Blocking Nonspecific Primer Amplification in Loop-Mediated Isothermal Amplification with TrueLAMP

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Abstract

Loop-mediated isothermal amplification (LAMP) is a rapid and equipment-minimal nucleic acid amplification method, but its broader adoption has been hindered by nonspecific primer amplification that generates false positives in no-template controls and sample reactions. We developed TrueLAMP, a polymerase formulation containing a proprietary inhibitory component that selectively blocks primer-driven amplification while preserving target-specific amplification. Using SARS-CoV-2 RNA and virions as model systems, TrueLAMP was evaluated across multiple independent primer sets with both semi-real-time colorimetric imaging in a convection oven and endpoint colorimetric detection in a water bath. TrueLAMP achieved a limit of detection of 250 RNA copies per reaction with 96.9 % sensitivity and 100 % specificity. By eliminating false positives without increasing assay complexity, TrueLAMP enhances confidence in colorimetric LAMP assays and preserves their hallmark simplicity, enabling robust molecular detection using unmodified, off-the-shelf equipment suitable for decentralized, point-of-care, and resource-limited settings.

Keywords: Loop-mediated isothermal amplification (LAMP); colorimetric LAMP; nonspecific amplification; false positives; point-of-care diagnostics; TrueLAMP™.

Introduction

Loop-mediated isothermal amplification (LAMP) has become a prominent method for rapid nucleic acid detection due to its high amplification efficiency and operation under isothermal conditions, making it ideal for point-of-care and field testing [1–4]. Despite its potential as a research and diagnostic tool, the widespread use of LAMP has been limited by a persistent problem: nonspecific amplification occurring even when no template DNA or RNA is present. Such spurious amplification leads to false positives in no-template controls (NTCs) and test samples, undermining assay reliability [5–7].

Confronted by these false positives, researchers often suspect contamination and spend considerable time and resources addressing it—replacing reagents and consumables, cleaning workspaces and equipment, implementing separate preparation and reaction areas, or incorporating uracil-DNA glycosylase (UDG) and dUTP systems to prevent cross-contamination. When nonspecific amplification persists, practitioners frequently redesign and rescreen new primer sets. The recurring problem frustrates users and often leads to the abandonment of LAMP-based projects.

Numerous additives—such as betaine, DMSO, tetramethylammonium chloride (TMAC), and pullulan—have been explored to improve LAMP specificity, but none consistently prevent primer-driven amplification [7–10]. Many reports describe partial improvements based on short reaction times, yet when incubations extend beyond 40–60 minutes, nonspecific amplification typically reappears.

Several recent strategies have sought to overcome LAMP's nonspecific amplification by coupling the reaction to sequence-specific nucleases such as CRISPR/Cas12a or programmable Argonaute (pAgo) proteins. In these hybrid systems, the nuclease is activated only when the correct target sequence is bound by a complementary guide RNA or DNA, thereby rejecting off-target or primer-derived amplicons and

improving assay specificity [11–14]. While effective, such approaches introduce additional reagents, guide design steps, and detection layers, increasing assay complexity and cost.

We hypothesized that nonspecific amplification in LAMP is an intrinsic property of the reaction chemistry itself, arising from primer–primer interactions and secondary structures that can be extended by Bst-type polymerases. With their strong strand-displacement and low-fidelity activity, these polymerases can elongate short, mismatched primer duplexes and generate spurious amplicons. Mechanistic studies have confirmed this pathway, demonstrating that false-positive signals in NTCs result from the amplification of primer dimers, self-priming hairpins, or other mispriming events [15–17].

Here, we describe TrueLAMP, a polymerase formulation that includes a proprietary inhibitor designed to selectively block nonspecific primer amplification without compromising target-specific amplification. Unlike conventional additives that provide only a brief window of inhibition—typically lasting minutes—TrueLAMP achieves durable suppression of primer-driven amplification for hours. Furthermore, unlike LAMP-coupled CRISPR, pAgo, and other sequence-specific post-LAMP detection systems, TrueLAMP suppresses primer-intrinsic nonspecific amplification directly within the amplification reaction, preserving the simplicity, accessibility, and low cost that make isothermal amplification attractive for point-of-care applications. We evaluate TrueLAMP across multiple primer sets and demonstrate its effectiveness in blocking false positives while maintaining robust amplification of target nucleic acids. By maintaining assay simplicity while eliminating false positives, TrueLAMP directly addresses the reliability barrier that has prevented LAMP from realizing its full potential in decentralized and low-resource testing environments.

Materials and Methods

Reagents and Enzymes

TrueLAMP™ kits were obtained from Boltii Diagnostics Inc. (Salt Lake City, Utah), comprising TrueLAMP polymerase and 2× TrueLAMP buffer containing an inhibitor that suppresses nonspecific LAMP amplification. The inhibitor composition is proprietary and not disclosed. SARS-CoV-2 RNA (NR-52347) and heat inactivated SARS-CoV-2 virus (NR-52286) were obtained from BEI Resources, NIAID, NIH.

