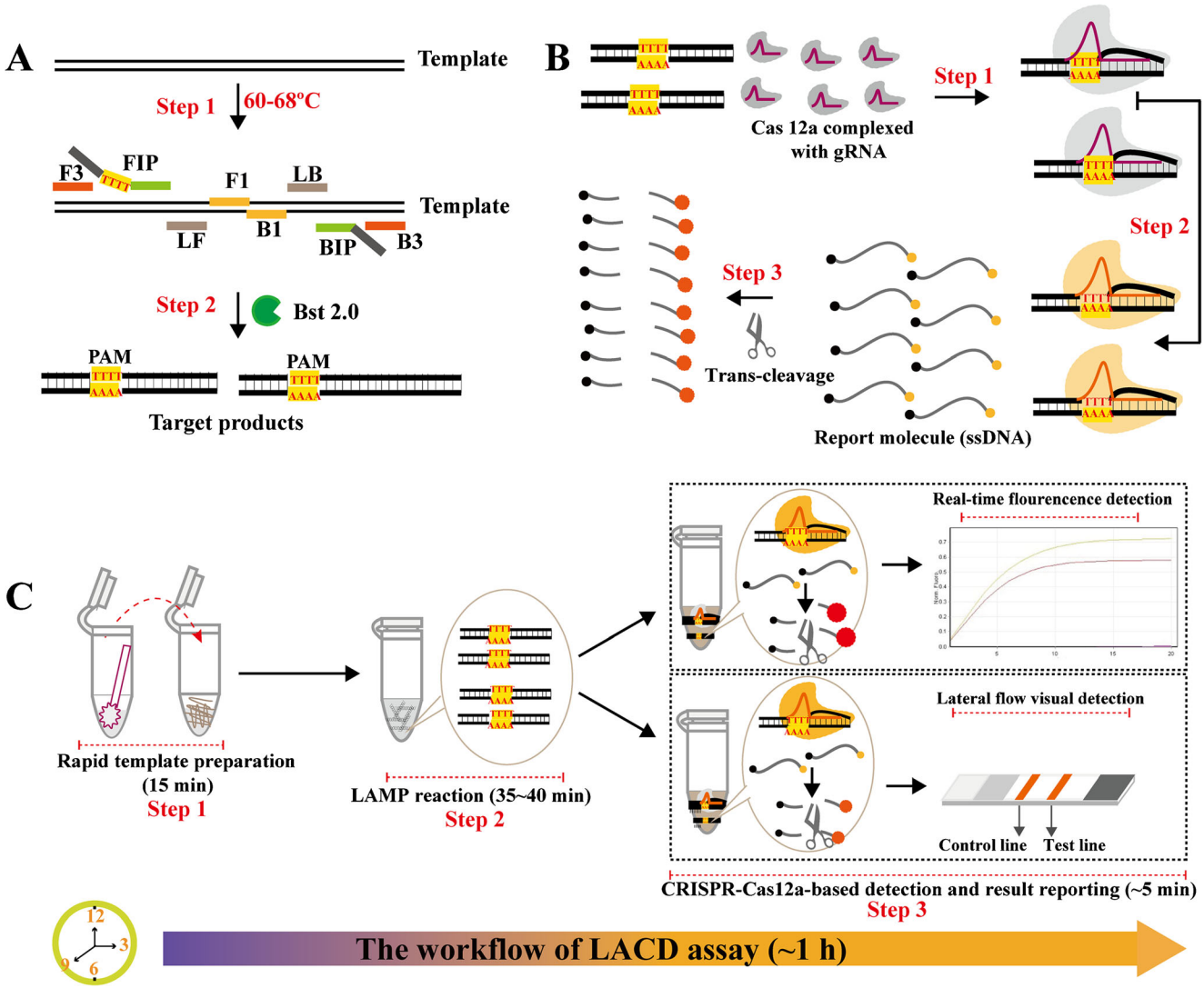

Fig. 1 Outline of LACD assay. (**A**) Schematic illustration of the principle for LAMP using modified FIP primer. The linker region from a conventional FIP primer was engineered with a PAM site (TTTT). After LAMP amplification with the modified FIP primer, the LAMP amplicons contain a CRISPR-Cas12a recognition site (TTTT) which was derived from the modified FIP primer. (**B**) Schematic illustration of the principle for CRISPR-Cas12a detection. LAMP products yielded from step 1, which contain many introduced PAM sites guide the CRISPR-Cas12a/gRNA complex to recognize target sites (step 1). Then, the Cas12a effectors are activated (step 2) and can nonspecifically cleave ssDNA (single-strand DNA) reporter molecules using trans-cleavage activity (step 3). These cleaved ssDNA reporters can release signals that can be detected using lateral flow biosensor and real-time fluorescence. (**C**) Schematic of the LACD workflow. LACD employs several closely linked steps: rapid template preparation (step 1), LAMP reaction (step 2), and CRISPR-Cas12a cleavage and signal detection (step 3)

