Pathogen research

Real-time LAMP for the detection of human pathogen DNA using SuperScript IV RT-LAMP Master Mix

Introduction

Early and fast detection of pathogenic bacteria and viruses plays a vital role in the management and surveillance of widespread human diseases. Therefore, assay developers across the globe urgently need cost-effective, sensitive, and accurate nucleic acid amplification methods. Loop-mediated isothermal amplification (LAMP) has emerged as a promising technology that offers numerous possibilities for simple, rapid, and sensitive identification of a selected target. LAMP technology allows for simple reaction preparation and nucleic acid amplification in 5–30 minutes under isothermal conditions. A typical LAMP reaction employs a set of 4–6 specially designed primers that facilitate new DNA synthesis through the creation of stem-loop structures, accelerating amplification of the target region. Molecular amplification of pathogen DNA or RNA targets is performed at a constant temperature of 65°C using a DNA polymerase with strong strand-displacement activity, like *Bst* DNA polymerase. LAMP requires no specialized equipment such as a thermal cycler and can be performed simply with a heat block or water bath for incubation of reactions.

Here we demonstrate the use of Invitrogen™ SuperScript™ IV RT-LAMP Master Mix for rapid real-time detection of genomic DNA (gDNA) from four different pathogens of humans, including *Salmonella enterica*, *Staphylococcus aureus*, *Mycoplasma pneumoniae*, and human adenovirus. Even though SuperScript IV RT-LAMP Master Mix contains a reverse transcriptase to generate cDNA in reverse transcription LAMP (RT-LAMP), it can be successfully applied to amplify bacterial or viral gDNA in less than 20 minutes with no protocol adjustments.

Cleaning instructions

The laboratory workspace should be properly cleaned prior to setting up real-time LAMP reactions, to avoid environmental and random template contamination. Clean the workspace and all equipment thoroughly using the following reagents in the given order:

1. 70% ethanol solution
2. Invitrogen™ DNA*Zap*™ PCR DNA Degradation Solutions (Cat. No. AM9890)
3. Invitrogen™ UltraPure™ DNase/RNase-Free Distilled Water (Cat. No. 10977049)
4. 70% ethanol solution

Important notes

- Use separate workspaces as well as specially dedicated equipment and devices for:
  - RT-LAMP reaction setup and amplification
  - Analysis of RT-LAMP products by endpoint detection
- Use only dedicated equipment and supplies for real-time LAMP. Use of pipette tips with aerosol filters is strongly recommended for preparing DNA samples and setting up real-time LAMP reactions.
- We recommend setting up LAMP mixtures in a laminar flow cabinet equipped with a UV lamp.
- Change gloves frequently—put on fresh gloves after cleaning the workspace and equipment, before DNA sample preparation, and after LAMP mixture setup.
- Always perform negative control reactions (e.g., no-template control, or NTC) to check for background amplification.
- Close tubes or wells with NTC reactions before adding DNA template to other reactions, to reduce the risk of sample cross-contamination.
- Prior to each real-time LAMP experiment, prepare a fresh 50 µM solution of Invitrogen™ SYTO™ 9 Green Fluorescent Nucleic Acid Stain by diluting the 5 mM SYTO 9 stain 1:100 in nuclease-free water.

Materials and methods

Reagents

- SuperScript IV RT-LAMP Master Mix, 2X (Cat. No. A51801)
- SYTO 9 Green Fluorescent Nucleic Acid Stain (Cat. No. S34854; supplied with SuperScript IV RT-LAMP Master Mix)
- Water, nuclease-free (Cat. No. R0581)
- 70% ethanol solution
- DNA*Zap* PCR DNA Degradation Solutions (Cat. No. AM9890)
- UltraPure DNase/RNase-Free Distilled Water (Cat. No. 10977049)

LAMP targets and primer sets

Target regions and primer sets used to detect bacterial and viral pathogens are described in Table 1.

Table 1. Targets and primer sets used for real-time LAMP.