Primer Sets

Four independent LAMP primer sets targeting the SARS-CoV-2 genomic RNA were used to assess the performance and generality of TrueLAMP: one proprietary set (N008) from Boltii Diagnostics and three published sets—'Lamb,' 'N27,' and 'M3.' The Lamb primer set corresponds to the SARS-CoV-2 RT-LAMP design by Lamb et al. (2020) [18], and the N27 and M3 primer sets were adopted from the LAMP optimization study by Huang, Tang, Ismail, and Wang (2022) [19], with full sequences available in the supplementary data (mmc1.pdf) of that publication. All primer sequences used here exactly match those reported. Final primer concentrations in the 10 µL reaction were 0.2 µM (F3/B3), 0.4 µM (LF/LB), and 1.6 µM (FIP/BIP).

LAMP Reactions

Reactions were carried out according to the manufacturer's instructions. Each 10 µL reaction contained 3.0 µL nuclease-free water, 5.0 µL 2× buffer, 0.05 µL polymerase, 1.0 µL 10× primer mix, and either 1.0 µL nuclease-free water (no-template control, NTC) or template (RNA or DNA or virions). Reactions were incubated at 65 °C for 90 min. Positive reactions turned yellow to orange, whereas NTCs remained red. Color changes were quantified by measuring magenta intensity as described below.

Semi-Real-Time Colorimetric LAMP Setup

A convection hybridization oven (Stratagene PersonalHyb, Model 401030) was set to 66.7 °C, corresponding to an internal reaction temperature of 65 °C at the tube position, confirmed using a liquid thermometer. Eight-strip PCR tubes were placed upright and photographed at 15-min intervals through the oven viewing window using a stationary smartphone fixed in place (**Figure 1**). Maintaining constant image geometry was essential, as changes in angle or distance affected numerical color values. Color intensity (maximum magenta or minimum green) of the reaction was quantified using a smartphone colorimeter app by sampling the region immediately below the liquid meniscus with the smallest available aperture. Magenta channel decrease (CMYK mode) or green channel increase (RGB mode) was used as the amplification readout, depending on app output format; both metrics report the same underlying red-to-yellow color transition during LAMP amplification.

Endpoint Colorimetric LAMP Incubation Modes

Three incubation modes were evaluated:

1. Direct 65 °C incubation: Reaction tubes were placed directly into a pre-equilibrated 65 °C water bath (Model 2LS, Fisher Scientific, Pittsburgh, PA).
2. Slow-ramp condition (informative intermediate case): Reaction tubes were placed in a 60 °C water bath, after which the bath thermostat was adjusted to 65 °C. Due to gradual heat transfer, the reaction temperature passed through ~60–63 °C for several minutes before reaching 65 °C. This was not adopted as a standard protocol but served to reveal the importance of early low-temperature reverse transcription.
3. Defined two-step RT-LAMP (generalizable protocol): Reactions were incubated at 60 °C for 10 min, then increased to 65 °C. This creates a controlled low-temperature pre-phase that is independent of device-specific ramp rates and enables reproducible performance in ovens, water baths, incubators, or thermocyclers.

All reactions used identical reagent compositions and endpoint detection procedures.

Analytical Sensitivity and Limit of Detection

To estimate the limit of detection (LOD), SARS-CoV-2 RNA was serially diluted from 1000 to 16 copies per reaction. Each dilution was tested in triplicate. The lowest dilution yielding positive amplification in all three replicates was used for LOD confirmation. For LOD confirmation, 21 replicates were performed, and the LOD was defined as the lowest concentration producing at least 20 of 21 positive reactions ($\geqslant$ 95 %).

Statistical Analysis

Quantitative colorimetric data were analyzed using descriptive statistics (mean ± standard deviation). Group comparisons of magenta intensity over time were performed using the parametric Welch's t-test

[20] and the non-parametric Mann–Whitney U test [21]. Differences were considered statistically significant at $p < 0.05$. For binary outcome data, including sensitivity and specificity assays, Fisher's exact test [22] was used to assess categorical associations. Confidence intervals (95 % CI) for proportions were calculated using the Clopper–Pearson exact method [23]. All analyses and graphs were generated in GraphPad Prism (GraphPad Software, San Diego, CA).