trans-cleavage mixture was added to the sample pad of the biosensor, and further 2 drops of running buffer (100 mM PBS, PH7.4 with 1% Tween 20) were also added to the sample pad. The lateral flow readout was visualized in the form of red bands on the detection area within 2 min.

Sensitivity of the LACD assay

To determine the sensitivity of our LACD assay, purified genomic templates from the *M. tuberculosis* reference strain (H37Rv) were used to make serial dilutions (5 ng/μl, 500 pg/μl, 50 pg/μl, 5 pg/μl, 500 fg/μl, 50 fg/μl, 5 fg/μl, and 500 ag/μl). The serial dilutions were equivalent to 1×10^6 copies/μl, 1×10^5 copies/μl, 1×10^4 copies/μl, 1×10^3 copies/μl, 1×10^2 copies/μl, 1×10^1 copies/μl, 1×10^0 copies/μl, and 1×10^{-1} copies/μl, respectively. The copy number for each dilution was calculated based on the fact that 5 fg of genomic template was equivalent to a single copy of the H37Rv genome (assuming a molecular size of 4.4 Mbp for H37Rv) [18, 19]. Aliquots of 1 μl for each dilution were used as templates for our LACD diagnostic test. The experiments were performed three times to examine the reproducibility for our LACD assay.

