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

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Introduction

- **Dengue fever** is a mosquito-borne viral disease that affects **~400 million** people annually, primarily in tropical regions. With rising temperatures, the viral vector is **expanding** westward.[2]
- Current diagnostic methods (RT-PCR and ELISA) require specialized laboratory equipment, **limiting accessibility** in low-resource settings.[3]
- **Reverse Transcription Loop-mediated Isothermal Amplification (LAMP)** and **Lateral Flow Assays (LFA)** can be integrated to create a low-cost platform for **rapid dengue detection**.

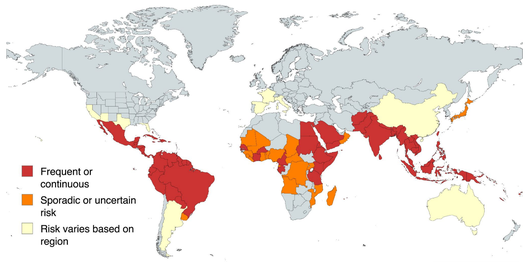

Figure 1. Global dengue prevalence map [4]

Study Design

Objective: Develop a **low-cost, reliable point-of-care diagnostic platform** that combines **one-pot RT-LAMP** with **multiplex LFA** to **detect and differentiate the four dengue virus serotypes**.

Motivation: An affordable, **rapid** serotype-specific **diagnostic assay** is needed to improve dengue detection in settings with **limited** laboratory access.

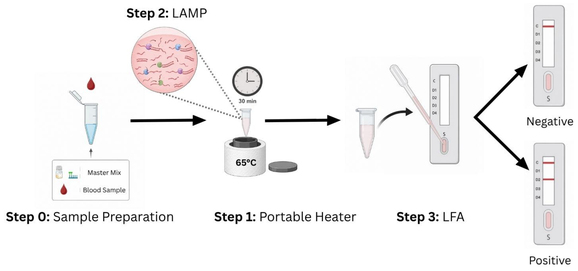

Figure 2. Assay Platform Design. **Step 0:** Add blood sample. **Step 1:** Amplify RNA using RT-LAMP in a portable heater (60-65 °C, 30-40 min). **Step 2:** Apply product to multiplex LFA. **Step 3:** Read positive (test + control) or negative (control only).

Portable Heater

- Custom **ABS** (acrylonitrile butadiene styrene) **inserts** housed in a **Beast 10 oz stainless steel tumbler** positions a PCR tube above a water-activated **Mg Fe alloy** (Meals Ready-to-Eat heater pack).[5,6]
- Two phase change materials (PCMs) were tested: **carbon black-encapsulated palmitic acid**[5] and **PureTemp 63X** (PureTemp LLC).[6]
- **0.2 M/2 M NaCl and 1:1 PCM to MRE ratio** were used.

$$Mg + 2H_2O \xrightarrow{Fe,\ NaCl} Mg(OH)_2 + H_2 + Heat$$

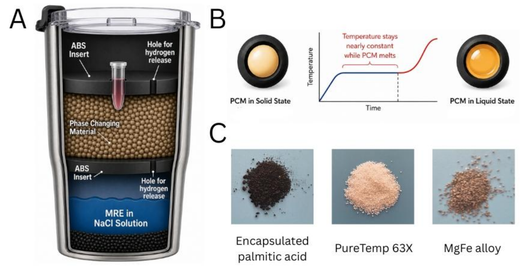

Figure 3. Experimental heating system. **(A)** Custom insert design. **(B)** PCM temperature-buffering behavior. **(C)** Representative photographs of the materials used.

Portable Heater Results

- Optimized the heater formulation by varying the masses of the **MgFe alloy and Fatty Acid,** the **NaCl** molarity**,** the **water volume**, and **insulation.**
- Higher salt concentrations (2 M NaCl) **accelerated** the MgFe corrosion reaction.
- **1:1 ratio** of MRE to Carbon Black Fatty Acid (2g each) in 2M NaCL produced the best heat output and maintained a **constant temperature** for longest

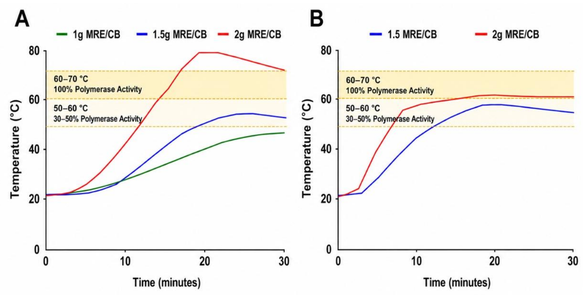

Figure 4. Temperature versus time for varying amounts of MgFe alloy and carbon black fatty acid (CB) in 0.2 M NaCl **(A).** and 2 M NaCl **(B)**. Yellow regions indicate polymerase activity range.[11]

LAMP

- **RT-LAMP** converts dengue viral **RNA into cDNA,** then **amplification** occurs at a **constant temperature (65°C)** using **4-6 primers** and **Bst 2.0 polymerase.** Amplification is typically detected through a **colorimetric or fluorescent signal.**[7]

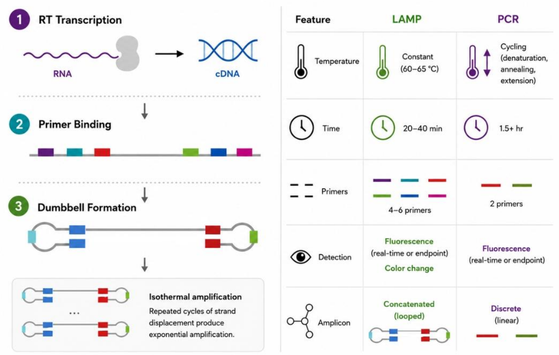

Figure 5. Left: RT-LAMP amplification workflow from reverse transcription to isothermal DNA amplification. **Right:** Comparison of RT-LAMP and PCR workflows.