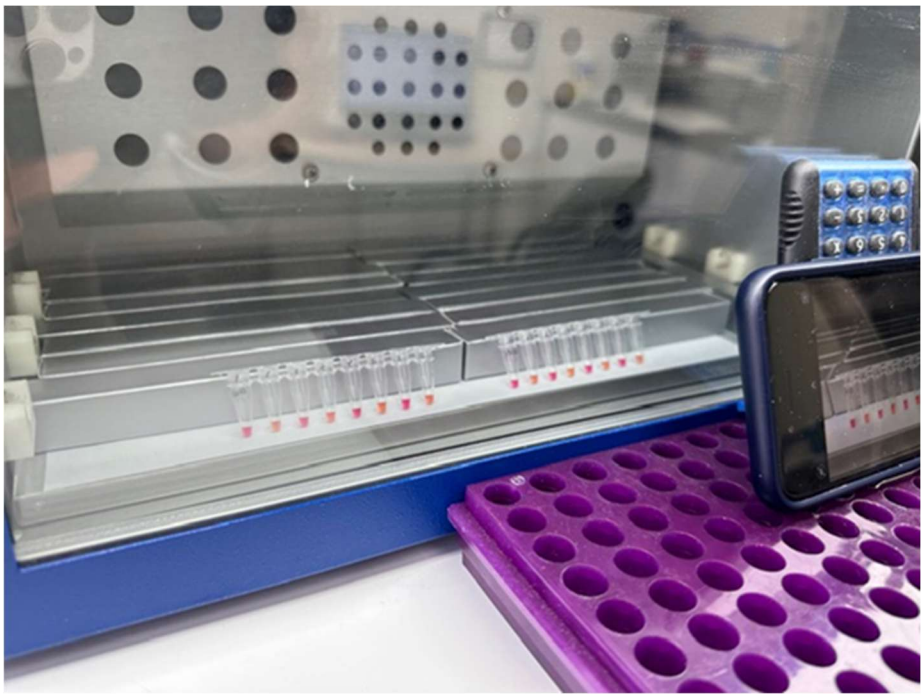

Figure 1. Semi-real-time colorimetric LAMP setup. The 8-strip LAMP reaction tubes were placed upright in a convection oven at 65 °C. The reaction tubes were photographed every 15 min through the oven's glass door using a stationary smartphone. Magenta intensity was quantified from each snapshot using a CMYK colorimeter app.

Results

Blocking Primer-Driven Nonspecific Amplification

To evaluate whether TrueLAMP blocks nonspecific, primer-driven amplification, NTC reactions with a nonspecific amplification–prone M3 primer set [19] were performed using either conventional Bst polymerase (without inhibitor) or TrueLAMP polymerase (with inhibitor) under identical conditions at 65 °C. Magenta color intensity was monitored every 15 minutes for 120 minutes (**Figure 2**). With conventional Bst polymerase, magenta intensity decreased progressively from 51.5 ± 1.3 at 15 min to 22.3 ± 1.5 at 120 min, indicating extensive color loss consistent with nonspecific primer amplification. In contrast, TrueLAMP reactions maintained stable red color throughout incubation, with magenta intensities between 55.3 ± 1.0 at 15 min and 54.5 ± 3.2 at 120 min. Statistical analysis (Welch's t-test) confirmed that TrueLAMP consistently maintained significantly higher magenta intensities than Bst polymerase at all time points ($p < 0.005$ at 15 min; $p < 0.0001$ from 30 min onward). Given the small

sample size (n = 4), a non-parametric Mann–Whitney U test was also performed, yielding an exact two-sided *p*-value of 1/35 (≈ 0.0286) for all time points. The agreement between both tests supports the conclusion that TrueLAMP completely suppresses nonspecific LAMP amplification in NTCs.

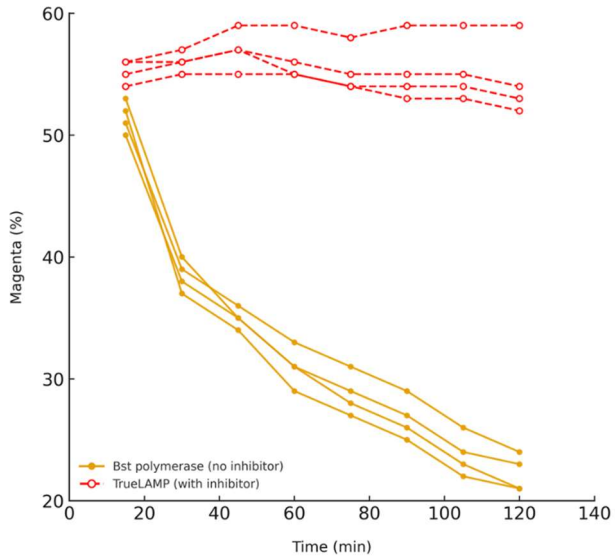

Figure 2. Suppression of nonspecific primer amplification by TrueLAMP. LAMP reactions with the nonspecific amplification-prone M3 primer set were performed in the absence of template using conventional Bst polymerase (without nonspecific LAMP inhibitor) or TrueLAMP polymerase (with nonspecific LAMP inhibitor) under identical conditions. Decline in magenta intensity indicates nonspecific primer amplification. TrueLAMP reactions showed no detectable color change over 120 min, confirming complete suppression of primer-driven amplification.