Pathogen	Strain	Target	Primer set
***Salmonella enterica* subsp. *enterica* (*ex* Kauffmann and Edwards) Le Minor and Popoff serovar Typhimurium (ATCC, Cat. No. 700720)**	LT2	*ttrRSBCA* locus	Custom-made at Thermo Fisher Scientific
***Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC, Cat. No. 43300)**	F-182	*nuc* gene	Custom-made at Thermo Fisher Scientific
***Mycoplasma pneumoniae* Somerson et al. (ATCC, Cat. No. 15531)**	FH strain of Eaton Agent [NCTC 10119]	*gyrB* gene	Ref. 1
Human adenovirus 41 (ATCC, Cat. No. VR-930)	Tak (73-3544)	*hexon* gene	Custom-made at Thermo Fisher Scientific

LAMP reaction setup

The reaction mix was prepared on ice according to Tables 2 and 3. The volume of individual real-time LAMP reactions was 25 µL. Eppendorf™ DNA LoBind™ 1.5 mL nuclease-free and nonstick tubes, along with sterile filtered pipette tips, were used to prevent aerosol contamination and ensure maximum sample recovery for improved reaction performance.

Each table lists components of the reaction mix required to perform a real-time LAMP assay for the detection of four different human pathogens individually. *S. enterica*, *S. aureus*, and human adenovirus targets were amplified according to Table 2, and the *M. pneumoniae* target was amplified according to Table 3. LAMP reactions must be prepared in separate reaction tubes or wells for each pathogen or each target gene.

Real-time LAMP

All real-time LAMP reactions for bacterial and viral DNA detection were performed in real-time PCR instruments (Applied Biosystems™ QuantStudio™ 6 Flex and 7 Flex Real-Time PCR Systems). One-step cycling conditions were applied to all real-time LAMP reactions according to Table 4. Reaction kinetics and specificity of amplified products were analyzed using Applied Biosystems™ QuantStudio™ Real-Time PCR Software for QuantStudio™ 6 and 7 Flex Systems. Specificity of the real-time LAMP amplicons was determined by melting curve analysis.

Table 2. Reaction setup for *S. enterica*, *S. aureus*, and human adenovirus gDNA detection.

Component of reaction mix	Detection of target DNA	No-template control (NTC)	Final concentration
SuperScript IV RT-LAMP Master Mix (2X)	12.5 µL	12.5 µL	1X
100 µM FIP/BIP primer	0.4 µL each	0.4 µL each	1.6 µM
100 µM F3/B3 primer	0.05 µL each	0.05 µL each	0.2 µM
100 µM LoopF/LoopB primer	0.1 µL each	0.1 µL each	0.4 µM
50 µM fresh solution of SYTO 9 Green Fluorescent Nucleic Acid Stain (optional)*	2.5 µL	2.5 µL	5 µM
Nuclease-free water	7.9 µL	8.9 µL	–
Close NTC tubes or wells prior to adding gDNA template.			
gDNA template	1 µL	–	–
Total volume	**25 µL**	**25 µL**	–

* For real-time fluorescence detection only.

Table 3. Reaction setup for *M. pneumoniae* gDNA detection.

Component of reaction mix	Detection of target DNA	No-template control (NTC)	Final concentration
SuperScript IV RT-LAMP Master Mix (2X)	12.5 µL	12.5 µL	1X
100 µM FIP/BIP primer	0.4 µL each	0.4 µL each	1.6 µM
100 µM F3/B3 primer	0.05 µL each	0.05 µL each	0.2 µM
50 µM fresh solution of SYTO 9 Green Fluorescent Nucleic Acid Stain (optional)*	2.5 µL	2.5 µL	5 µM
Nuclease-free water**	8.1 µL	9.1 µL	–
Close NTC tubes or wells prior to adding gDNA template.			
gDNA template	1 µL	–	–
Total volume	**25 µL**	**25 µL**	–

* For real-time fluorescence detection only.

** The nuclease-free water volume is scaled to obtain 25 µL total volume because only 4 primers (FIP/BIP, F3/B3) are used.

Table 4. One-step cycling protocol.

