Utilizing Lyophilized LAMP Reagents in Rapid Molecular Assays

Ece Alpaslan, Ph.D., Yinhua Zhang, Ph.D., Nathan A. Tanner, Ph.D., and Gregory C. Patton, Ph.D.

New England Biolabs, Inc. 240 Country Road, Ipswich, MA 01938, USA.

Introduction

Loop Mediated Isothermal Amplification (LAMP) has become a widely used method for detection of target nucleic acids (DNA and RNA) as it offers a robust and simple alternative to PCR. LAMP is particularly well suited to point of care (POC) applications such as COVID-19 diagnostics because only a single incubation temperature is required for nucleic acid amplification and the technology is compatible with simple detection strategies, including colorimetric or lateral flow readouts.

To further increase the utility of LAMP in POC molecular diagnostic workflows, elimination of cold chain requirements for reagent shipment and storage is desired and can often be accomplished by lyophilization. In this study, we investigate single and multiplex detection of several viral infectious diseases using lyophilized LAMP/RT-LAMP reagents.

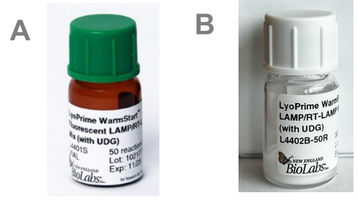


Figure 1: LyoPrime WarmStart LAMP/RT-LAMP Mix (with UDG)

(A) with fluorescent dye (NEB #L4401)
(B) without fluorescent dye (NEB #L4402)

Methods

Figure 2: LAMP Overview[1]

LAMP uses 4-6 primers recognizing 6-8 distinct regions of target DNA. A strand displacing DNA polymerase initiates synthesis and 2 of the primers form loop structures to facilitate subsequent rounds of amplification.

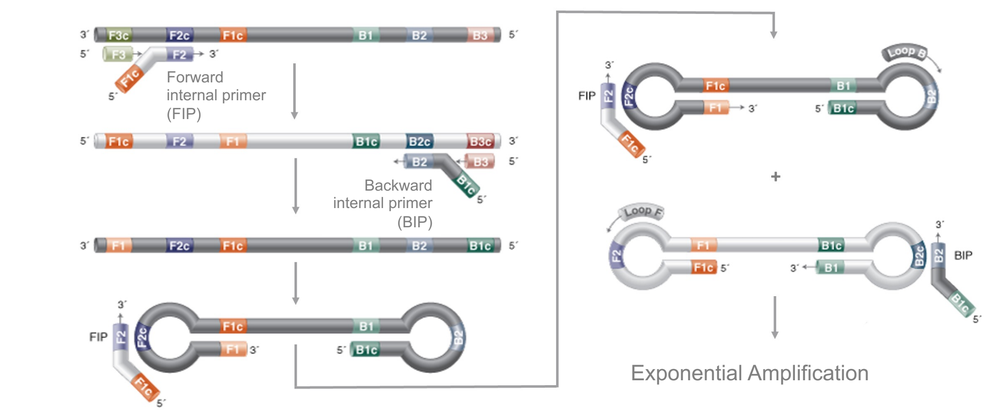
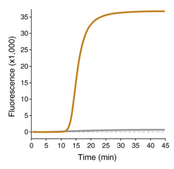


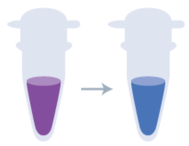
Figure 3: Commonly Used LAMP Detection Methods[2]

Real Time Fluorescence



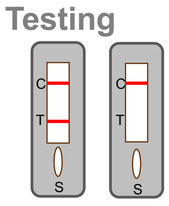
Amplification can be observed in real time using an intercalating fluorescent dye.

EndPoint Colorimetric Detection



Colorimetric dyes can be added into the reaction and color change can be visually observed post amplification.

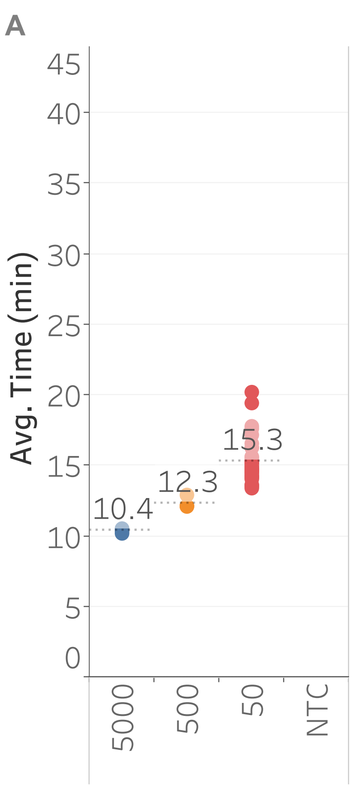
Lateral Flow Testing



Products can be detected on a lateral flow device after reaction completion.

