ASSAY FOR THE 45 MINUTE DETECTION OF HIV-1 RNA VIA MODIFIED RT-LAMP REACTION COUPLED WITH A GOLD NANOPARTICLE – PEPTIDE NUCLEIC ACID PROBE COLORIMETRIC REPORTER SYSTEM

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Abstract

The World Health Organization (WHO) developed recommendations to control the spread of HIV-1 to include increasing the percentage of HIV positive individuals who know their HIV-1 status from 70% to 90%. Key to this goal is development of a nucleic acid based assay capable of rapidly detecting HIV-1 in non-laboratory settings. This assay detects HIV-1 RNA utilizing a modified Reverse Transcriptase-Loop Mediated Isothermal Amplification (RT-LAMP) assay followed by a novel, colorimetric Gold Nanoparticle / Peptide Nucleic Acid (AuNP/PNA) probe reporter system. This study demonstrates the proof of principle for a rapid, sequence specific, colorimetric RT-LAMP assay for the detection of HIV-1.

Proposed Assay Overview

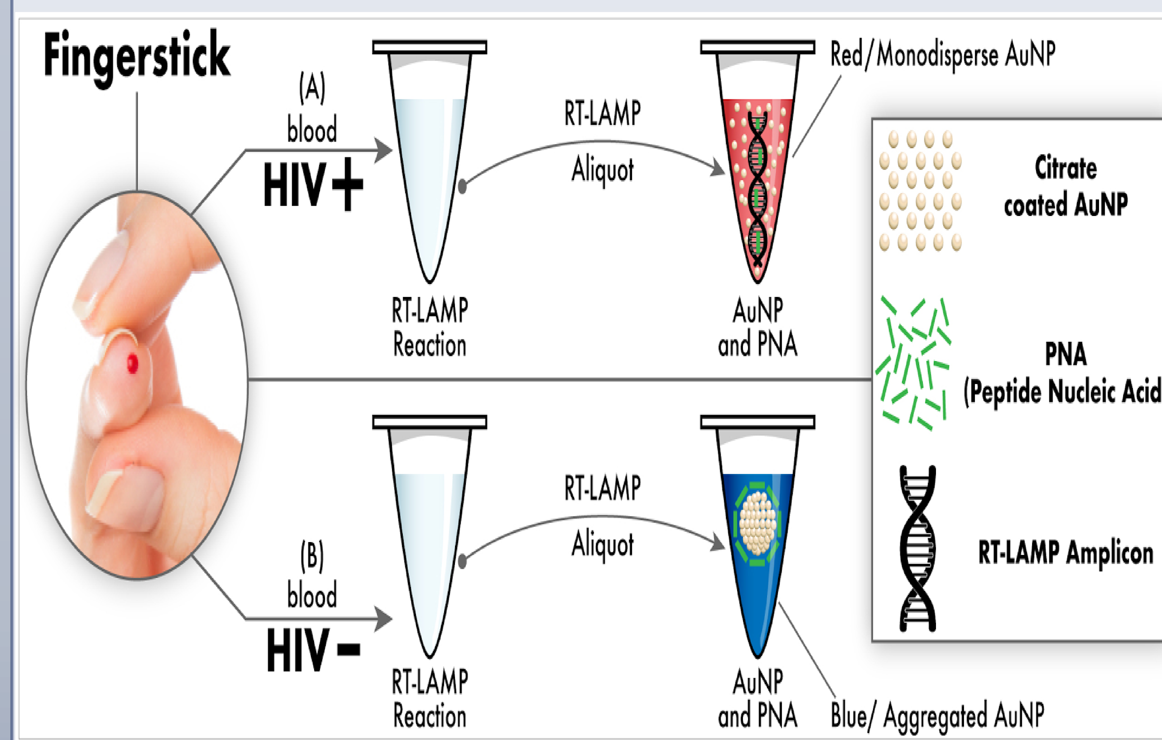

Figure 1: Proposed rapid, molecular HIV-1 assay. Finger stick blood from an individual is placed into the RT-LAMP reaction tube. After the RT-LAMP reaction, an aliquot is removed from the RT-LAMP tube and added to a second tube containing AuNPs and PNA. A) HIV Positive: Upon addition of the aliquot, the PNA will hybridize with the RT-LAMP generated HIV dependent amplicon allowing the AuNPs to remain monodispersed resulting in a red solution. B) HIV Negative: In the absence of the HIV dependent sequence, the PNA is free to induce AuNP aggregation resulting in a blue solution.

