Inhibition of Non-specific Amplification in Loop-Mediated Isothermal Amplification via Tetramethylammonium Chloride

MinJu Jang1 · Sanghyo Kim1

Received: 25 May 2022 / Revised: 24 June 2022 / Accepted: 7 July 2022 / Published online: 27 July 2022
© The Korean BioChip Society 2022

Abstract

Loop-mediated isothermal amplification (LAMP) may be used in molecular and point-of-care diagnostics for pathogen detection. The amplification occurs under isothermal conditions using up to six primers. However, non-specific amplification is frequently observed in LAMP. Non-specific amplification has the potential to be triggered by forward and reverse internal primers. And the relatively low reaction temperature (55–65 °C) induces the secondary structure via primer–primer interactions. Primer redesign and probe design have been recommended to solve this problem. LAMP primers have strict conditions, such as Tm, GC contents, primer dimer, and distance between primers compared to conventional PCR primers. Probe design requires specialized knowledge to have high specificity for a target. In polymerase chain reaction (PCR), some chemicals or proteins are used for improving specificity and efficiency. Therefore, we hypothesized that additives can suppress the non-specific amplification. In this study, tetramethylammonium chloride (TMAC), formamide, dimethyl sulfoxide, Tween 20, and bovine serum albumin have been used as LAMP additives. In our study, TMAC was presented as a promising additive for suppressing non-specific amplification in LAMP.

Keywords Additive · Loop-mediated isothermal amplification · Non-specific amplification · Tetramethylammonium chloride

1 Introduction

Polymerase chain reaction (PCR) is a well-established technique and is widely used in molecular biology and clinical medicine. This technique specifically amplifies a target region of a DNA sequence using two primers and Taq polymerase [1, 2]. It is carried out through three steps, such as denaturation, annealing, and extension. The process occurs as the temperature continuously changes. Complicated equipment and highly sophisticated technology are required to satisfy this. Therefore, PCR is limited to laboratories or hospitals [3, 4]. Loop-mediated isothermal amplification was devised to improve this problem. This technique can amplify nucleic acids in point-of-care testing (POCT). Therefore, it is widely used as an alternative to PCR [5–7].

Loop-mediated isothermal amplification (LAMP) rapidly amplifies nucleic acids using six primers. LAMP primers specifically recognize the target and improve high specificity and sensitivity. The LAMP primer consists of forward outer primer (F3), backward outer primer (B3), forward inner primer (FIP), backward inner primer (BIP), forward loop primer (LF), and backward loop primer (LB) [8–10]. FIP and BIP (approximately 40–45 nt) form the loop structure of LAMP amplicon. F3 and B3 (approximately 17–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene. LF and LB (approximately 15–21 nt) separate the amplified DNA strands generated by FIP and BIP into single-stranded DNA. These are involved in extending the target gene.
conditions, such as Tm, GC contents, primer dimer, and distance between primers [9]. In particular, LF and LB may not be designed according to the target sequence. FIP and BIP are highly complicated sequences containing the F2/B2 and F1c/B1c regions. Therefore, they have the possibility of forming a primer dimer. Probe design must be designed to have high specificity and therefore requires expertise [5, 9]. Some chemicals and proteins enhance specificity in PCR and isothermal amplification assays [26–30]. We hypothesized that some chemicals and proteins could suppress non-specific amplification. Five additives, such as tetramethylammonium chloride (TMAC), formamide, dimethyl sulfoxide (DMSO), Tween 20, and bovine serum albumin (BSA), are used for experiments.

In this study, TMAC was proposed as an alternative to overcome the technical limitations of LAMP. To the best of our knowledge, the correlations between TMAC and non-specific amplification have not been reported in the literature. We report a novel alternative to suppress non-specific amplification without primer redesign and probe design. As a result, expected that LAMP will have high specificity and be utilized in various fields.

2 Experimental Section

2.1 Materials

Isothermal amplification buffer (10x, containing 2 mM MgSO4 and 0.1% Tween 20), 10 mM deoxynucleotide (dNTP) solution, WarmStart RTx Reverse Transcriptase, and Bst 2.0 WarmStart DNA polymerase were purchased from New England BioLabs (Ipswich, MA, USA). SYBR Green I nucleic acid gel stain, Tween 20, dimethyl sulfoxide, formamide, and tetramethylammonium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). The LAMP primers were synthesized by Bioneer (Seoul, Korea). Diethylpyrocarbonate-treated water (DEPC-water) was purchased from Thermo Fisher Scientific (Waltham, MA). BSA was purchased from MP Biomedicals (Seoul, Korea). SARS-CoV-2 (NR-55245) was provided by BEI Resource (Manassas, Virginia, USA). RNA was isolated from the influenza viruses using the QIAamp Viral RNA Mini kit purchased from Qiagen (Hilden, Germany).