Robust Amplification Across Multiple Primer Sets

To assess the reproducibility and primer-set independence of TrueLAMP performance, semi-real-time colorimetric LAMP amplification of SARS-CoV-2 RNA (500 copies) and NTCs was compared using four independent primer sets (N008, Lamb, M3, and N27) across four replicate experiments at 65 °C (**Figure 3**). In all cases, NTC reactions maintained stable magenta color with no significant change throughout the 90-min reaction period (mean intensity 61–64 %), confirming suppression of nonspecific amplification. In contrast, RNA-containing reactions exhibited rapid decreases in magenta intensity, beginning at 15 min—when an orange hue became perceptible through the oven window—and reaching more than a 50 % reduction by 30 min. Statistical analysis confirmed significant differences between RNA and NTC reactions (Welch's t-test: $p < 0.05$ for all primer sets at 15 min and $p < 0.0001$ after 30 min; Mann–Whitney U test: $p < 0.05$ for M3 and N27 at 15 min and for all primer sets after 30 min). These results demonstrate that TrueLAMP reliably amplifies target RNA while fully blocking background primer amplification, independent of primer design or target locus.

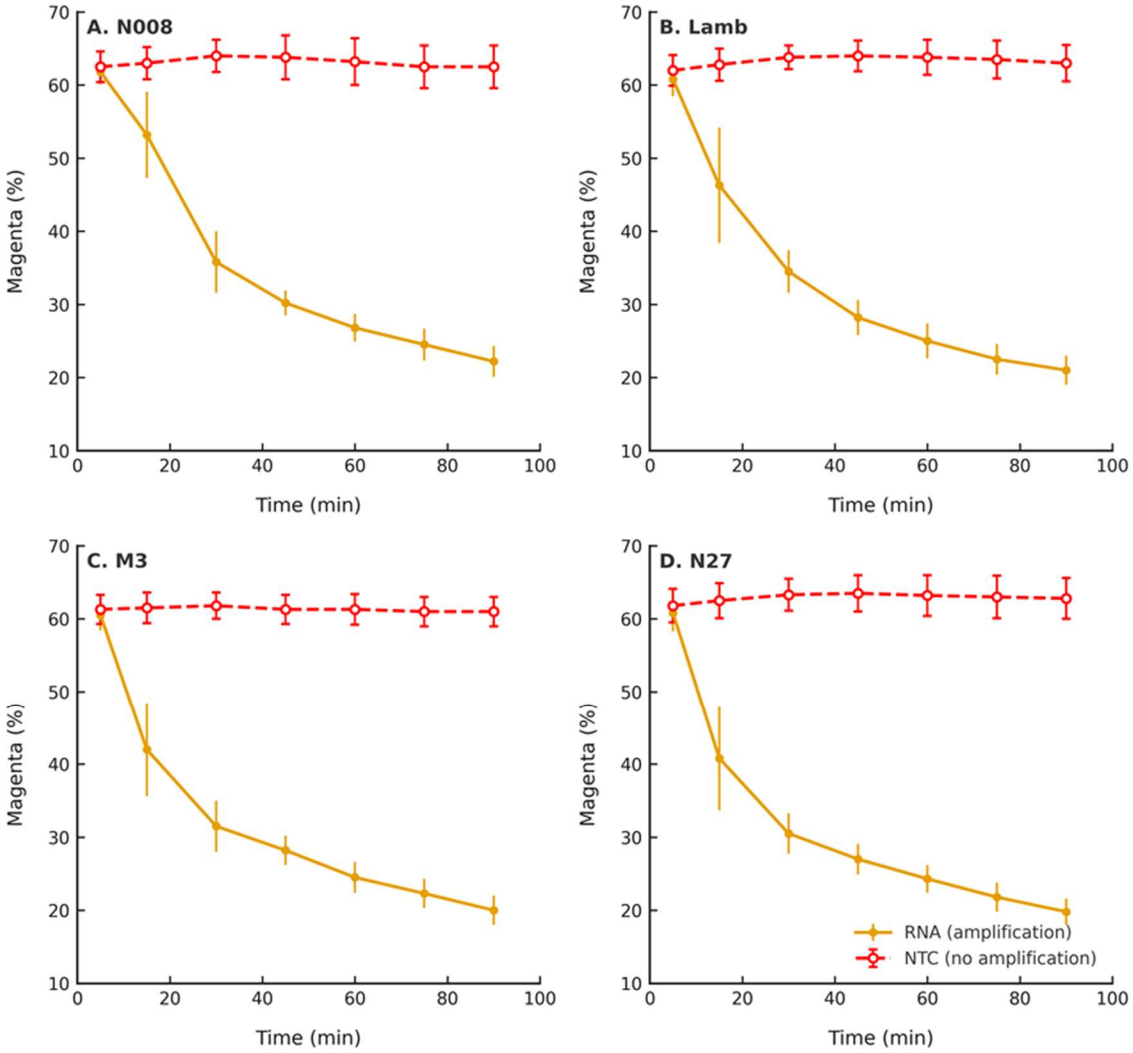

Figure 3. Amplification of SARS-CoV-2 RNA with four independent primer sets. Semi-real-time colorimetric LAMP reactions were performed at 65 °C using TrueLAMP with the N008, Lamb, M3, and N27 primer sets. RNA reactions (500 copies of RNA, orange, solid lines, filled circles) showed strong amplification kinetics, while NTCs (red, dashed lines, open circles) remained stable. Each curve represents the mean ± SD of four replicates.