Materials and Methods

Colorimetric RT-LAMP Reaction

Colorimetric RT-LAMP methods utilized the WarmStart Colorimetric LAMP 2X Master Mix (New England BioLabs).

Turbidimetric RT-LAMP Reaction

Real-time turbidimetry of the RT-LAMP reaction was performed using the LoopAmp RealTime Turbidimeter LA-500 (Eiken Chemical Co.)

Primer Concentrations

Concentrations of 1.6 µM FIP, 1.6 µM BIP, 0.2 µM F3, 0.2 µM B3, 0.4uM Loop F and 0.4uM Loop R primers for LAMP reactions were performed in a 25 µL reaction at 65°C for 60 minutes.

Table 1: Oligos

Target	Primer	Sequence (5' to 3')	Tm (◦C)
HIV-1 p24 (Curtis, 2008)	FIP (Poly T Linker)	CAGCTTCCTCATTGATGGTTTCttttTAACACCATGCTAAACACAGT	64.9
	BIP (Poly T Linker)	TGTTGCACCAGGCCAGATAAttttGTACTGGTAGTTCCTGCTATG	66.3
	FIP (PNA Linker)	CAGCTTCCTCATTGATGGTTTCgtcgtcatgcttatggTAACACCATGCTAAACACAGT	68.7
	BIP (PNA Linker)	TGTTGCACCAGGCCAGATAAgtcgtcatgcttatggGTACTGGTAGTTCCTGCTATG	69.8
	F3	ATTATCAGAAGGAGCCACC	51.5
	B3	CATCCTATTTGTTCCTGAAGG	50.8
	Loop F	TTTAACATTTGCATGGGCTGCTTGAT	56.3
	Loop R	GAGATCCAAGGGGAAGTGA	53.6
HIV-1 Protease (Curtis, 2008)	FIP (Poly T Linker)	GGTTTCCATCTTCCTGGCAAATTttttCTCTATTAG- ATACAGGAGCAGA	64.7
	BIP (Poly T Linker)	TGATAGGGGGAATTGGAGGTTTttttCCTATAGCTTTATGTCCACAGA	65.3
	FIP (PNA Linker)	GTTTCCATCTTCCTGGCAAATTgtcgtcatgcttatggCTCTAT TAGATACAGGAGCAGA	68.1
	BIP (PNA Linker)	TGATAGGGGGAATTGGAGGTgtcgtcatgcttatggTTCCTATAG CTTTATGTCCACAGA	68.9
	F3	AAAGATAGGGGGGCAACT	53.3
	B3	GTTGACAGGTGTAGGTCCTA	53.3
	Loop F	TATTTCTTCTAATACTGTATC	43.7
	Loop R	TATCAAAGTAAGACAGTA	41
HIV-1 Integrase	FIP (Poly T Linker)	ATTACTACTGCCCCTTCACCTTttttAGAAATCCACTTTGGAAAGGAA	66.1
	BIP (Poly T Linker)	AGTGACATAAAAGTAGTGCCAAGAAttttTCATCACCTGCCATCTGT	66.3
	FIP (PNA Linker)	ATTACTACTGCCCCTTCACCTTgtcgtcatgcttatggAGAAATCCACTTTGGAAAGGA A	71.6
	BIP (PNA Linker)	AGTGACATAAAAGTAGTGCCAAGAAgtcgtcatgcttatggTCATCACCTGCCATCTG T	71.1
	F3	GGACAGCGGGTTTATTACA	57.4
	B3	TGTCTACTTGCCACACAA	55.2