2.2 Primer Design for SARS-CoV-2

RT-LAMP primers were designed with Primer Explorer V5 software (http://primerexplorer.jp/lampv5e/l/). The RdRp region of ORF1 ab was selected to specifically recognize SARS-CoV-2. This region is known for highly conserved genes and World Health Organization is recommended for use [31, 32]. Six genes (MT232870.1, MT127116.1, MT066158.1, MT050415.1, MT050417.1, and MN970004.1) were arbitrarily selected from the NCBI database. The formation of primer dimers was evaluated to minimize false positives. Cross-reactivity was analyzed in silico with pathogens with potential for human respiratory disease. The designed primer sequences are shown in Table 1.

2.3 RT-qLAMP Reaction Optimization

2.3.1 RT-qLAMP Assay

The final volume was 20 µL. RT-qLAMP reagents consist of 10x isothermal amplification buffer [20 mM Tris–HCl, 10 mM (NH4)2SO4, 50 mM KCl, 2 mM MgSO4, and 0.1% Tween 20], 0.3 mM each of dNTP, 4 U WarmStart RTx Reverse Transcriptase, 8 U Bst 2.0 WarmStart DNA polymerase, 1x SYBR Green I and primer mix. The primer mix consists of 0.2 µM F3 and B3, 1.6 µM FIP and BIP, and 0.4 µM LF and LB. After preparing the LAMP reagent, 5 µL template RNA is added. For negative sample, use the DEPC-water instead of template RNA. The RT-qLAMP assays were carried out in 0.2 ml PCR tubes or 0.2 ml 96-well plates in a Bio-Rad T100 thermocycler (Hercules, CA, USA). The reaction was performed continuously at 60 °C for 60 min. The fluorescence data were recorded every 1 min. All experiments were terminated by enzymatic inactivation at 80 °C and were repeated three times. All data were expressed as the mean Ct and standard deviation of three experiments.

2.3.2 Analyzation of the Sensitivity for SARS-CoV-2 Primer

Limit of detection (LoD) was analyzed to evaluate the sensitivity of the primers. A standard curve was obtained through RT-qPCR by serial dilution of the RdRp plasmid. The copies number of the RNA was determined by comparing the standard curve. RNA was serially diluted 10-fold (6.8 × 10^6 to 6.8 × 10^1 copies/µL) based on the determined copy number and used for RT-qLAMP.

Table 1 Specific LAMP primer for SARS-CoV-2

| Primer | length | Sequence (5’ to 3’) |
|--------|--------|---------------------|
| F3     | 18     | TTTGTCAAGCCTGTCACGG |
| B3     | 18     | ACAGCACGTGCAGAGAT |
| FIP    | 45     | GACACTCATAAAGTCTGTGTT |
|        |        | GTTAAATGCACATTATTATC |
|        |        | TACGTA |
| BIP    | 46     | AGAGATGTGACACAGACTTT |
|        |        | GTGTCATTGACAAATGTT |
|        |        | TACGCAA |
| LF     | 21     | TAACGGCTATTCATACAGGC |
| LB     | 18     | AATGAGTTTACGCATA |


2.3.3 Evaluation of Overall Reactivity by Additive Treatment

Five additives were tested to suppress the non-specific amplification. Various concentrations of additive were used in the experiment (20–60 mM TMAC, 2.5–7.5% (v/v) formamide, 2.5–7.5% (v/v) DMSO, 0.5–2% (v/v) Tween 20, and 0.1 to 0.5 mg/ml BSA). Samples without additives were called control. The template (6.8 × 10^3 copies/µL) was prepared at a concentration 10 times higher than the detection limit. The results were analyzed using the threshold cycle (Ct) value. Ct is defined as the point where the fluorescence signal crosses a threshold [33]. A low value of Ct is observed with a large amount of target. The concentration of target can be reliably inferred via this value [34]. ΔCt is the difference between the Ct values of the positive and negative samples. The value is an indication to infer the reliability of the results. ΔCt was used to evaluate the correlation between additive and non-specific amplification. The values were calculated using Eq. 1. Ct\textsuperscript{(negative)} is the threshold of condition without template. Ct\textsuperscript{(positive)} is the threshold of condition with template. Ct\textsuperscript{(negative)} and Ct\textsuperscript{(positive)} were measured according to the additive. In addition, Ct\textsuperscript{(positive)} was analyzed to evaluate LAMP reactivity. The data were collected via RT-qLAMP.