Limit of Detection

To determine the analytical sensitivity of TrueLAMP, SARS-CoV-2 RNA was serially diluted (1000–16 copies per reaction) and amplified using the Lamb primer set at 65 °C in a convection oven. Reactions containing ⩾250 copies consistently produced positive amplification, whereas reactions with 125 copies or fewer yielded inconsistent or no amplification after 90 minutes. To confirm the LOD, three independent 8-tube strips—each containing one NTC and seven 250-copy RNA samples—were amplified under identical conditions with the Lamb primer set. After incubation, all NTCs remained red, and 20 of 21 RNA reactions (95 %) turned yellow, confirming successful amplification (**Figure 4**). The detection

probability at 250 copies per reaction was 95 % (95 % CI: 77–99 %), establishing 250 copies as the LOD for the Lamb primer set using TrueLAMP.

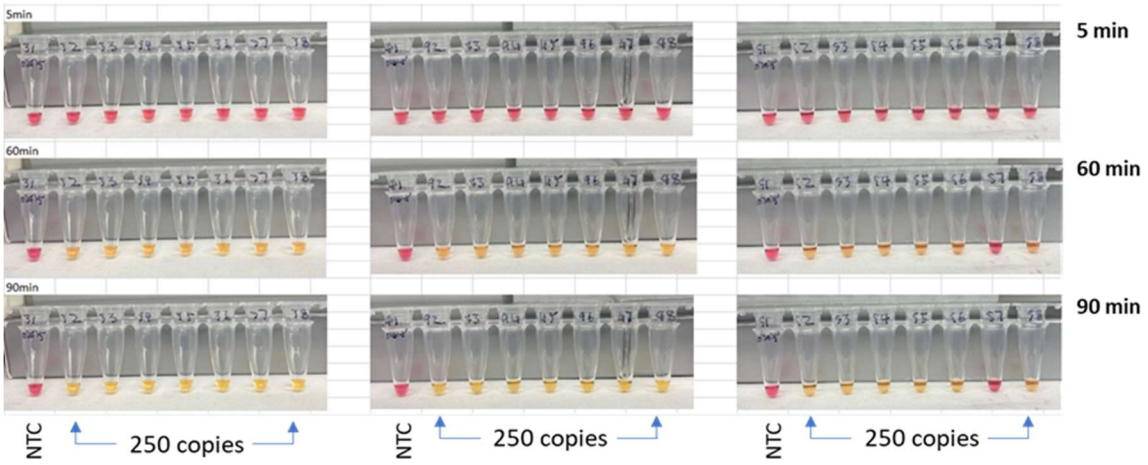

Figure 4. Limit of detection (LOD) of TrueLAMP using the Lamb primer set. Serial dilutions of SARS-CoV-2 RNA (1000–16 copies/µL) were tested at 65 °C using TrueLAMP with the Lamb primer set. Reactions containing ≥250 copies consistently produced positive amplification, while lower concentrations yielded inconsistent results. Triplicate 8-strip confirmations showed 20/21 positive reactions (95% detection probability; 95% CI 77–99%), establishing a LOD of 250 copies per reaction.

Effect of Incubation Modality on RT-LAMP

Having established TrueLAMP's sensitivity in semi-real-time RT-LAMP using a convection oven, we next examined its performance in endpoint RT-LAMP using a water bath. Direct 65 °C incubation resulted in reduced sensitivity in the water bath (apparent LOD > 1000 copies), whereas oven incubation under otherwise identical conditions achieved 95 % detection at 250 copies (**Table 1**). This difference suggests that rapid heat transfer in the water bath, which brings the reaction to equilibrium at 65 °C much faster than in a convection oven, may suppress reverse transcriptase activity during the early reaction phase. The slow-ramp water-bath condition (60 → 65 °C) yielded higher sensitivity because the reaction transiently passed through ~60–63 °C, favoring reverse transcription. To establish an instrument-independent approach, a defined two-step 60 °C 10 min → 65 °C RT-LAMP protocol was introduced. Under this controlled condition, all 21 of 21 reactions containing 250 virions were positive in both water bath and oven, and NTCs remained negative (**Table 1**). However, extending the 60 °C phase to ~60 min resulted in NTC amplification, confirming that the enhancement is time-dependent. A brief (10–15 min) 60 °C pre-phase enhances reverse transcription while maintaining TrueLAMP's suppression of nonspecific amplification.

Analytical Sensitivity and Specificity

To further evaluate the analytical performance of TrueLAMP, RT-LAMP reactions were performed at 65 °C using the N27 primer set with 250 copies of SARS-CoV-2 RNA. TrueLAMP detected 250 copies in 31 of 32 RNA reactions and produced no amplification in any of 34 NTCs (**Tables 2** and **3**). The

Table 1. RT-LAMP limit of detection (LOD) using convection oven vs water bath incubation*.

