Development of a reverse transcription recombinase polymerase amplification (RT-RPA) assay targeting long terminal repeat (LTR) regions of the HIV-1 genome to enable HIV-1 RNA viral quantification at point-of-care (POC)

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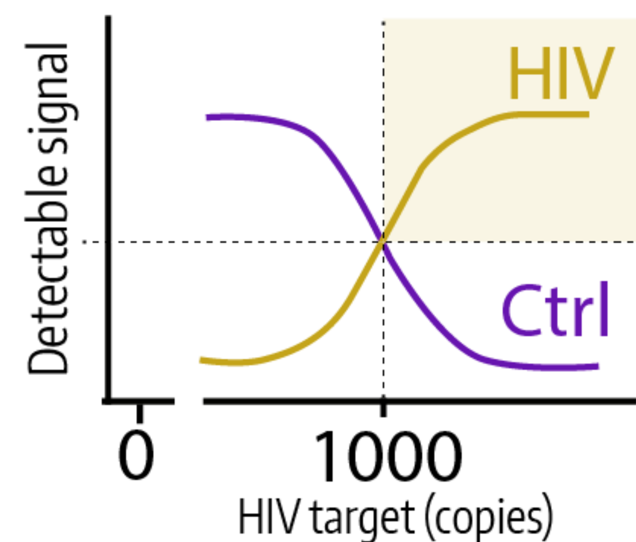
Background

Monitoring HIV RNA levels is essential for assessing viral load (**VL**) suppression and effective treatment in people living with HIV.

Undetectable U = U Untransmissible

Our toolbox:



A **semi-quantitative** assay for HIV RNA using RT-RPA. Plot was simplified from data in **[1]**.

POC **Harmony device**, a mobile operated, all-in-one heater/ reader for COVID testing **[2]**.

This study aims to:

- Investigate new primer/probe design to target long terminal repeat (**LTR**) HIV-1 region, used in clinical VL RT-PCR assay.
- Test performance of new LTR assay in our POC Harmony device.