\[
\Delta C_t(\text{additive}) = C_t(\text{additive}, \text{negative}) - C_t(\text{additive}, \text{positive})
\]  

3 Results and Discussion

3.1 Evaluation of LoD for SARS-CoV-2 Primer

LoD is defined as the lowest concentration at which a positive or negative sample can be reliably distinguished [35, 36]. In this study, the lowest concentration belonging to the 95% confidence level for the detection of the target was determined as LoD. Positive signal was confirmed at all concentrations except 6.8 × 10^2 copies/µL and 6.8 × 10^1 copies/µL in 20 out of 20 trials (Fig. 1). For 6.8 × 10^2 copies/µL, a positive signal was obtained only in 19 out of 20 trials. For 6.8 × 10^1 copies/µL, a positive signal was obtained only in 15 out of 20 trials. Therefore, LoD was determined to be 6.8 × 10^2 copies/µL, which is the lowest concentration among the concentrations belonging to the 95% confidence level.

3.2 Analysis of Non-specific Amplification According to Additive

3.2.1 TMAC

ΔC_t(TMAC) and Ct(TMAC, positive) were obtained through RT-qLAMP. This value was compared with ΔC_t(control) and Ct(control, positive) (Fig. 2). ΔC_t(Control) was measured to be 11.19 (Fig. 2(A)). ΔC_t(TMAC) was measured to be 29.5, 37, and 44.46 with increasing TMAC concentration. Non-specific amplification was not observed with 60 mM TMAC (Fig. S1). Therefore, the Ct(60 mM TMAC, negative) value of 60 was assumed. TMAC effectively suppressed non-specific amplification all concentration. It can be inferred that the inhibition of non-specific amplification according to the TMAC concentration. Ct\textsuperscript{(positive)} was measured to evaluate the reactivity (Fig. 2(B)). Ct\textsuperscript{(TMAC, positive)} is obtained increased value according to increasing concentration. There was no significant difference between Ct\textsuperscript{(20 mM TMAC, positive)} and Ct\textsuperscript{(control, positive)}; Ct\textsuperscript{(40 mM TMAC, positive)} was delayed by 2 min, and Ct\textsuperscript{(60 mM TMAC, positive)} was delayed by 4 min. A high concentration of TMAC has the potential to suppress the LAMP. There is no significant effect on the overall reaction. This result is caused by the non-polar characteristic of TMAC. TMAC preferentially binds to the hydrated AT base pair via the attraction of the non-polar arm of the
alkylammonium ion [18, 37]. Thus, it contributes to the stability of GC base pairs and adjacent AT base pairs [18]. This property increases the DNA melting temperature and reduces the mismatch of DNA or RNA [18, 37]. LAMP reaction is affected by 40 mM and 60 mM TMAC. However, Ct(20 mM TMAC, positive) has a similar value to Ct(control, positive). Also ΔCt(20 mM TMAC) increased by 2.63 times compared to ΔCt(control). ΔCt(40 mM TMAC) increased by 1.25 times and ΔCt(60 mM TMAC) increased by 1.2 times compared to the previous concentration. 20 mM TMAC exerts a dramatic effect without suppressing LAMP reaction. Therefore, the optimal concentration was determined the 20 mM TMAC.

The effects of TMAC were confirmed through concentration screening in non-specific amplification. Therefore, further experiments were performed at concentrations above 60 mM TMAC. In the positive sample, the standard deviation of 20 mM to 60 mM TMAC was measured to be less than 0.4, but the standard deviation increased to about 1.8 in 80 mM and 100 mM TMAC (Fig. S1(B)). The reproducibility decreased with increasing concentration in positive samples. Non-specific amplification was not observed even in 80 mM and 100 mM TMAC (Fig. S1(C)). Although non-specific amplification was not observed, reactivity was significantly decreased and reproducibility was not ensured. Therefore, concentrations above 80 mM are of limited use.