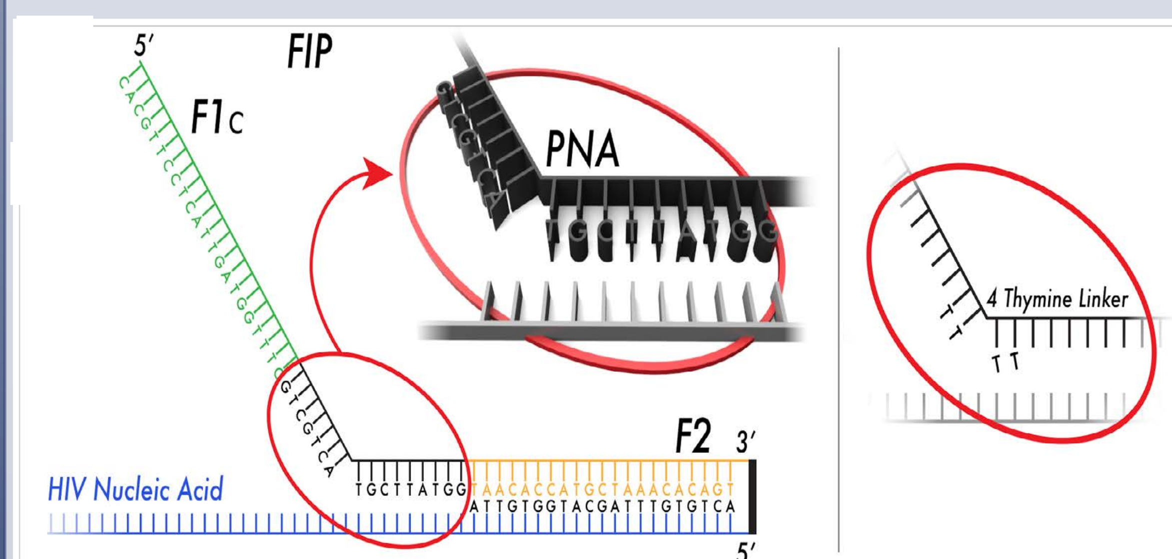

Figure 2: Modification to FIP/BIP Primers

Materials and Method (continued)

AuNP/PNA Probe Detection

Colorimetric detection of RT-LAMP amplicon was achieved with a two-step hybridization and detection procedure.

Hybridization: A 10 µL pre-incubation mixture containing 1.5 µM of PNA (17-mer), Phosphate Buffer Saline (2 mM phosphate, 27.4 mM NaCl, and 0.54 mM KCl, at pH 7.4) and 1 µL of RT-LAMP DNA amplicon was heated at 95°C for 5 minutes.

Detection: A 10 µL aliquot of 30 nm AuNP was added for a final volume of 20 µL. The colorimetric results were visualized after 1 minute.

