A Multiplex RT-LAMP-Lateral Flow Platform For Comprehensive Dengue Serotype Detection

Vertically Integrated Projects @Maryland

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Intent & Motivation

Objective: To develop a diagnostic tool that uses one-pot RT-LAMP setup for amplification and a multiplex LFA system to detect and differentiate all four dengue serotypes.
Motivation: To create a low-cost, reliable, and stable point-of-care diagnostic tool for dengue that allows early detection and serotype differentiation

Background

- Dengue fever is a **mosquito-borne viral disease** that affects **350 million people annually**, primarily in **tropical regions,** and is expanding globally due to rising temperatures and urbanization.[1]
- Current diagnostics (RT-PCR, ELISA) require specialized lab equipment, **limiting accessibility in low-resource settings**.[2]
- **Reverse-transcription loop-mediated isothermal amplification (RT-LAMP)** enables rapid viral RNA amplification at a constant temperature.[3]
- **Lateral flow assays (LFAs)** provide a simple, paper-based visible line when the target molecule is detected.[4]
- Integrated RT-LAMP with gold nanoparticle (AuNP)-based LFAs offers a **sensitive, low-cost, point-of-care dengue diagnostics for under-resourced regions.**

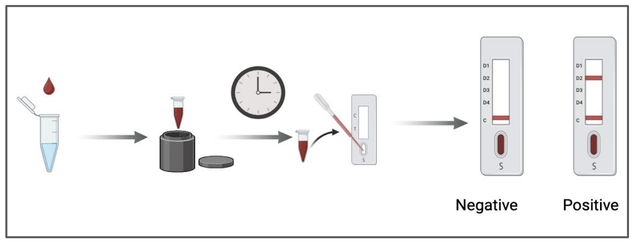

Figure 1. Blood is collected and added to a tube with freeze-dried buffer, followed by RT-LAMP amplification at ~60–65 °C for 30–40 min. The amplified products are transferred to an LFA strip for serotype-specific visual detection, where positive results show test lines along with a control line, and negative results show only the control line.

References & Acknowledgements

QR code links to references

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Methods

Reverse-Transcription LAMP

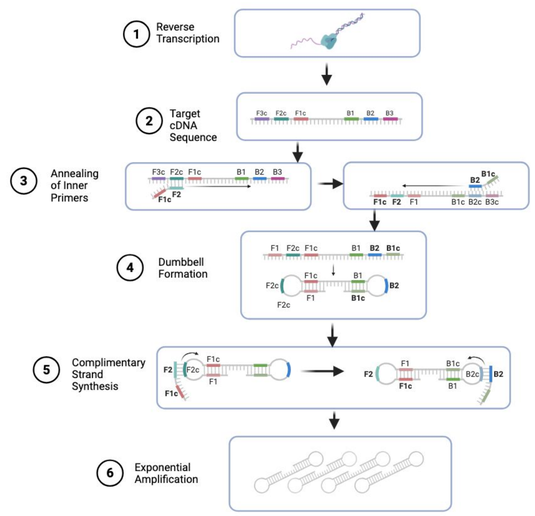


Figure 2: RT-LAMP Process. Binding sites for the LAMP primers are highlighted in the figure.

- Target RNA is converted to cDNA and amplified by multiplex LAMP targeting all four dengue serotypes[5]
- LAMP utilizes 4-6 primers and Bst 2.0 polymerase
- Colorimetric and fluorescent LAMP were performed for initial testing

One Pot Heating System

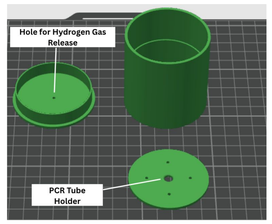


Figure 3: 3D Printed One-Pot Chemical Heating system (1 square = 1 cm²)

- Custom 3D-printed acrylonitrile butadiene styrene (ABS) lid and base hold a test tube over a Mg-Fe alloy chamber that generates and maintains 60-65°C[6] for LAMP upon water activation

Multiplex LFA Layout

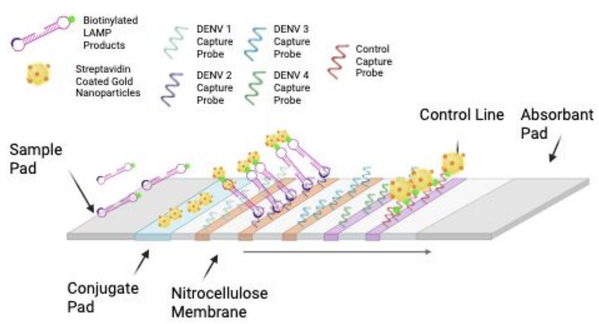


Figure 4: Layout of the multiplex LFA for dengue serotype detection

- An amplified sample is applied to a pad that isolates LAMP products
- LAMP products bind to complementary capture probes immobilized on the test lines, that are designed by 2D modeling of LAMP products to ensure complementary binding
- Streptavidin-coated gold nanoparticles bind to biotin-tagged LAMP products to produce a visible signal
- A control probe is included in the control line to confirm proper function
- High salt concentrations enable probe binding without antibodies use[7]

Results & Discussion

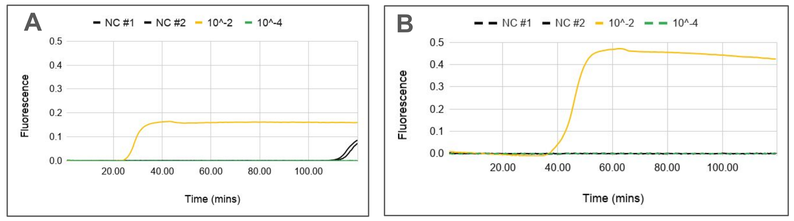


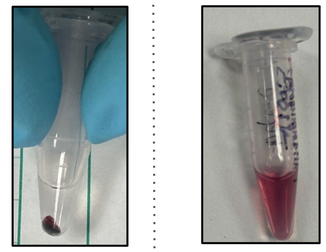
Figure 5: Fluorescent LAMP ~ Optimization of LAMP amplification conditions for dengue detection using synthetic DNA.

- The new primer mix with betaine **(A)** demonstrates earlier amplification in the 10^{-2} sample (0.0775 nM) compared to the old primer mix **(B)**. The 10^{-4} sample (0.000775 nM) did not show clear amplification and was dominated by background noise. Negative controls exhibited minor increases in fluorescence, likely due to non-specific amplification or experimental variability.

Figure 6: Streptavidin Gold Nanoparticle (AuNP) Synthesis and Conjugation Optimization

- 12 nm AuNPs were synthesized via citrate reduction
- Conjugation was optimized by adjusting pH, BSA, and streptavidin concentration
- The reaction was scaled up 5x for downstream use

Streptavidin AuNPs



Pellet (**before** resuspension)

Conjugates (AuNP + Streptavidin)

Discussion:

- Successful fluorescence was seen in the 0.0775 nM sample with betaine added in under 40 minutes.
- Streptavidin AuNP synthesis was achieved at 10 mg/µL, with proteins remaining reddish before and after 10% NaCl addition, confirming successful conjugation

Future Work

- Evaluate one-pot heating system stability
- Validate LFA performance using salt-mediated immobilization
- Conduct cost analysis of system components
- Expand testing to all dengue serotypes beyond DENV-2