### 3.2.2 Formamide

ΔCt(formamide) value was calculated to analyze the correlation between formamide and non-specific amplification (Fig. 3(A)). ΔCt(2.5% formamide) increased by 0.94 compared to ΔCt(control). Ct(2.5% formamide, negative) was measured to be 25.78, Ct(control, negative) was measured to be 22.71 (Fig. S2). There was no significant difference. However, 5% formamide was observed to suppress non-specific amplification. ΔCt(5% formamide) was measured to be 15.6, and ΔCt(7.5% formamide) was measured to be 19.25. All ΔCt(formamide) values were calculated to analyze the correlation between formamide and non-specific amplification.
was increased compared to \( \Delta C_t(\text{control}) \). \( C_t(5\% \text{ formamide, negative}) \) was measured to be 33.55 and \( C_t(7.5\% \text{ formamide, negative}) \) was measured to be 39.42 (Fig. S2). Formamide suppressed non-specific amplification. However, \( C_t(5\% \text{ formamide, positive}) \) and \( C_t(7.5\% \text{ formamide, positive}) \) were measured to be 17.95 and 20.17 (Fig. 3(B)). The detection rate was significantly delayed compared to control. This result is caused by the involvement of formamide on the secondary structure. Formamide binds to the minor or major groove and induces instability in the double helix [38]. Formamide interferes with secondary structure formation. However, the LAMP amplicon has a secondary structure and the amplification efficiency is inhibited by formamide. Consequently, formamide is limited to use.

### 3.2.3 DMSO

\( \Delta C_t \) value was calculated according to the DMSO concentration (Fig. 4(A)). \( \Delta C_t(2.5\% \text{ DMSO}) \) was not significantly different from \( \Delta C_t(\text{control}) \). A high concentration of DMSO increases the \( \Delta C_t \) value (\( \Delta C_t(5\% \text{ DMSO}) \) is 13.3 and \( \Delta C_t(7.5\% \text{ DMSO}) \) is 15.26. However, it is delayed up to 4.07 and cannot be effectively suppressed. \( C_t(\text{positive}) \) has a higher value as the concentration of DMSO increases (Fig. 4(B)). A high concentration of DMSO inhibits the activity of Bst polymerase [22]. DMSO was excluded by suppressing the LAMP. Amplification curves of control and DMSO-treated conditions are shown in Supplementary Information Fig. S3.

#### 3.2.4 Tween 20

\( \Delta C_t(\text{Tween 20}) \) is decreased the value as an increased Tween 20 concentration (Fig. 5(A)). \( \Delta C_t(0.5\% \text{ Tween 20}) \) and \( \Delta C_t(1\% \text{ Tween 20}) \) had increased values than \( \Delta C_t(\text{control}) \). \( \Delta C_t(2\% \text{ Tween 20}) \) was measured as 11.18 and was similar to \( \Delta C_t(\text{control}) \). However, it can be inferred that Tween 20 is not effective for non-specific amplification by comparing \( C_t(\text{Tween 20, negative}) \). \( C_t(0.5\% \text{ Tween 20, negative}) \) was measured to be 23.43, and \( C_t(1\% \text{ Tween 20, negative}) \) was measured to be 20.81 (Fig. S4). Non-specific amplification was detected similarly to \( C_t(\text{control, negative}) \). \( C_t(2\% \text{ Tween 20, negative}) \) was measured to be 18.31 and detected earlier than the control. \( C_t(\text{Tween 20, positive}) \)
tends to increase at high concentration (Fig. 5(B)). Appropriate concentrations such as 0.5% or 1% enhance the reaction. However, Fig. S4 shows that high concentrations of Tween 20 simultaneously enhance non-specific amplification. Tween 20 increases the probability of DNA or RNA mismatches [39, 40]. Therefore, it is recommended to use only a small amount of the LAMP reagent rather than being used as an additive.