Number of cycles	Step	Temperature	Time
60	Amplification	65°C	30 seconds
1	Inactivation	95°C	2 minutes
–	Melt curve	60–95°C	–

Results and discussion

To demonstrate that SuperScript IV RT-LAMP Master Mix is suitable for accurate detection of bacterial and viral DNA of four widespread human pathogens, *S. enterica*, *S. aureus*, *M. pneumoniae*, and human adenovirus, were used. The LAMP reactions were carried out on QuantStudio 6 Flex and 7 Flex Real-Time PCR Systems to determine amplification kinetics. Reaction speed and sensitivity were evaluated using serially diluted gDNA at concentrations of 500–2,600, 500–5,000, or 500–10,000 copies per reaction, depending on the pathogen being analyzed. The reaction specificity of amplified products was analyzed using QuantStudio Real-Time PCR Software for QuantStudio 6 and 7 Flex Systems.

S. enterica

The sequence selected for designing real-time LAMP primers was a region within the *ttrRSBCA* locus. The real-time LAMP reaction using SuperScript IV RT-LAMP Master Mix amplified the *S. enterica* target region in less than 12 minutes (Figure 1A). As predicted, amplification signals were detected earlier when the template input was higher. Negative results were obtained for all 8 NTC replicates in the 60-cycle reaction.

S. aureus

A real-time LAMP reaction using SuperScript IV RT-LAMP Master Mix was run to detect the *nuc* gene, which encodes a virulence factor of *S. aureus*. The results presented in Figure 1B show that the amplification signals of the target region were detected in less than 10 minutes, regardless of gDNA input. No amplification signals were observed with the NTC replicates in 30-minute reactions.

M. pneumoniae

A set of four real-time LAMP primers, including two inner and two outer primers, was used against the *gyrB* gene, which is highly conserved among *M. pneumoniae* species [1]. SuperScript IV RT-LAMP Master Mix amplified the *M. pneumoniae* target region in less than 20 minutes (Figure 1C). No amplification was detected in NTC replicates in 30-minute reactions.

Human adenovirus

A high-efficiency primer set targeting the human adenovirus *hexon* gene was custom-designed. Results presented in Figure 1D demonstrate that SuperScript IV RT-LAMP Master Mix detected human adenovirus gDNA in less than 10 minutes. NTC replicates showed no amplification signals in 30-minute reactions.