Results

Figure 4: Real-Time Fluorescence Detection of SARS-CoV-2



B

SARS-CoV-2 RNA copies per reaction	5,000	500	50	NTC
User 1	4/4	4/4	36/36	0/4
User 2	4/4	4/4	36/36	0/4
User 3	4/4	4/4	35/36	0/4

Lyophilized Fluorescent LAMP/RT-LAMP Mix with UDG was utilized in a RT-LAMP reaction with either positive samples (human total RNA + synthetic SARS-CoV-2 RNA at 5,000, 500 or 50 copies per reaction) or no template (NTCs) as indicated.

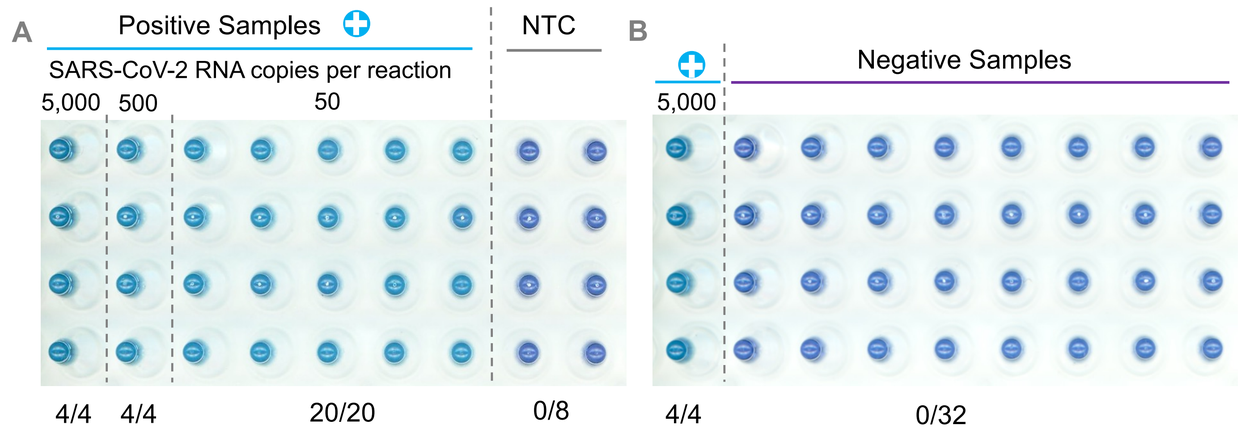
(A) All positive samples gave a time to result of less than 25 minutes, including all replicates that contained 50 copies of RNA per reaction (n = 36).

(B) Summary of results from analogous testing conducted by 3 users in 3 experiments total. At 50 copies per reaction, >99% (107/108) of reactions were detected. No NTCs were observed within 45 minutes.

Results Continued

Figure 5: Endpoint Colorimetric Detection (A&B)

Lyophilized LAMP/RT-LAMP Mix with UDG (without fluorescent dye) was used to amplify synthetic SARS-CoV-2 RNA using a dual primer mix that targets the N and E genes in the presence of 0.12 mM eriochrome black T, a colorimetric indicator.



The assay was carried out using either positive samples (human total RNA plus synthetic SARS-CoV-2 RNA at 5,000, 500 or 50 copies per reaction), negative samples (human total RNA alone) or no template (NTCs), as indicated. The 25 µl reactions were incubated at 65°C for 45 minutes, cooled to room temperature and then visually inspected.

(A) All positive samples changed color change from purple to blue, indicating amplification of the target, including all samples containing 50 copies of RNA per reaction (n = 20).

(B) No color change was observed for numerous replicates of negative samples (n = 32).

Figure 5: Endpoint Colorimetric Detection (C-E)

Other non-pH-based detection modalities were tested with the lyophilized LAMP mix using 500 copies of synthetic SARS-CoV-2 RNA as input. The mix is compatible with (C) calcein dye at 25 µM using UV-detection, but not visual detection with (D) 120 µM hydroxynaphthol blue (HNB). When both HNB and calcein dyes were used simultaneously at 1:4 ratio, amplification was clearly visible by eye for the positive targets (E).