### 3.2.5 BSA

$\Delta C_t$ value was calculated to analyze the non-specific amplification according to the concentration (Fig. 6(A)). The $\Delta C_t$ value decreases with increasing concentration of BSA. That is, non-specific amplification is observed to be accelerated. $\Delta C_t(0.1 \text{ mg/ml BSA})$ and $\Delta C_t(0.25 \text{ mg/ml BSA})$ have increased values than $\Delta C_t(\text{control})$. $C_t(0.1 \text{ mg/ml BSA, negative})$ was measured to be 23.86, and no significant effect was observed (Fig. S5). In addition, $C_t(0.25 \text{ mg/ml BSA, negative})$ was obtained an increased value than $C_t(\text{control, negative})$. $C_t(0.5 \text{ mg/ml BSA, negative})$ was observed decreased value than $C_t(\text{control})$. Non-specific amplification was accelerated to be 18.84. The $C_t$ value decreases with increasing concentration of BSA (Fig. 6(B)). BSA contributes to stabilizing the activity of polymerase. This improves the amplification efficiency [41]. But, BSA causes DNA or RNA mismatches during nucleic acid amplification similar to Tween 20. Thus, non-specific amplification accelerated at all concentration conditions. BSA cannot be considered an appropriate additive by accelerating the overall reaction and non-specific amplification.

### 4 Conclusion

Non-specific amplification is observed due to technical limitations of LAMP. To solve this problem, five additives were treated in the experiment and TMAC was presented as a promising additive. The inhibitory effect of non-specific amplification showed a concentration-dependent for TMAC. Non-specific amplification was dramatically suppressed with an increasing concentration of TMAC. Surprisingly, non-specific amplification was eliminated in 60 mM TMAC. TMAC is decreased slightly reactivity with a high concentration. However, positive sample was detected within 20 min under all concentrations. In conclusion, TMAC can be used by modifying the concentration according to the purpose. For the purpose of rapid detection in a short time, it is recommended to treat a low concentration of TMAC. In contrast, a high concentration of TMAC is recommended to completely eliminate non-specific amplification in studies or detection requiring high specificity.

Consequently, we report a novel alternative that can suppress non-specific amplification without primer redesign and probe design in LAMP. TMAC is expected to have the potential to be flexibly applied in various fields through the trade-off between detection time and specificity.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s13206-022-00070-3.

**Acknowledgements** This work was supported by the Gachon University research fund of 2020(GCU-202008480009) and Research Investment for Global Health Technology Fund (RIGHT Fund) (RF-TAA-2020-D02).

**Author contributions** MinJu Jang: conceptualization, methodology, validation, form analysis, investigation, data curation, visualization, writing original draft. Sanghyo Kim: Supervision, Funding acquisition.

**Declarations**

**Conflicts of interest** There are no conflicts to declare.
References

1. Yuce, M., Kurt, H., Mokkapati, V.R.S.S., Budak, H.: Employment of nanomaterials in polymerase chain reaction: insight into the impacts and putative operating mechanisms of nano-additives in PCR. RSC Adv. 4, 36800–36814 (2014)

2. Mühl, H., Kochem, A.-J., Disqué, C., Sakka, S.G.: Activity and DNA contamination of commercial polymerase chain reaction reagents for the universal 16S rDNA real-time polymerase chain reaction detection of bacterial pathogens in blood. Diagn. Microbiol. Infect. Dis. 66, 41–49 (2010)

3. Delidow, B.C., Lynch, J.P., Peluso, J.J., White, B.A.: Polymerase Chain Reaction: Basic Protocols. In: PCR Protocols, pp. 1–30. Humana Press, New Jersey (1993)

4. Erlich, H.A.: Polymerase chain reaction. J. Clin. Immunol. 9, 11 (1989)

5. Notomi, T., Mori, Y., Tomita, N., Kanda, H.: Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. J Microbiol. 53, 1–5 (2015)

6. Jawla, J., Kumar, R.R., Mendiratta, S.K., Agarwal, R.K., Singh, P., Saxena, V., Kumari, S., Bopy, N., Kumar, D., Rana, P.: On-site paper-based loop-mediated isothermal amplification coupled lateral flow assay for pig tissue identification targeting mitochondrial CO I gene. J. Food Compos. Anal. 102, 104036 (2021)

7. Schmidt, J., Berghaus, S., Blessing, F., Herbeck, H., Blessing, J., Schierack, P., Rödiger, S., Roggenbuck, D.: A semi-automated, isolation-free, high-throughput SARS-CoV-2 reverse transcriptase (RT) loop-mediated isothermal amplification (LAMP) test. Sci Rep. 11, 21385 (2021)

8. Nagamine, K., Hase, T., Notomi, T.: Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol. Cell. Probes 16, 223–229 (2002)