Incubation condition	RNA (copies)	Positive detections	95 % CI (exact)	LOD (copies)	p Value (vs p_0 = 0.95)
1. Oven (65 °C)	250	20/21 (95 %)	77–99 %	250	0.66 (pass)
2. Water bath (65 °C)	1000	14/21 (67 %)	43–85 %	> 1000	4.9×10^{-5} (fail)
3. Water bath (60→65 °C)	250	21/21 (100 %)	84–100 %	250	> 0.99 (pass)
4. Oven (60 °C 10 min→65 °C)	250#	21/21 (100 %)	84–100 %	250	> 0.99 (pass)
5. Water bath (60 °C 10 min→65 °C)	250#	21/21 (100%)	84–100 %	250	> 0.99 (pass)

* Twenty-one replicates were tested per condition with 3 no-template controls (all negative) using the Lamb primer set. LOD defined as concentration giving ⩾ 95 % detection (⩾ 20 / 21 positives). # Heat-inactivated SARS-CoV-2 virions (NR-52286) were used as template for these reactions.

Table 2. Analytical results of TrueLAMP for the detection of SARS-CoV-2 RNA.**

Condition	NTC (n = 34)	RNA (n = 32)	Total
Negative amplification	34	1	35
Positive amplification	0	31	31
Total	34	32	66

**Assays were performed using the N27 primer set at 1× LOD (250 copies per reaction) in 65 °C oven.

Table 3. Diagnostic performance metrics of TrueLAMP for SARS-CoV-2 RNA detection.**

Metric	Estimate	95 % CI	Notes
Sensitivity	96.9 % (31/32)	83.8 – 99.9 %	Clopper–Pearson exact
Specificity	100 % (34/34)	91.6 – 100 %	Clopper–Pearson exact
Positive predictive value (PPV)	100 % (31/31)	90.8 – 100 %	
Negative predictive value (NPV)	97.1 % (34/35)	85.1 – 99.9 %	
Accuracy	98.5 % (65/66)	91.8 – 100 %	
Fisher's exact test (p)	2.2×10^{-17}	—	Two-sided
Diagnostic odds ratio	$\gg 1$	—	Strong testing accuracy

**Assays were performed using the N27 primer set at 1× LOD (250 copies per reaction) in 65 °C oven.

resulting analytical sensitivity was 96.9 % (exact 95 % CI, 83.8–99.9 %), and specificity was 100 % (exact 95 % CI, 91.6–100 %). Positive and negative predictive values were 100 % and 97.1 %, respectively, with an overall accuracy of 98.5 %. Fisher's exact test confirmed a highly significant association between RNA and NTC outcomes ($p < 0.0001$), demonstrating robust detection and complete blocking of false positives.

Discussion

Loop-mediated isothermal amplification has long been recognized as a promising method for decentralized nucleic acid detection, but routine use has been hindered by nonspecific primer-driven amplification, which produces false positives even in NTCs. These artifacts arise when Bst-type polymerases extend short primer–primer or self-priming structures, generating amplicons unrelated to the intended target. The selective suppression of this primer-intrinsic amplification is therefore essential for enabling reliable LAMP-based diagnostics.

By selectively blocking primer-driven amplification, TrueLAMP effectively prevents false positives without compromising speed or efficiency for true targets (**Figures 2** and **3**). Its inherent selectivity enables clear colorimetric distinction between true positives and NTCs, even after extended incubation for hours. This property allows convenient endpoint colorimetric testing in the field without the need for precise timing or continuous monitoring, and may also eliminate the need for the subjective threshold time (Tt) used in conventional real-time LAMP, providing a more direct and definitive readout.

The absence of amplification in NTCs across multiple primer sets indicates that TrueLAMP is broadly applicable to diverse primer compositions and targets while preserving reaction temperature, kinetics, and simplicity. This compatibility ensures seamless integration with existing LAMP workflows and makes TrueLAMP readily adaptable to laboratory, field, or educational use with only basic equipment, such as a digital oven, as demonstrated in our semi-real-time colorimetric imaging setup (**Figure 1**). Such visual monitoring using unmodified consumer devices highlights the accessibility of TrueLAMP for low-infrastructure laboratories, mobile testing units, and educational settings. To our knowledge, this represents the first demonstration of a semi-real-time colorimetric LAMP using unmodified, off-the-shelf equipment, highlighting its accessibility for resource-limited settings.

The sensitivity differed among incubation modes (65 °C, 60→65 °C, and 60 °C 10 min→65 °C) (**Table 1**). In the water bath, direct 65 °C incubation reduced sensitivity because the reaction rapidly equilibrated to 65 °C, limiting reverse transcriptase activity. In contrast, gradual heating unintentionally provided a short interval in the 60–63 °C range, allowing cDNA synthesis before the reaction reached 65 °C. Introducing a defined 10-min dwell at 60 °C reproduced this beneficial phase in a time-controlled, device-independent manner, restoring full sensitivity in both water bath and oven. The effect is time-dependent: a brief (10–15 min) 60 °C pre-phase enhances reverse transcription without permitting primer-driven amplification, whereas prolonged residence (~60 min) allows nonspecific amplification even in TrueLAMP reactions. Thus, the critical operational factor is the controlled duration near 60 °C, not the heating device itself. This principle enables consistent RT-LAMP performance across ovens, water baths, incubators, and thermocyclers, improving robustness for laboratory, portable, and field applications.