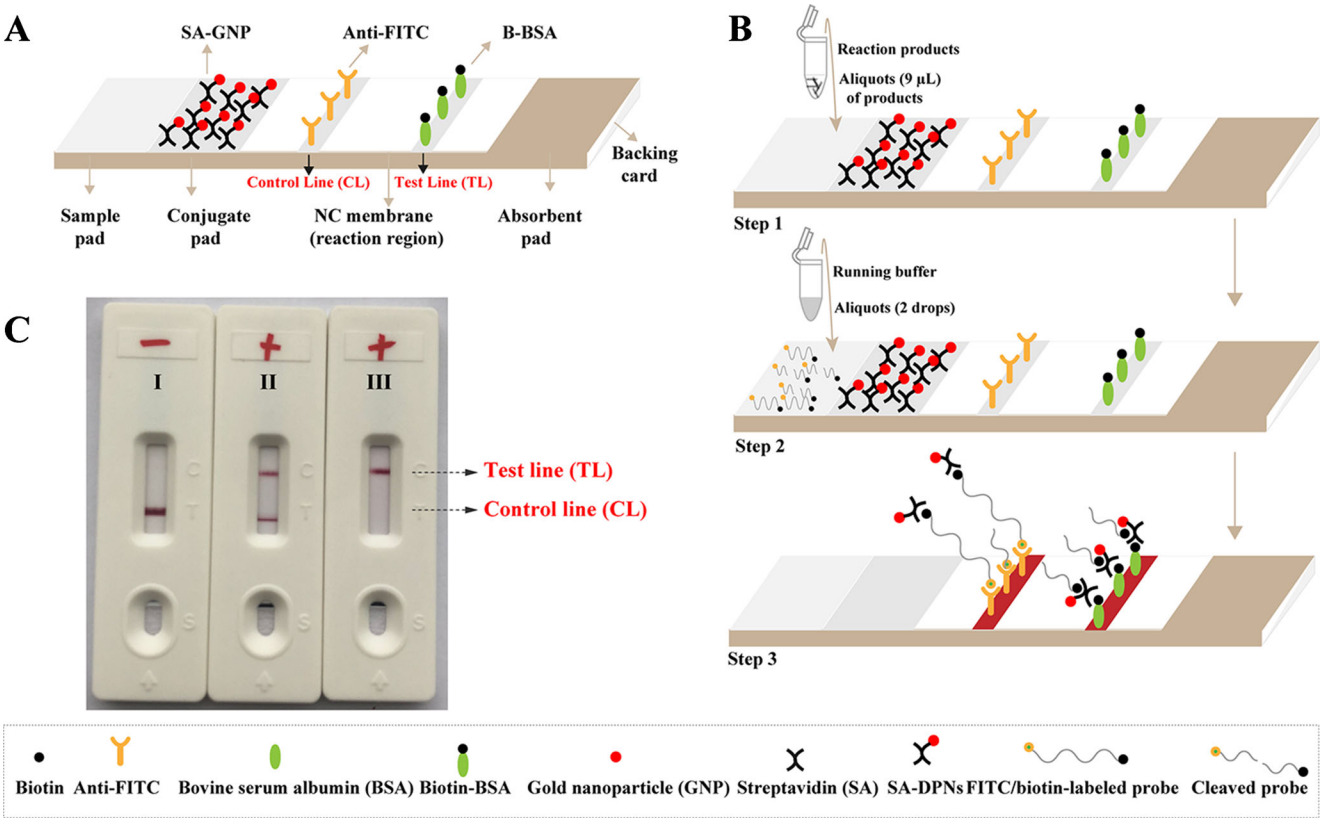

Fig. 2 The principle of LFB for visualization of LACD products. (**A**) The details for LFB. (**B**) The principle of LFB for LACD products. Step 1: 9 μl of cleaved products are deposited onto the LFB sample pad; step 2: two drops of running buffer are also added to the LFB sample region; step 3: indicating the LACD results by LFB. (**C**) Interpretation of the LACD results. I and II, positive results for the LACD test (CL and TL appear on the LFB, or only TL appears on the LFB) and III, negative (only the control line appears on the LFB)

Specificity of the LACD assay

The analytical specificity of our LACD assay was examined by testing the LACD assay on at least 5 ng of templates extracted from 37 mycobacterial strains and 15 non-mycobacterial species (Table S2).

Applicability of LACD assay to clinical samples

To evaluate our LACD assay on clinical samples, 44 sputum specimens, which were derived from patients with suspected pulmonary infection, were used. These suspected clinical sputum samples were detected using smear microscopy for the presence or absence of acid-fast bacilli and culture and Xpert MTB/RIF tests for the presence or absence of MTC. Genomic templates were extracted from the clinical sputum samples using the boiling method as described by Afghani and Stutman [20]. Five-microliter templates were used for LACD assay, and the detection results were compared with smear microscopy test, regular culture method, and Xpert MTB/RIF test.

Result and discussion

LACD design

As shown in Fig. 1 and Fig. S1, six primers (F3, B3, FIP, BIP, LF, and LB), which recognize 8 sites in the target template, initiate LAMP. LAMP amplification is completed within 40–60 min at a fixed temperature (60–68 °C) (Fig. 1A, step 1). In the conventional LAMP system, the core primers FIP and BIP include three regions: a region at the 3′-end (16–20 bp) that is complementary to the target template for primer extension, a region at the 5′-end (20–25 bp) that is reverse complementary to the target template for exponential amplification, and an additional region (0–4 bp) in the middle of these two regions that is used as a linker (Fig. 1A and Fig. S1). Importantly, the 3′- and 5′-terminal regions of FIP or BIP are target sequence dependent, while the middle linker region is target sequence independent. Hence, in our LACD design, the conventional FIP primer is engineered with the addition of TTTT bases to the linker region. By using the engineered FIP primer, the LAMP products contain a newly acquired PAM (protospacer adjacent motif) site (TTTT), which can be used to activate and locate the CRISPR-Cas12a system to the target site (Fig. 1A

and Fig. S1). The engineered FIP primer allows the amplified LAMP products to meet the requirements for CRISPR-Cas12a-based detection. Hence, our LACD assay can be used to detect any nucleic acid sequences, even sequences which do not contain any suitable PAM sites for CRISPR-Cas12a/gRNA complex, as long as they meet the requirements for LAMP primer design.

In the CRISPR-Cas12a-based detection stage (Fig. 1B), the LAMP products, which contain PAM sites (TTTT) derived from the engineered FIP primer, are used to locate and activate the CRISPR-Cas12a effector (Fig. 1B, step 1 and step 2). The binding of the CRISPR-Cas12a/gRNA complex to a guide-complementary LAMP amplicon enables rapid cleavage of ssDNA (single-strand DNA reporter molecules) within an extremely short time (Fig. 1B, step 3). In summary, the LACD assay consists of LAMP amplification of the target genome, followed by CRISPR-Cas12a-based detection of predefined target templates, and finally, digestion of a report molecule (ssDNA) confirms detection of the target marker. Careful attention is required when opening the LAMP reaction tube during the CRISPR detection stage as it may introduce LAMP products into the environment, increasing the risk of carryover contamination.

