# Optimisation of LAMP Assay for HIV Diagnosis in Resource-limited Settings

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## Introduction

- **HIV** is a +ssRNA retrovirus that causes immunosuppression by targeting CD4+ T cells, leading to AIDS. It is managed with antiretroviral therapy (ART).[1]
- **HIV is diagnosed** via antibody, antigen/antibody, and nucleic acid tests.[2]
- **Nucleic Acid Tests (NATs)** offer early infant detection because infants born to HIV-positive mothers may possess maternal antibodies. NATs directly detect the virus's genetic material (RNA or DNA), unlike antibody tests, which rely on the body producing antibodies, a process that can take weeks or months.
- **LAMP (Loop-mediated isothermal amplification)** amplifies RNA and DNA via UV, turbidity and colour change. This assay uses a colorimetric version changing from pink to yellow upon successful HIV detection.

## Objectives

- Optimize a cost-effective, accessible colorimetric LAMP for sensitive and specific HIV detection in resource-limited, point-of-care settings, omitting nucleic acid extraction by using aspirated plasma.
- Colorimetric LAMP was used because of it's rapid and nncomplicated nature.

## LAMP Procedure

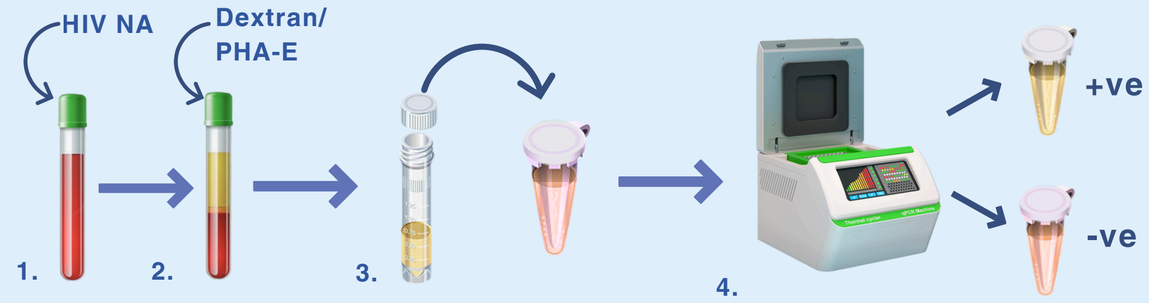
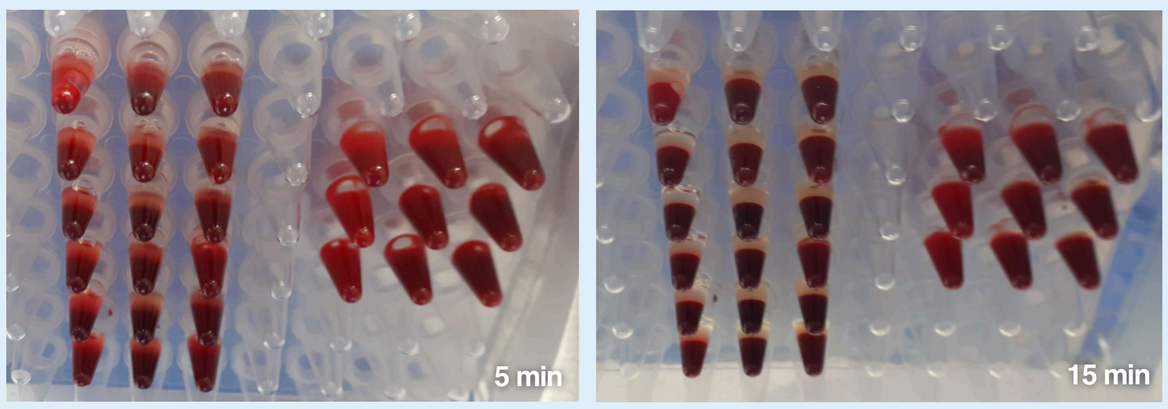


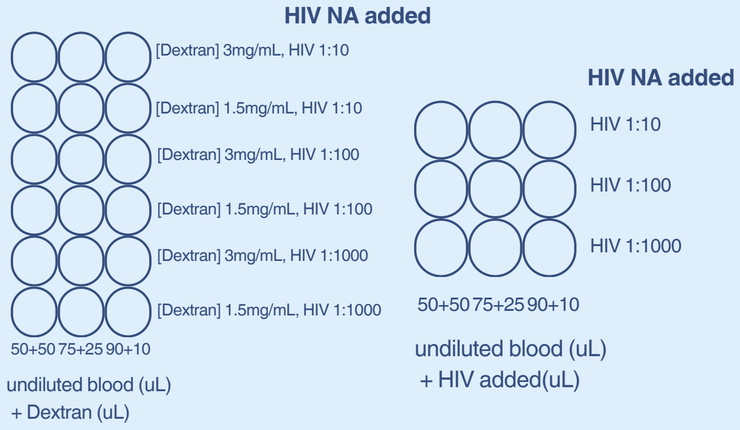

**Figure 1. Schematic Representation of LAMP with aspirated plasma**

1. Blood was collected in K2EDTA tubes and spiked with HIV RNA & DNA.
2. Erythrocytes were separated using either Dextran sedimentation or PHA-E aggregation.
3. 1 µL of aspirated plasma was added to WarmStart Colorimetric LAMP 2X Master Mix and ACe-IN primer mix optimized to detect different HIV-1 subtypes.[3] Additives $MgCl_2$, $MgSO_4$, DMSO and BSA were added to reverse inhibition of LAMP by plasma.
4. The reaction mix was incubated at 63°C for 20-60 minutes.