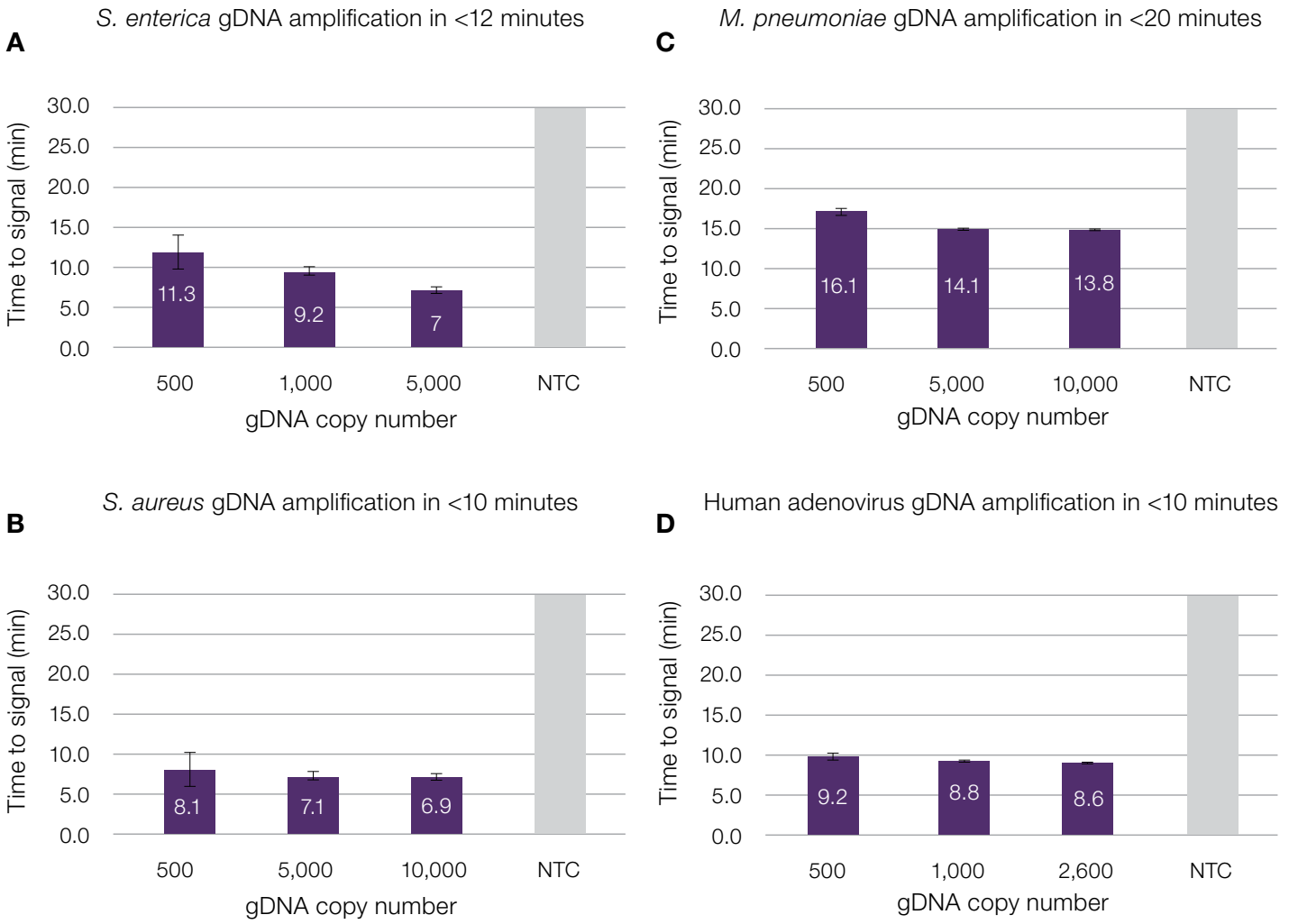

Figure 1. Detection of four different bacterial and viral gDNA targets in less than 20 minutes via real-time LAMP using SuperScript IV RT-LAMP Master Mix. Amplification kinetics were demonstrated using different gDNA copy number inputs: **(A)** 500, 1,000, and 5,000 copies of *S. enterica* gDNA; **(B)** 500, 5,000, and 10,000 copies of *S. aureus* and **(C)** *M. pneumoniae* gDNA; and **(D)** 500, 1,000, and 2,600 copies of human adenovirus gDNA. Error bars represent standard deviation of reaction speed (time to signal), calculated from multiple technical replicates (n = 4) for each tested sample.

Conclusions

SuperScript IV RT-LAMP Master Mix showed superior amplification speed, excellent technical repeatability, and ease of use when used to detect DNA from bacterial and viral pathogens of humans. Real-time LAMP reactions incorporating SuperScript IV RT-LAMP Master Mix detected all four gDNA targets in less than 20 minutes. These results demonstrate that SuperScript IV RT-LAMP Master Mix can be used in the development of highly accurate, sensitive, and rapid LAMP assays for the detection of a broad range of pathogen DNA targets.

Ordering information

Product	Cat. No.
DNA*Zap* PCR DNA Degradation Solutions	AM9890
UltraPure DNase/RNase-Free Distilled Water	10977049
SuperScript IV RT-LAMP Master Mix (2X)	A51801
SYTO 9 Green Fluorescent Nucleic Acid Stain	S34854
Water, nuclease-free	R0581

Reference

1. Arfaatabar M, Noori Goodarzi N, Afshar D et al. (2019) Rapid detection of *Mycoplasma pneumoniae* by loop-mediated isothermal amplification (LAMP) in clinical respiratory specimens. *Iran J Public Health* 48:917–924.

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