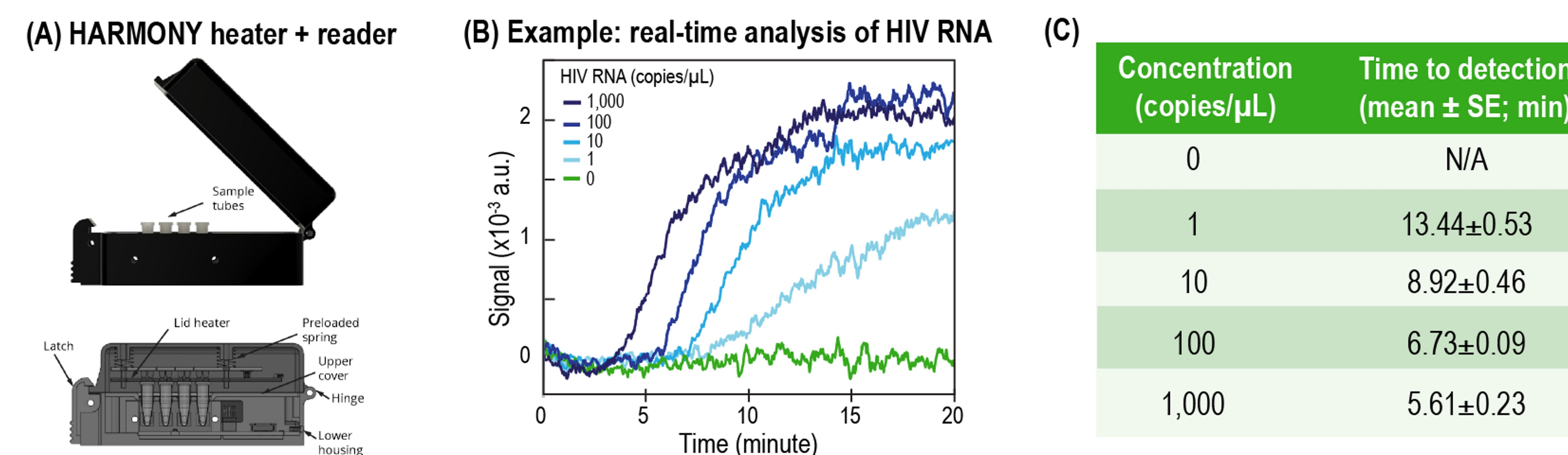
Materials and Methods

- ***In-silico* design of primers and exonuclease probes:** Target LTR regions of HIV-1, as used in a clinically-validated RT-PCR **[3]**. 90% coverage of HIV-1 (M group) sequences from LANL with specifications to meet RPA requirements **[4]**.
- **Experimental set-up**: exo-RT-RPA reactions were prepared according to manufacturer's protocol **[4]**, supplemented with Omniscript RT. We ran exo-RT-RPA in the presence of 0-1000 HIV RNA copies/μL (n=4) using Harmony device (37°C for 30 min).
- **Analysis:** Time to detection was reported as means ± standard errors.
- **Testing sites**: Initial development was performed at UW and six pilot runs were performed at University of Pretoria in South Africa.

POC **Harmony device**, operated **In South Africa (2023)**

Results, Discussion, and Conclusions

Our RT-RPA *LTR* assay for HIV-1 RNA is sensitive and rapid



Concentration (copies/μL)	Time to detection (mean ± SE; min)
0	N/A
1	13.44±0.53
10	8.92±0.46
100	6.73±0.09
1,000	5.61±0.23

Figure 1. Point-of-care assay to detect LTR gene in HIV RNA. (A) Diagram of how the RT-RPA reactions were carried out by our in-house device. RT-RPA reactions in the presence of FAM-based exonuclease probe were analyzed by a low-cost customized heater/reader "Harmony" previously used to analyze RT-LAMP reactions. Here the reactions were incubated at 42 °C for 30 minutes. **(B)** Examples of real-time analysis of HIV RNA at 0, 1, 10, 100, or 1000 copies/μL using our new LTR RT-RPA assay operated by Harmony. **(C)** Time to detection (mean ± SE; n=4) in minutes at which signal across the threshold of 0.005 a.u.

- Newly-developed RT-RPA *LTR* exhibited a high analytical sensitivity, detecting as low as **1 copy/μL of HIV RNA**.
- A key advantage: its rapidity, with a remarkably short time to **detection of < 15 minutes** Fig. 1 B and C vs ~1.5 hours of wait-time in typical RT-PCR assays.
- RT-RPA *LTR* assay relies on a **$250 heater/ reader device**, vs $5k+ machine for RT-qPCR.
- **Next step:** incorporating target mimicking internal control to enable the semi-quantitation of HIV RNA.

Our pilot on-site testing in South Africa was promising

- **A pilot test:** 5 participants at University of Pretoria performed RPA on blinded samples (1 water and 2 LTR DNA template at 10 copies/μL).
- All participants ran the RPA assay on Harmony, **generating 100% accurate results** with similar time to detection to those obtained in the US.
- **Next step:** testing RNA derived from clinical specimens on-site in South Africa.

Acknowledgments

Collaborators:

- **Lutz lab members**: Robert Atkinson, Michael Roller, Daniel Leon, Dr. Amy Oreskovic, Qin Wang, Dr. Ian Hull, and Jack Henry Kotnik for their previous contributions to the development and evaluation of Harmony device for SARS-CoV-2 RNA detection.
- **Drs. Theresa Rossouw and Lisa Frenkel** for clinical insights in HIV.
- We thank **researchers at the University of Pretoria** who participated in setting up the RT-RPA assay and provided feedback. These participants will continue to collaborate with the UW team to prepare a follow-up peer-reviewed manuscript.

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References

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[4] https://www.twistdx.co.uk/product/twistamp-exo/

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