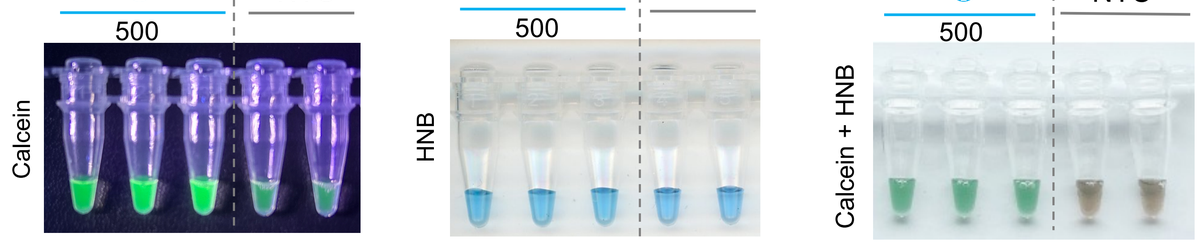


Figure 6: Lateral Flow Testing

Lyophilized Fluorescent LAMP/RT-LAMP Mix was utilized in a RT-LAMP reaction using positive samples (1 ng human total RNA + synthetic SARS-CoV-2 RNA at 1,000, 100 or 50 copies per reaction) as indicated using 1X SARS-CoV-2 primers (5'-Biotin-FIP, 5'-FAM-LoopF) and 0.5X ACTB primers (5'-Dig-FIP, 5'-FAM-LoopF). Reaction was run at 65°C for 30 minutes. 1:100 diluted reaction products were run on Milenia HybriDetect 2T strips.

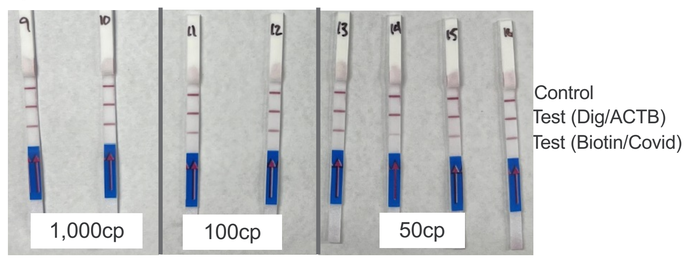
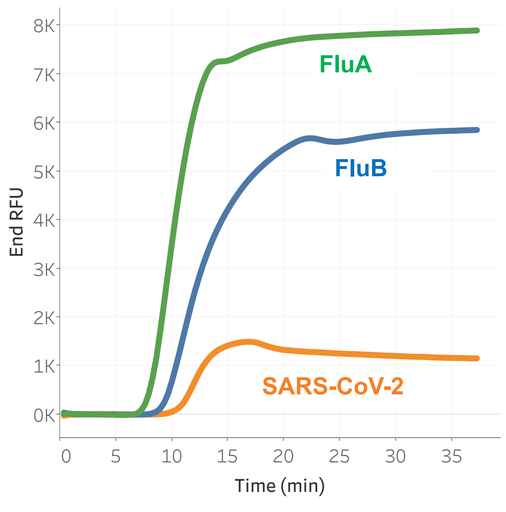


Figure 7: Multiplexing LAMP to Detect Respiratory Viruses



TaqMan-like hybridization probes are easy to design and have been shown to be effective in LAMP/RT-LAMP reactions.[3] Compatibility of the lyophilized LAMP/RT-LAMP Mix (no dye) was evaluated using previously designed probes for three common respiratory RNA viruses: FluA (Cy5), FluB (FAM) and SARS-CoV-2 (HEX).

Figure 7 shows multiplex detection of all three RNA viruses on a Biorad-CFX real-time PCR instrument. Samples contained 1,000 copies of each RNA template and data was collected in triplicate. The amplification curves represent the average of the three replicates. Positive amplification was observed within 15 minutes for each target.

Conclusions

LAMP is a robust, simple, isothermal amplification strategy that continues to enable testing beyond traditional laboratory settings. Lyophilized reagents extend the utility of LAMP in POC settings regardless of the detection strategy and will be an important factor in the extension of this powerful molecular diagnostic tool to decentralized, field, and at-home testing applications.

References

1. Loop mediated Isothermal Amplification: Application Overview www.neb.com/applications/dna-amplification-pcr-and-qpcr/isothermal-amplification/loop-mediated-isothermal-amplification-lamp
2. Augustine R., *et al*. "Loop-Mediated Isothermal Amplification (LAMP): A Rapid, Sensitive, Specific, and Cost-Effective Point-of-Care Test for Coronaviruses in the Context of COVID-19 Pandemic" Biology (Basel), 2020 Jul 22 ;9(8):182.
3. Zhang Y., Tanner N. A., "Efficient multiplexing and variant discrimination in reverse-transcription loop mediated isothermal amplification with sequence-specific hybridization probes" BioTechniques, 2022 Nov 5; 73(5).

Poster # B-243