The LACD methodology is a novel CRISPR-based biosensing platform. It integrates LAMP with CRISPR-Cas12a-based detection and was successfully established for rapid and reliable detection of nucleic acids (Fig. 1). To perform the LACD assay, only simple instruments such as a water bath or heating block are required to perform LAMP amplification at a fixed temperature and CRISPR-Cas12a-based detection at 37 °C. This alleviates the need for sophisticated apparatus (e.g., thermocycling) and may be more suitable than PCR-based techniques for nucleic acid detection, especially in resource-poor settings.

MTC-LACD assay

To demonstrate the feasibility of our LACD assay, we established a TB diagnostic test called MTC-LACD, which uses the principle of LACD. We designed a set of LAMP primers targeting *IS6110*, a specific gene marker for MTC (Fig. S2). The FIP primer was modified with a PAM site (TTTT) at the linker region, and a specific gRNA was also designed to detect the *IS6110* sequence in MTC (Fig. 1, Fig. S1, Fig. S2, and Table S1). The entire diagnostic test for our MTC-LACD assay, including rapid template extraction (15 min, Fig. 1C, step 1), LAMP amplification (approximately 40 min, Fig. 1C, step 2), and CRISPR-Cas12a-based detection (within 5 min, Fig. 1C, step 3), can be completed within 60 min. Thus, our protocol provides a rapid turnaround time compared to conventional PCR-based assays. The detection results for LACD assay can be visualized using real-time fluorescence or a lateral flow biosensor.

The MTC genome has a high-GC content and does contain any suitable PAM sites (TTTN) for CRISPR-Cas12a effector in its specific gene marker (*IS6110*). Therefore, development of CRISPR-Cas-based assays for TB diagnosis is difficult. However, this limitation was successful overcome using our LACD platform. Instead of using conventional FIP primers, the engineered FIP primers, which were modified to contain a PAM site (TTTT) at the linker region, were added to the LAMP mixture for amplification. After LAMP, the resulting amplicons were used for CRISPR-Cas12a-mediated detection. A crucial factor in our LACD assay was to successfully design the guide RNA CRISPR-Cas12a-mediated detection, enabling the target nucleic acid sequence to correspond to the unique gRNA. Although LAMP amplicons are complex, the PAM site can assist the CRISPR-Cas12a/gRNA complex to precisely target all LAMP amplicons generated from the engineered FIP primer. Thus, the MTC *IS6110* sequence was able to be detected by our LACD platform (Table S3).

The principle of real-time fluorescence and LFB for visualization of LACD results

In the LACD assay, the ssDNA molecules were modified with a fluorophore at the 5′-end and with a dark quencher at the 3′-end. The binding of the Cas12a/gRNA complex to a guide-complementary amplicon enables activation of the *trans*-activity of Cas12a (Fig. 1B, step 1). The activated Cas12a rapidly digests the ssDNA which releases the quencher resulting in increased fluorescent signaling (Fig. 1B, steps 2 and 3). The real-time LACD assay can be conducted in a RotorGene system (Qiagen, Hilden, Germany) using the following setting: 15 cycles of denaturation at 37 °C for 10 s and extension at 37 °C for 30 s.

The details for LFB are displayed in Fig. 2A. After CRISPR-Cas12a reaction, 9 μl of cleaved products was deposited onto the sample pad of the biosensor (Fig. 2B, step 1), followed by two drops of running buffer which were also added to the sample region (Fig. 2B, step 2). The running buffer, which travels along the biosensor through capillary action, rehydrates the indicator reagents (SA-GNPs) in the conjugate sample. The uncleaved ssDNA reporter molecules are captured by anti-FITC antibodies immobilized on the control line (CL), and the biotin in the reporter molecule can bind SA-GNPs for visualization (Fig. 2B, step 3). When the CRISPR-Cas12a effectors are activated, the reporter molecules are digested and cleaved, separating FITC and biotin. As a result, the SA-GNPs/biotin complexes are captured by B-BSA that are immobilized on the test line (TL). This indicates reported a positive result (Fig. 2B, step 3). The interpretation of the LACD method using biosensor is shown in Fig. 2C and Fig. S3.