LAMP Results

- Amplification of a synthetic random target sequence (gBlock DNA) observed through a **color change from pink to yellow** in positive reactions, and **visualized on a gel**.
- Higher template concentrations amplified **earlier (20-30 min)**, while lower concentrations required **up to 40 min** for a complete color change.
- No amplification observed in **negative control**.

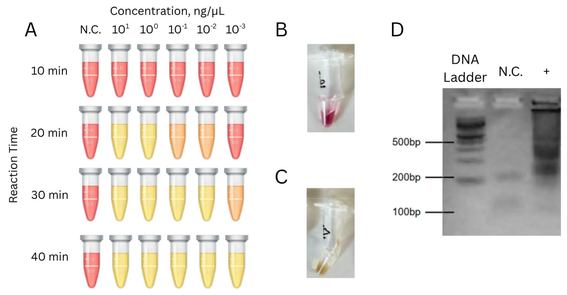

Figure 6. **(A)** Concentration-dependent colorimetric LAMP assay. **(B)** Positive reaction. **(C)** Negative control. **(D)** Gel of biotinylated LAMP products after 20 minutes. Positive sample (+) = 10ng/µL random target.

Lateral Flow Assay (LFA)

- **Capture probes** complementary to serotype-specific biotinylated RT-LAMP products generate visible test lines using **streptavidin-coated** 40 nm BioReady **Gold Nanospheres** (NanoComposix).[8]
- $CaCl_2$-mediated **salt immobilization** was evaluated for **capture probe** immobilization on nitrocellulose.[9]
- The antibody-free, paper-based platform enables **rapid**, **low-cost**, **point-of-care** dengue detection with a built-in **control line**.[10]

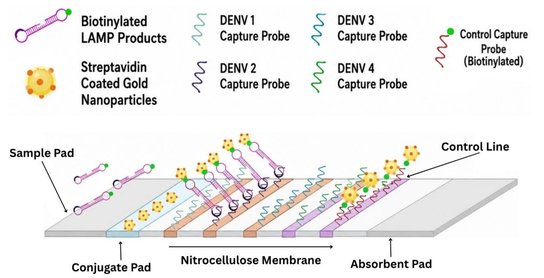

Figure 7. Multiplex Lateral Flow Assay Setup

LFA Results

- Identified optimal **strip design**, immobilization protocol, drying conditions, and LFA buffer to maximize assy performance.
- **LFA buffer** includes 160 mM Tris-HCl (pH 8), 0.6M Sodium Acetate, 0.6% Tween 20, and Streptavidin-coated Gold Nanoparticles (AuNP).
- Demonstrated reproducible control line detection, confirming **successful probe immobilization** and **gold nanoparticle labeling.**
- Further optimization required for successful integration of LAMP products into LFA platform.

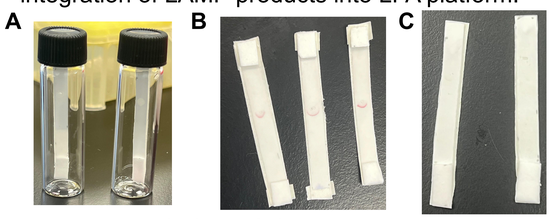

Figure 8. LFA Platform. **(A)** LFA reaction setup. Control Capture DNA (80µM) immobilized using 0.8M $CaCl_2$. **(B)** LFA results tested using varying $CaCl_2$ concentrations (left to right: 0.5M, 0.8M, 1.5M). **(C)** Unsuccessful LFA LAMP reaction (no red spot)

Conclusion

- The addition of **fatty acids** improved thermal stability of the **portable heater**, providing more consistent RT-LAMP reaction temperatures for reliable amplification.
- The colorimetric **RT-LAMP** assay **successfully detected** the random target sequence at concentrations as low as 10^{-3} ng/µL after 40 minutes.
- The **lateral flow assay** successfully immobilized the **control capture probe** and demonstrated consistent test dot formation, confirming successful conjugation of streptavidin-coated gold nanoparticles to the biotinylated capture probe.

Future Work

- **Optimize** the portable heater to maintain **60-70°C for 30-40** minutes and finalize the design of 3D-printed parts.
- Validate successful **detection** of RT-LAMP amplification products using the multiplex **LFA platform**.
- Integrate the optimized portable heater with the RT-LAMP/LFA workflow to show a **fully instrument-free** diagnostic system.
- Evaluate **cost** and **shelf life** of the final product.

Acknowledgements & References

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Scan for References