TrueLAMP achieved a LOD of 250 RNA copies per reaction with the Lamb primer set (**Figure 4**) and comparable results with other SARS-CoV-2 primer sets, including N008, M3, and N27, as well as a human RNP primer set for DNA detection. The similar detection sensitivities observed across these independently designed primer sets—targeting distinct loci and nucleic acid types—suggest that TrueLAMP may provide a more uniform performance across diverse LAMP designs. This consistency is encouraging, as it implies that primer design may become less critical for achieving high sensitivity with TrueLAMP. The detection sensitivity achieved by TrueLAMP is comparable to or better than previously reported colorimetric LAMP assays using SARS-CoV-2 RNA as the template [24–26], despite the inclusion of the inhibitory component. This demonstrates that TrueLAMP's selectivity did not compromise sensitivity. However, users should still empirically evaluate the LOD of their own primer sets under intended conditions, as broader validation will be needed to confirm the generality of TrueLAMP's uniform performance across all LAMP primer designs.

TrueLAMP's diagnostic performance (**Tables 2** and **3**) underscores its reliability: across replicate assays at 1× LOD, it achieved 96.9 % analytical sensitivity and 100 % specificity, with a statistically significant separation between positive and NTC reactions ($p = 2.2 \times 10^{-17}$, Fisher's exact test). These results demonstrate excellent diagnostic discrimination and concordance with molecular assay standards, further confirming the analytical robustness of TrueLAMP.

In summary, TrueLAMP resolves one of the most persistent issues in isothermal amplification—primer-driven nonspecific amplification—while retaining the hallmark speed, simplicity, and sensitivity of LAMP. It establishes a reliable and adaptable foundation for molecular diagnostics and point-of-need testing, particularly in resource-limited settings.

Limitations and Future Directions

This study focused on SARS-CoV-2 RNA and SARS-CoV-2 virions as a model system using four primer sets under controlled laboratory conditions. Additional validation across independent laboratories, primer designs, and diverse RNA/DNA targets will be needed to confirm the generality of TrueLAMP's performance. Collaboration with external partners to evaluate TrueLAMP on clinical and environmental samples will further establish its real-world consistency and potential regulatory readiness. Beyond analytical validation, future work should emphasize field deployment and usability in decentralized or resource-limited settings. This includes assessing TrueLAMP's performance in portable or battery-powered heating systems typical of point-of-care use. Integration with low-cost sample preparation and smartphone-based result interpretation could enable complete molecular testing workflows outside of conventional laboratories. These directions will help realize TrueLAMP's potential as a reliable, affordable, and instrument-minimal diagnostic platform adaptable to diverse public health, veterinary, and environmental applications.

Conclusion

TrueLAMP overcomes a fundamental limitation of LAMP by preventing primer-driven nonspecific amplification while maintaining fast, robust, and primer-set-independent target amplification. The method supports semi-real-time and endpoint colorimetric detection using unmodified laboratory and field-

portable equipment, and performs consistently across independent primer designs and RNA and DNA targets. By enabling accurate molecular detection without advanced instrumentation and without false-positive interference, TrueLAMP expands the practical reach of LAMP-based diagnostics into decentralized, mobile, and resource-limited environments.

Acknowledgments

The following reagents were obtained through BEI Resources, NIAID, NIH: NR-52347: Quantitative PCR Control RNA from heat-inactivated SARS-CoV-2, and NR-52286: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat Inactivated.

Competing Interests Statement

Z.C. is the inventor of TrueLAMP and founder of Boltii Diagnostics Inc., which manufactures the TrueLAMP kit. The author declares no additional competing interests.

References

1. Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Ono, T., & Hasegawa, Y. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, *28*(12), Article e63.

2. Mori, Y., & Notomi, T. (2009). Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. *Journal of Infection and Chemotherapy*, *15*(2), 62–69.

3. Moore, K. J. M., Cahill, J., Aidelberg, G., Lall, D., Chan, P., & Mason, C. E. (2021). Loop-Mediated Isothermal Amplification (LAMP) detection of SARS-CoV-2 and myriad other applications. *Journal of Biomolecular Techniques*, *32*(3), 228–275.

4. Njiru, Z. K. (2012). Loop-mediated isothermal amplification technology: towards point-of-care diagnostics. *PLoS Neglected Tropical Diseases*, *6*(6), Article e1572.

5. Jang, M. J., & Kim, S. (2022). Inhibition of non-specific amplification in loop-mediated isothermal amplification. *Applied Biochemistry and Biotechnology*, *194*(10), 4169–4183.

6. Habibzadeh, P., Mofatteh, M., Silawi, M., & Mousavi, S. E. (2021). Molecular diagnostic assays for COVID-19: an overview. *Critical Reviews in Clinical Laboratory Sciences*, *58*(6), 467–488.