Optimal conditions for LACD assay

In this report, we first confirmed the feasibility of our *IS6110*-LAMP primers. As shown in Fig. 3, the *IS6110*-LAMP primers can successfully amplify the H37Rv (MTC reference strain) templates and thus can be used to develop LACD for MTC detection. We also determined the optimal isothermal amplification temperature of our MTC-LACD method, and a reaction temperature of 67 °C was selected for the assay (Fig. S4). We then optimized the cleavage time for CRISPR-Cas12a detection and compared the results generated by the CRISPR-Cas12a technique when using LFB and real-time fluorescence for detection. MTC-LACD assays were conducted using H37Rv templates (50 pg) and the CRISPR-Cas12a readout results assessed by LFB and a real-time fluorescence instrument at 0, 2.5, 5, 7.5, and 10 min. A visual signal using LFB was detectable within 5 min (Fig. S5A), and a fluorescent signal was detected within 1 min (Fig. S5B). Thus, an incubation time of 5 min was used for the CRISPR-Cas12a reaction (Fig. 1C, step 3, and Fig. S5).

Sensitivity of LACD assay for MTC detection

Using different dilutions of genomic templates extracted from pure cultures of MTC H37Rv, the limit of detection for our MTC-LACD assay was determined. The limit of detection (LoD) was defined as the lowest dilution that was detectable by our MTC-LACD assay. For the LFB biosensor, the limit of detection for MTC-LACD was 50 fg of genomic DNA (~10 copies) per reaction (Fig. 4A). As shown in Fig. 4B, the limit of detection using real-time fluorescence for MTC-LACD was also 50 fg of genomic DNA per reaction, which was in agreement with our LFB biosensor result.

Due to its high sensitivity, LAMP was chosen as the technique for nucleic acid amplification for our LACD platform. Previous reports have shown that LAMP is 10 to 100 times more sensitive than conventional PCR assays and other isothermal amplification techniques (e.g., CPA, PSR, or HDA) [1]. Our data also demonstrated that LACD was highly sensitive (Fig. 4) and able to successfully detect templates down to ~10 copies (50 fg of MTC genomic template) per test. Using LFB (Fig. 4A), the limit of detection was in agreement with real-time fluorescence (Fig. 4B), and LFB may be more suitable as the monitoring technique in field settings. Apart from high sensitivity, LAMP also has high amplification efficiency and only requires a short reaction time (40 min) for nucleic acid amplification. Forty minutes was the recommended cut-off reaction time for LACD testing. Thus, the entire LACD test from template preparation to result readout can be completed in approximately 1 h (Fig. 1C).

Specificity of LACD assay for MTC detection

Among the mycobacterial species tested using our LACD assay, four species including *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, and *Mycobacterium microti* are members of the MTC, while 17 species were unrelated to the MTC (Table S2). The positive results were only yielded from templates extracted from the four mycobacterial species belonging to the MTC. Thus, the analytical specificity of our MTC-LACD assay was 100% (Table S2). In addition, no cross-reactions to non-MTC strains were produced. This data suggests that the MTC-LACD assay was highly specific for MTC detection.

Our LACD protocol achieves detection of nucleic acids through two sequential reactions: (1) rapid amplification of nucleic acid using LAMP assay and (2) detection of the resulting LAMP amplicons using CRISPR-Cas12a-mediated collateral reporter cleavage. During the LAMP reaction stage, a set of 6 primers, which specifically recognizes 8 regions of the target sequence, ensured the high specificity for nucleic acid detection. In the second step, the LAMP amplicons are rapidly and precisely detected using CRISPR-Cas12a. Importantly, CRISPR-Cas12a-medated detection showed extremely high specificity (single nucleotide target specificity) for target sequence detection by using a target-dependent gRNA and a PAM site. This further ensured the reliability and specificity of our LACD test. The high specificity of the LACD assay is demonstrated by the results in this study which showed no false-positive results yielded from nontarget templates (Table S2). The LACD assay also did not