Results

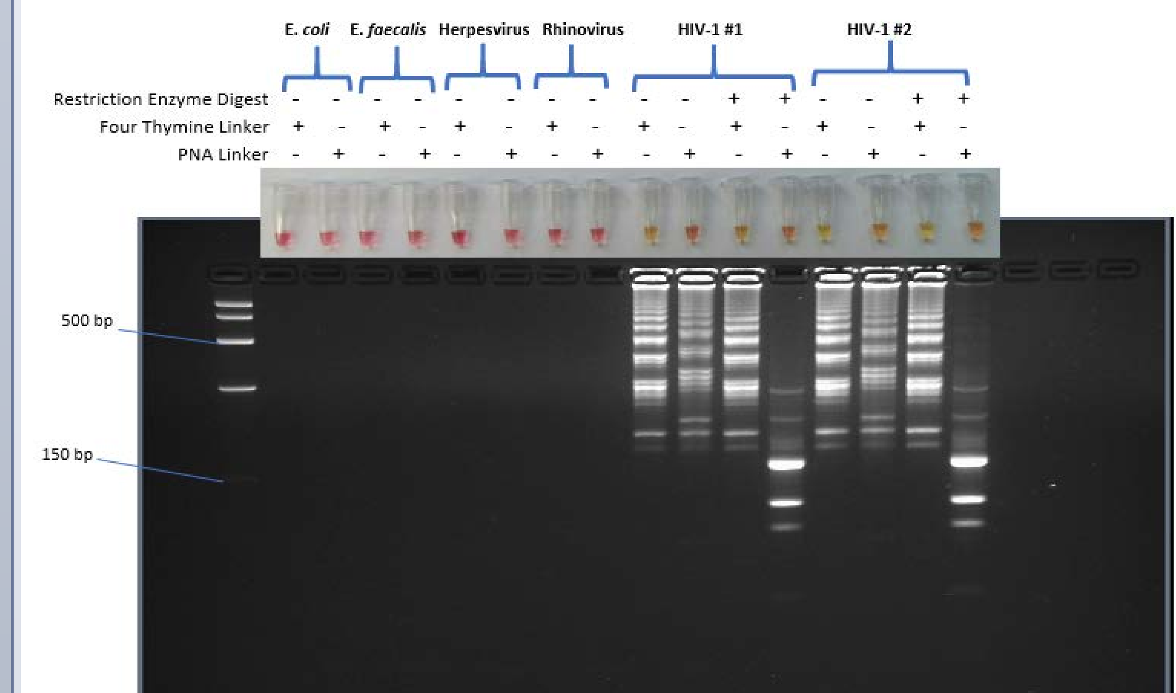

Figure 3: Evidence for generation of the PNA linker sequence using HIV-1 protease gene target. Using the restriction enzyme, MsI1, specific for a sequence within the generated target that is complementary to the PNA. Only RT-LAMP products containing the sequence were cut eliminating the production of the laddering migrations seen in LAMP reactions.

Table 2: Time to positive for detection of HIV-1 Integrase by modified RT-LAMP using Eiken LA-5000 Real-Time turbidimeter.

HIV Target	Time to Positive	Average T_t
HIV-1 Integrase	33:12	33:00 +/- 0.21
HIV-1 Integrase	32:48	
HIV-1 Integrase	33:06	

*Samples used for AuNP/PNA Probe Detection, see figure 4.

Results (continued)

AuNP/PNA Probe Detection of HIV-1 Dependent Sequence

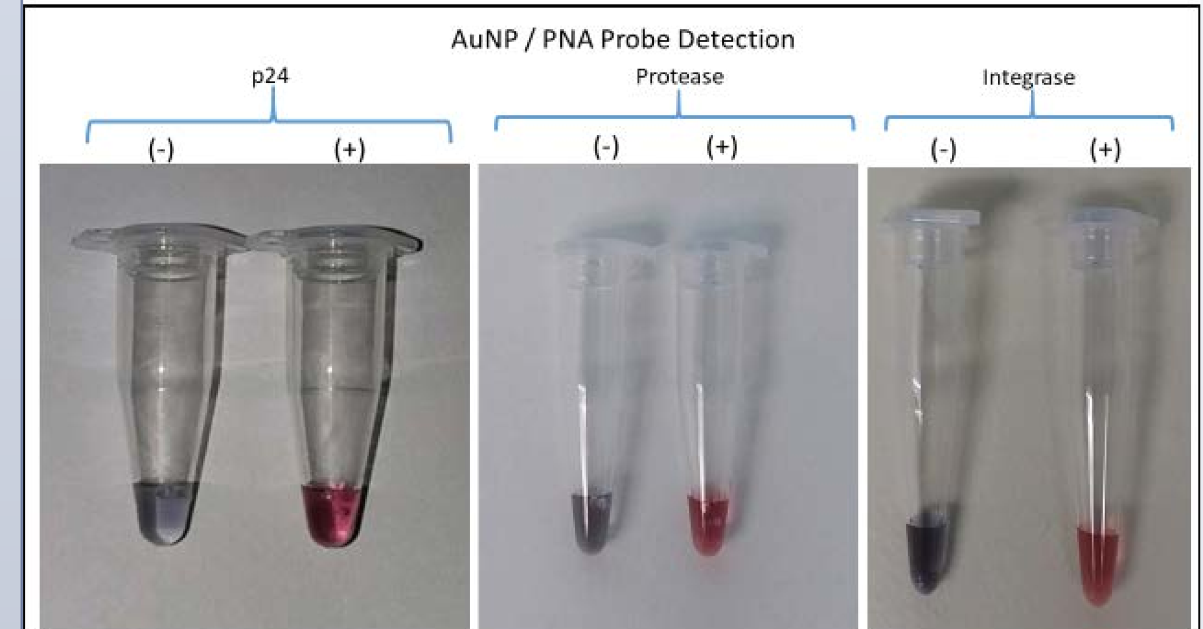

Figure 4: Colorimetric AuNP/PNA probe detection differentiates between the polyT (-) linker and PNA (+) linker sequences. All six of the RT-LAMP reactions were positive for HIV by turbidimetry. Above are representative for HIV-1 detection. RT-LAMP with AuNP/PNA probe colorimetric detection results have been seen in as soon as 40 minutes.

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Acknowledgements

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