9. Notomi, T.: Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 28, 63e–663 (2000)

10. Park, G.-S., Koo, K., Baek, S.-H., Kim, S.-J., Kim, S.i., Kim, B.-T., Maeng, J.-S.: Development of reverse transcription loop-mediated isothermal amplification assays targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). J. Mol. Diagn. 22, 729–735 (2020)

11. Lee, J.W., Nguyen, V.D., Seo, T.S.: Paper-based molecular diagnostics for the amplification and detection of pathogenic bacteria from human whole blood and milk without a sample preparation step. BioChip J. 13, 243–250 (2019)

12. Kim, J.H., Kang, M., Park, E., Chung, D.R., Kim, J., Hwang, E.S.: A simple and multiplex loop-mediated isothermal amplification (LAMP) assay for rapid detection of SARS-CoV. BioChip J. 13, 341–351 (2019)

13. Choopara, I., Suea-Ngam, A., Teethaisong, Y., Howes, P.D., Schmelcher, M., Leelavathanichkul, A., Thunyaharn, S., Wongswaeng, D., deMello, A.J., Dean, D., Somboona, N.: Fluorometric paper-based, loop-mediated isothermal amplification devices for quantitative point-of-care detection of methicillin-resistant staphylococcus aureus (MRSA). ACS Sens. 6, 742–751 (2021)

14. Xu, W., Li, Q., Cui, X., Cao, M., Xiong, X., Wang, L., Xiong, X.: Real-time loop-mediated isothermal amplification (LAMP) using self-quenching fluorogenic probes: the application in Skipjack Tuna (Katsuwonus pelamis) authentication. Food Anal. Methods. 15, 658–665 (2022)

15. Liu, W., Huang, S., Liu, N., Dong, D., Yang, Z., Tang, Y., Ma, W., He, X., Ao, D., Xu, Y., Zou, D., Huang, L.: Establishment of an accurate and fast detection method using molecular beacons in loop-mediated isothermal amplification assay. Sci Rep. 7, 40125 (2017)

16. Chou, P.-H., Lin, Y.-C., Teng, P.-H., Chen, C.-L., Lee, P.-Y.: Real-time target-specific detection of loop-mediated isothermal amplification for white spot syndrome virus using fluorescence energy transfer-based probes. J. Virol. Methods 173, 67–74 (2011)

17. Kuboki, N., Inoue, N., Sakurai, T., Di Cello, F., Grab, D.J., Suzuki, H., Sugimoto, C., Igarashi, I.: Loop-mediated isothermal amplification for detection of african trypanosomes. J Clin Microbiol. 41, 5517–5524 (2003)

18. Özyaz, B., McCalla, S.E.: A review of reaction enhancement strategies for isothermal nucleic acid amplification reactions. Sens Actuators Rep. 3, 100033 (2021)

19. Ali, M.M., Li, F., Zhang, Z., Zhang, K., Kang, D.-K., Ankrum, J.A., Le, X.C., Zhao, W.: Rolling circle amplification: a versatile tool for chemical biology, materials science and medicine. Chem. Soc. Rev. 43, 3324 (2014)

20. Yamanaka, E.S., Tortajada-Genaro, L.A., Pastor, N., Maqueira, Á.: Polymorphism genotyping based on loop-mediated isothermal amplification and smartphone detection. Biosens. Bioelectron. 109, 177–183 (2018)

21. Kong, F., Outhred, A.C., Iredell, J.R., Verweij, J.J., Chen, S.C.-A., James, G., Lee, R., Watts, M.R., Ginn, A.N., Sultana, Y.: A loop-mediated isothermal amplification (LAMP) assay for strongyloides stercoralis in stool that uses a visual detection method with SYTO-82 fluorescent dye. Am. J. Trop. Med. Hyg. 90, 306–311 (2014)

22. Wang, D.-G., Brewster, J., Paul, M., Tomasula, P.: Two methods for increased specificity and sensitivity in loop-mediated isothermal amplification. Molecules 20, 6048–6059 (2015)

23. Hardinge, P., Murray, J.A.H.: Reduced false positives and improved reporting of loop-mediated isothermal amplification using quenched fluorescent primers. Sci Rep. 9, 7400 (2019)

24. Tani, H., Teramura, T., Adachi, K., Tsuneda, S., Kurata, S., Nakamura, K., Kanagawa, T., Noda, N.: Technique for quantitative detection of specific dna sequences using alternately binding probing probe competitive assay combined with loop-mediated isothermal amplification. Anal. Chem. 79, 5608–5613 (2007)