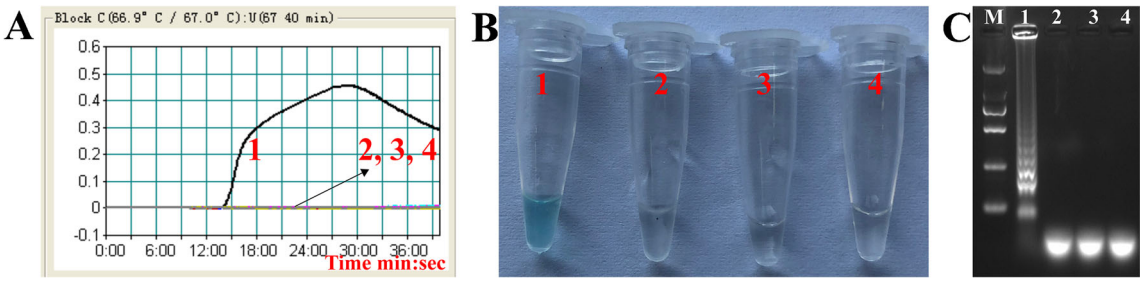

Fig. 3 Validation of the feasibility of *IS6110*-LAMP primer set. (**A**) Real-time turbidity used to monitor the *IS6110*-LAMP reaction. (**B**) Visual detection reagent applied to monitor the *IS6110*-LAMP reaction. (**C**) Agarose gel electrophoresis used to monitor the *IS6110*-LAMP reaction. Signal 1/tube 1/lane 1, positive amplification; signal 2/tube 2/lane 2, negative amplification (*Listeria monocytogenes*); signal 3/tube 3/lane 3, negative amplification (Salmonella); and signal 4/tube 4/lane 4, blank control (DW)