7. Kim, S. H., Lee, S. Y., Kim, U., Oh, S. W., & Kim, S. (2023). Diverse methods of reducing and confirming false-positive results of loop-mediated isothermal amplification assays: A review. Analytica Chimica Acta, 1280, Article 341693.

8. Mok, Y. K., Cho, Y., & Kim, B. (2016). Comprehensive evaluation of molecular enhancers of loop-mediated isothermal amplification. *Analytical Biochemistry*, *502*, 60–67.

9. Gao, Y., Huang, W., Wu, B., Li, M., & Huang, R. (2019). Pullulan reduces the non-specific amplification of loop-mediated isothermal amplification. *Analytical Letters*, *52*(14), 2262–2274.

10. Jang, M. J., & Kim, S. (2022). Inhibition of non-specific amplification in loop-mediated isothermal amplification via tetramethylammonium chloride. *BioChip Journal*, *16*(3), 326–333.

11. Zhang, M., Zhao, Q., Sun, W., Zhu, L., Li, H., & Wang, W. (2021). CRISPR/Cas12a-assisted ligation-initiated loop-mediated isothermal amplification for sequence-specific detection. *Analytical Chemistry*, *93*(15), 6195–6202.

12. Trung, N. T., Phan, T. T. H., Hoang, L., Toan, L. T., Hoang, D. B., & Doan, T. T. T. (2022). CRISPR-Cas12a combination to alleviate the false-positive in LAMP assay for Neisseria meningitidis. *BMC Infectious Diseases*, *22*(1), Article 429.

13. Yu, Z., Shao, Y., Zhang, Y., & Zhang, Y. (2025). LAMP Assay Coupled with a PfAgo (Pyrococcus furiosus Argonaute) System for the Rapid Detection of Porcine Epidemic Diarrhea Virus. *ACS Synthetic Biology*, *14*(3), 689–698.

14. Wang, L., Fan, L., Wang, D., Ma, Q., Hu, J., Wang, X., & Wang, L. (2025). A TtAgo-mediated LAMP System for highly sensitive and specific detection of rotavirus in pediatric diarrheal samples. *Frontiers in Cellular and Infection Microbiology*, *15*, Article 1682786.

15. Meagher, R. J., Priye, A., Light, Y. K., Wang, J., & Hughes, G. L. (2018). Impact of primer dimers and self-amplifying hairpins on qPCR, dPCR, and LAMP assay performance. *Analytical Chemistry*, *90*(1), 1013–1020.

16. Wang, D., Hsieh, K., Zec, H. C., & Ying, B. (2015). Identification of primer–dimer artifacts in loop-mediated isothermal amplification for pathogen detection. *Molecules*, *20*(10), 19518–19531.

17. Rolando, J. C., Jue, E., Schoepp, N. G., & Ismagilov, R. F. (2020). Real-time, digital LAMP with commercial microfluidic chips reveals the role of false positives and primer–dimer formation in LAMP. *Nucleic Acids Research*, *48*(16), Article e93.

18. Lamb, L. E., Bartolone, S. N., Ward, E., & Chancellor, M. B. (2020). Rapid detection of SARS-CoV-2 using reverse transcription loop-mediated isothermal amplification (RT-LAMP). *PLoS One*, *15*(6), Article e0234682.

19. Huang, X., Tang, G., Ismail, N., & Wang, X. (2022). Developing RT-LAMP assays for rapid diagnosis of SARS-CoV-2 in saliva. *EBioMedicine*, *75*, Article 103736.

20. Welch, B. L. (1947). The generalization of 'Student's' problem when several different population variances are involved. *Biometrika*, *34*(1-2), 28–35.

21. Mann, H. B., & Whitney, D. R. (1947). On a Test of Whether one of Two Random Variables is Stochastically Larger than the Other. *Annals of Mathematical Statistics*, 18(1), 50-60.

22. Fisher, R. A. (1922). On the interpretation of χ^2 from contingency tables, and the calculation of P. *Journal of the Royal Statistical Society, Supplement*, *1*(1), 87–94.

23. Clopper, C. J., & Pearson, E. S. (1934). The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika*, *26*(4), 404–413.

24. Rabe, B. A., & Cepko, C. (2020). SARS-CoV-2 detection using isothermal amplification and a rapid, inexpensive protocol for sample inactivation and purification. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(40), 24450–24458.

25. García-Bernalt Diego, J., de Salazar, A., Muñoz-Fontela, C., & de la Torre, J. C. (2021). Validation of a colorimetric RT-LAMP for rapid SARS-CoV-2 RNA detection. *Diagnostics*, *11*(3), Article 438.

26. Alhamid, A., Abu-Farha, M., Alfouzan, L., Abu-Khdair, R., Al-Abdulkarim, A., Alharbi, O., Althawadi, S. & Alfouzan, W. (2023). Rapid colorimetric detection of SARS-CoV-2 by loop-mediated isothermal amplification assay. *Scientific Reports*, *13*(1), Article 4221.