25. Khumwan, P., Pengpanich, S., Kampeera, J., Kamsong, W., Karunan, C., Sappat, A., Srilohusain, P., Chairapraset, A., Tuantranont, A., Kiathipathomchai, W.: Identification of S315T mutation in katG gene using probe-free exclusive mismatch primers for a rapid diagnosis of isoniazid-resistant Mycobacterium tuberculosis by real-time loop-mediated isothermal amplification. Microchem. J. 175, 107108 (2022)

26. Shahbazi, E., Mollasalehi, H., Minai-Tehrani, D.: Development and evaluation of an improved quantitative loop-mediated isothermal amplification method for rapid detection of Morganella morganii. Talanta 191, 54–58 (2019)

27. Lorenz, T.C.: Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. J. Virol. Methods 202, 3998–4012 (2015)

28. Zhang, Y., Ren, G., Buss, J., Barry, A.J., Patton, G.C., Tanner, N.A.: Enhancing colorimetric loop-mediated isothermal amplification speed and sensitivity with guanidine chloride. Biotechniques 69, 178–185 (2020)

29. Sekikawa, T., Kawasaki, Y., Katayama, Y., Iwashori, K.: Suppression of Bst DNA polymerase inhibition by nonionic surfactants and its application for cryptosporidium parvum DNA detection. Japanese J. Wat. Treat. Biol. 44, 203–208 (2008)

30. Ghaith, D.M., Abu Ghazaleh, R.: Carboxamide and N-alkylcarboxamide additives can greatly reduce non specific amplification of loop-mediated isothermal amplification and its application for cryptosporidium parvum DNA detection. Korean J Healthc Assoc Infect Control Prev. 298, 63–65 (2020)

31. Jiang, J., Bong, J.-H., Kim, H.-R., Park, J.-H., Lee, C.K., Kang, M.-J., Kim, H.O., Pyun, J.-C.: Anti-SARS-CoV-2 nucleoprotein antibodies derived from pig serum with a controlled specificity. BioChip J. 15, 195–203 (2021)
33. Rao, X., Huang, X., Zhou, Z., Lin, X.: An improvement of the 2^(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. 13 (2014)

34. Rabaan, A.A., Tirupathi, R., Sule, A.A., Aldali, J., Mutair, A.A., Alhumaid, S., Mazaheed, Gupta, N., Koritala, T., Adhikari, R., Bilal, M., Dhawan, M., Tiwari, R., Mitra, S., Emran, T.B., Dhama, K.: Viral dynamics and real-time RT-PCR Ct values correlation with disease severity in COVID-19. Diagnostics. 11, 1091 (2021)

35. Krajden, M., Ziemann, R., Khan, A., Mak, A., Leung, K., Hendricks, D., Comanor, L.: Qualitative detection of hepatitis C virus RNA: comparison of analytical sensitivity, clinical performance, and workflow of the Cobas Amplicor HCV Test Version 20 and the HCV RNA transcription-mediated amplification qualitative assay. J Clin Microbiol. 40, 2903–2907 (2002)

36. Pum, J.: A practical guide to validation and verification of analytical methods in the clinical laboratory. In: Advances in Clinical Chemistry, pp. 215–281. Elsevier, Amsterdam (2019)

37. Melchior, W.B., Hippel, P.H.V.: Alteration of the relative stability of dA dT and dG dC base pairs in DNA. Proc. Natl. Acad. Sci. U.S.A. 70, 298–302 (1973)

38. Chakrabarti, R.: The enhancement of PCR amplification by low molecular weight amides. Nucleic Acids Res. 29, 2377–2381 (2001)

39. Gao, X., Sun, B., Guan, Y.: Pullulan reduces the non-specific amplification of loop-mediated isothermal amplification (LAMP). Anal Bioanal Chem. 411, 1211–1218 (2019)

40. Xiao, Z.-X., Cao, H.-M., Luan, X.-H., Zhao, J.-L., Wei, D.-Z., Xiao, J.-H.: Effects of additives on efficiency and specificity of ligase detection reaction. Mol Biotechnol. 35, 129–133 (2007)

41. Farell, E.M., Alexandre, G.: Bovine serum albumin further enhances the effects of organic solvents on increased yield of polymerase chain reaction of GC-rich templates. BMC Res Notes. 5, 257 (2012)

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.