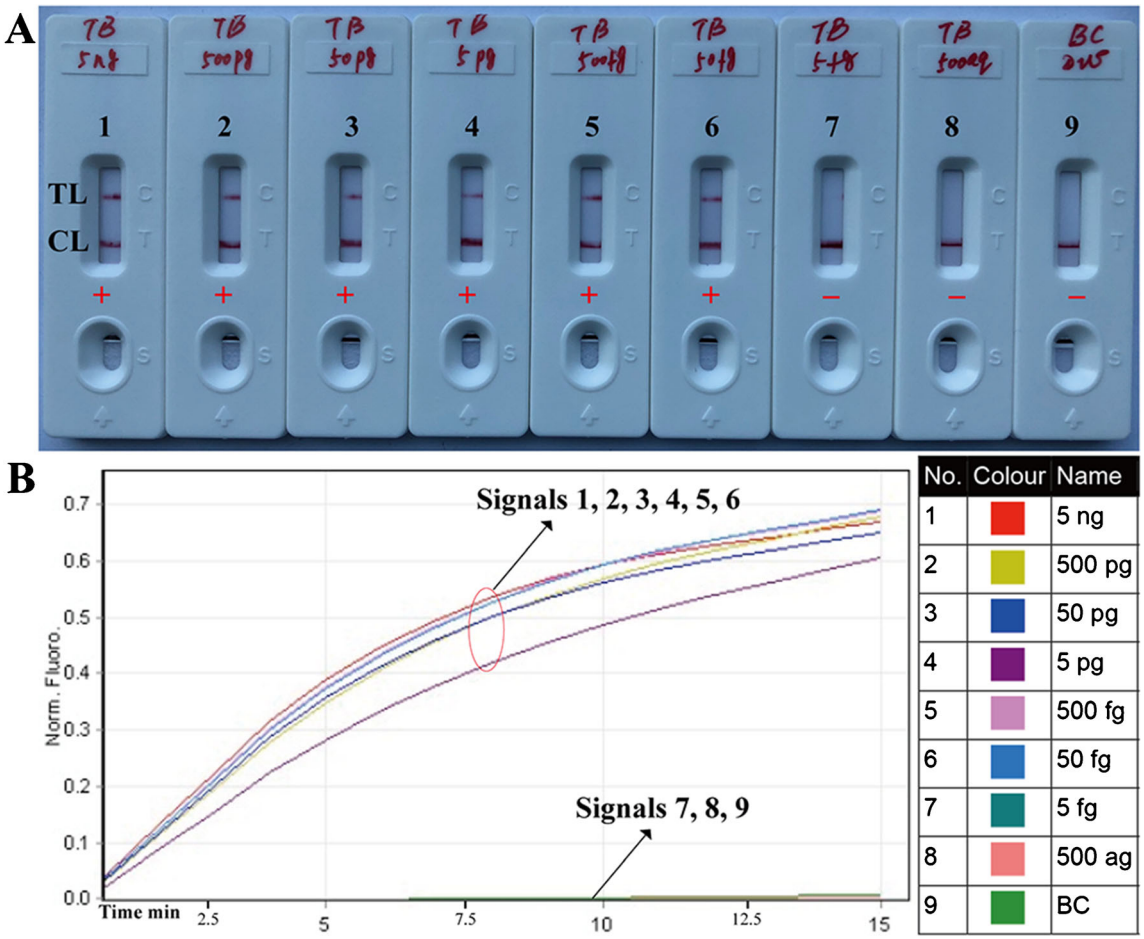

Fig. 4 Sensitivity of LACD assay for TB detection. (**A**) Lateral flow biosensor was used for reporting the LACD results. Biosensors 1–9 represent the amount of input template (H37Rv genomic DNA) from 5 ng, 500 pg, 50 pg, 5 pg, 500 fg, 50 fg, 5 fg, 500 aq, and blank control (DW). Positive results were obtained using 5 ng, 500 pg, 50 pg, 5 pg, 500 fg, and 50 fg templates. (**B**) Real-time fluorescence analysis was used for reporting LACD results. Signals 1–9 represent the amount of input template (H37Rv genomic DNA) from 5 ng, 500 pg, 50 pg, 5 pg, 500 fg, 50 fg, 5 fg, 500 aq, and blank control (DW). Positive results were obtained using 5 ng, 500 pg, 50 pg, 5 pg, 500 fg, and 50 fg templates

cross-react with non-MTC templates. All positive results were from members of the MTC tested, whereas other non-MTC strains showed negative results. Therefore, our LACD technique is a highly specific biosensing platform for nucleic acid detection.

Applicability of LACD assay to clinical samples

A total of 44 double-blinded clinical sputum samples were tested by Guizhou CDC using conventional culture, AFB smear, and Xpert MTB/FIR tests. For MTC-LACD, after 40-min LAMP amplification at 67 °C, all results were visualized using CRISPR-Cas12a-based biosensor and real-time fluorescence analysis and compared with those generated from conventional culture, AFB smear test, and Xpert MTB/FIR detection. The MTC-LACD assay detected 35/44 (79.5%) MTC in sputum samples from TB patients compared to 20 (45.5%) for conventional culture, 16 (36.4%) for AFB smear examination, and 27 (61.4%) for Xpert MTB/FIR test (Table 1). Some clinical samples that were negative using conventional culture, AFB smear test, and Xpert MTB/FIR detection had a low amount of target pathogen. These sputum samples with a low MTC pathogen load could still be detected using our LACD protocol. These results demonstrate that the LACD technique can be used as a valuable diagnostic tool for nucleic acid detection.

As a proof of concept for LACD, MTC was selected to illustrate the use of our LACD assay. Furthermore, to evaluate

Table 1 Comparison of different methods used for MTC detection in clinical TB patients

Detection methods	Clinical TB patients (n=44)	
	Positive	Negative
AFB smear	16 (36.4%)	28
Culture	20 (45.5%)	24
Xpert MTB/FIR	27 (61.4%)	17
LACD	35 (79.5%)	9

the potential clinical applications for LACD, we employed LACD to detect TB in clinical samples. The results suggest that our LACD displayed higher sensitivity (35/44) in sputum specimens over AFB smear test (16/44), conventional culture assay (20/44), and Xpert MTB/FIR examination (27/44). Some samples that were negative using conventional culture, AFB smear, and Xpert MTB/FIR tests contained a low load of target pathogen. However, these sputum samples with low pathogen load were still diagnosable by our LACD protocol. These preliminary data validate that the LACD assay developed in this study can be used as a valuable diagnostic tool for nucleic acid detection. For point-of-care clinical or field diagnosis, we recommend using LFB to report the LACD readout. This is because LFBs are disposable and easy-to-use and can visually report the detection results within 2 min. They also do not rely on any instrument and are available for large-scale tests.

Conclusion

Here, a novel CRISPR-based diagnostic platform called LACD which combines LAMP with CRISPR detection for rapid, ultrasensitive, and highly specific detection of nucleic acids was developed. We validated the capability of our LACD protocol to detect MTC. The whole test procedure for MTC-LACD, including nucleic acid preparation, CRISPR detection, and result readout, can be completed within 1 h and provides the advantages of high specificity, sensitivity, and rapid results and only requires simple equipment. Moreover, the LACD assay, as a proof-of-concept technique, can be reconfigured to detect various sequences by redesigning the LAMP primers.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00604-021-04985-w.

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Declarations

Conflict of interest The authors declare no competing interests.

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