## Results

### Sedimentation of Erthycyotes via Dextran (HIV +ve)



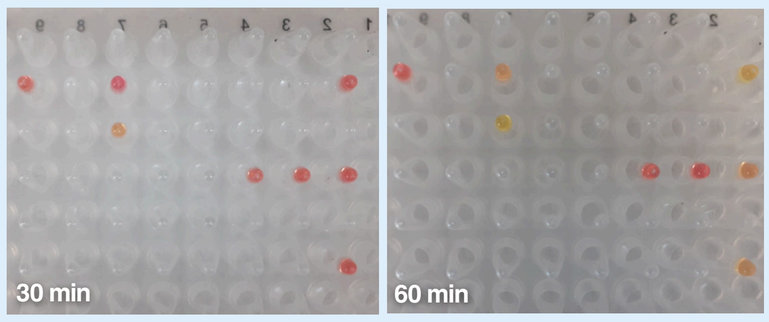




- similar sedimentation using Dextran 3mg/L & 1.5mg/mL
- supernatant can be taken off at 15 min
- 90+10 is the best combination: prevent blood dilution

### Reversing Plasma Inhibition in LAMP

- aspirated plasma from whole blood treated with dextran was used



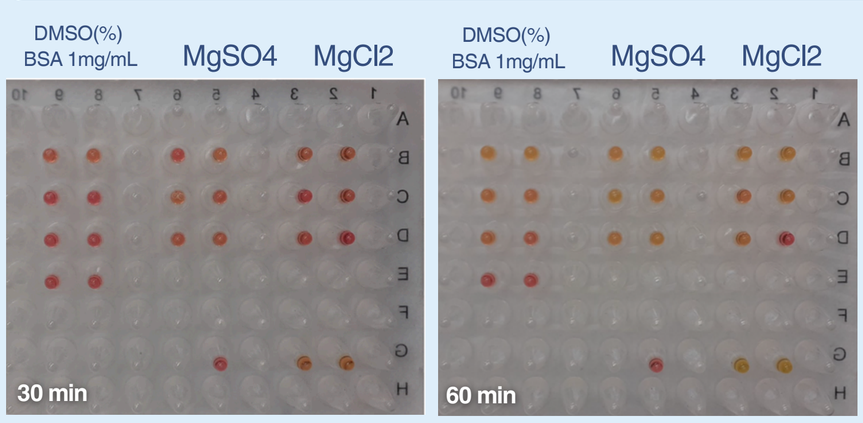

B2: MgCl2 2.5nM

D2: DMSO 1%, BSA 1mg/ML
D3: DMSO 3%, BSA 1mg/ML
D4: DMSO 5%, BSA 1mg/ML

F2: MgCl2 2.5nM, DMSO 3%, BSA 1mg/ML

B7: plasma + HIV 1:100 + PBS
B8: HIV 1:100 in H2O
B9: H2O

- MgCl2 reverses plasma inhibition LAMP to the largest extent




Row B
- MgCl2, MgSO4: 2.5nM/ DMSO: 2%

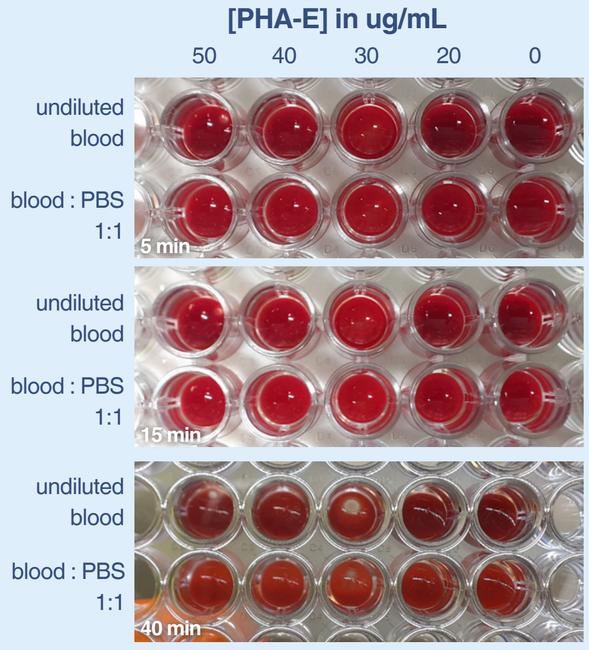
Row C
- MgCl2, MgSO4: 3.5nM/ DMSO: 5%

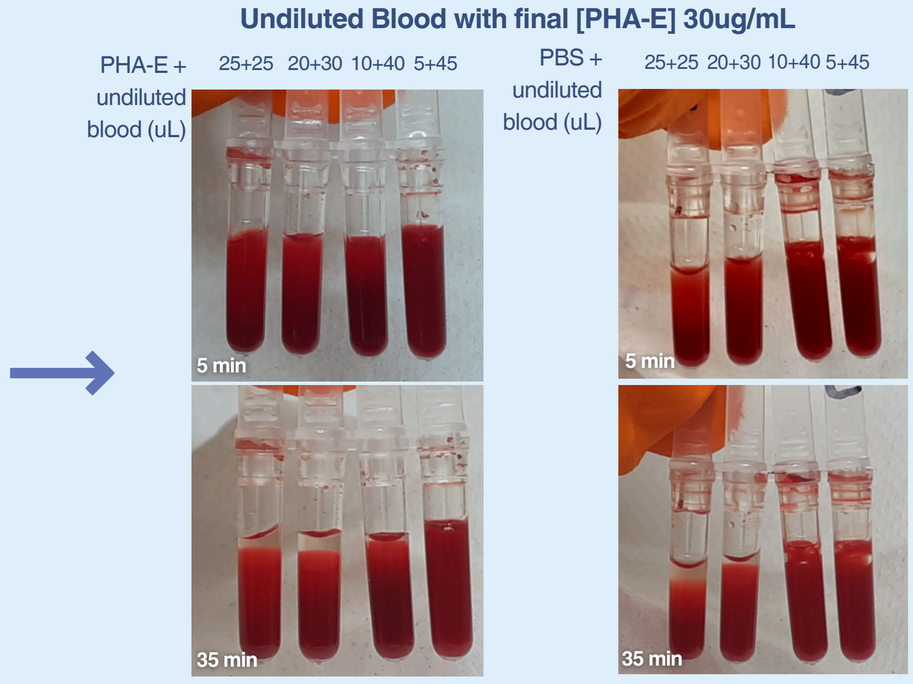
Row D
- MgCl2, MgSO4: 4.5nM/ DMSO: 7%

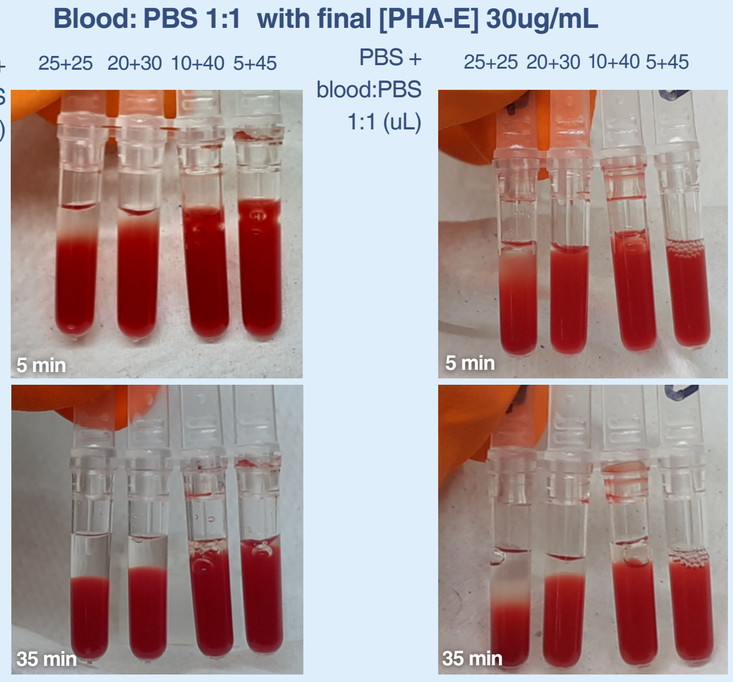
Row E
- DMSO: 10%

- Best reagents to reverse plasma inhibition: MgSO4 2.5nM/ 2% DMSO + 1mg/mL BSA

### Aggregation of Erthycyotes via PHA-E (HIV-ve)




- best PHA-E concentration: 30ug/mL in undiluted blood

- PHA-E aggregates erthryocytes best at 30ug/mL at a ratio of plasma:PHA-E 3:2 & 1:1 at 40 min
- PHA-E also facilitates plasma aspiration similar to dextran but with longer incubation time

## Future Directions

- test LAMP using plasma spirated from whole blood spiked with HIV ,with added PHA-E, to evaluate the efficacy of PHA-E aggregation
- spike blood cell line 8E5 infected with HIV, then test these cells in qPCR & LAMP

## References

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3. Ocwieja KE, Sherrill-Mix S, Liu C, Song J, Bau H, Bushman FD. A reverse transcription loop-mediated isothermal amplification assay optimized to detect multiple HIV subtypes. PLoS One. 2015;10(2):e0117852.

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