Supplementary Information

A Rapid Variant-Tolerant Reverse Transcription Loop-Mediated Isothermal Amplification Assay for Point of Care Detection of HIV-1

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Table S1. Primers and probes used in the study.

Aim	Primers	Sequence (5'-3')	Ref.
Mutant construction	F3-Mu-A(F)	CACTTTGGAAAGGACCAGAAAAGCTCCTCT	This study
	F3-Mu-A(R)	TTGGTCCTTTCCAAAGTGGATTTCTGCTGT	
	F3-Mu-G(F)	CACTTTGGAAAGGACCAGGAAAGCTCCTCT	
	F3-Mu-G(R)	CTGGTCCTTTCCAAAGTGGATTTCTGCTGT	
	F1-Mu-A(F)	GGGGCAGTAGTAATACAAAATAATAGTGAC	
	F1-Mu-A(R)	TTTGTATTACTACTGCCCCTTCACCTTTCC	
	F1-Mu-C(F)	GGGGCAGTAGTAATACAACATAATAGTGAC	
	F1-Mu-C(R)	CTTGTATTACTACTGCCCCTTCACCTTTCC	
	LB-Mu-A(F)	ATGATTGTGTGGCAAGTAAACAGGATGAGG	
	LB-Mu-A(R)	TTACTTGCCACACAATCATCACCTGCCATC	
	LB-Mu-C(F)	ATGATTGTGTGGCAAGTACACAGGATGAGG	
	LB-Mu-C(R)	GTACTTGCCACACAATCATCACCTGCCATC	
	FIP-Mu-A(F)	AAGCTCCTCTGGAAAGGTAAAGGGGCAGTA	
	FIP-Mu-A(R)	CCTTTCCAGAGGAGCTTTGCTGGTCCTTTT	
	FIP-Mu-C(F)	AAGCTCCTCTGGAAAGGTCAAGGGGCAGTA	
	FIP-Mu-C(R)	CCTTTCCAGAGGAGCTTTGCTGGTCCTTTG	
	FIP-Mu-2G(F)	AAAGCTCCTCTGGAAAGGGGAAGGGGCAGT	
	FIP-Mu-2G(R)	CCCTTTCCAGAGGAGCTTTGCTGGTCCTTT	
	FIP-Mu-2C(F)	AAAGCTCCTCTGGAAAGGCGAAGGGGCAGT	
	FIP-Mu-2C(R)	GCCTTTCCAGAGGAGCTTTGCTGGTCCTTT	
	FIP-Mu-3A(F)	CAAAGCTCCTCTGGAAAGATGAAGGGGCAG	
	FIP-Mu-3A(R)	TCTTTCCAGAGGAGCTTTGCTGGTCCTTTC	
	FIP-Mu-3C(F)	CAAAGCTCCTCTGGAAAGCTGAAGGGGCAG	
	FIP-Mu-3C(R)	GCTTTCCAGAGGAGCTTTGCTGGTCCTTTC	
	FIP-Mu-5T(F)	AGCAAAGCTCCTCTGGAATGGTGAAGGGGC	
	FIP-Mu-5T(R)	ATTCCAGAGGAGCTTTGCTGGTCCTTTCCA	
	FIP-Mu-5G(F)	AGCAAAGCTCCTCTGGAAGGGTGAAGGGGC	
	FIP-Mu-5G(R)	CTTCCAGAGGAGCTTTGCTGGTCCTTTCCA	
RT-LAMP assay of HIV-1	AceIN-F3	CCMMTTTGGAAAGGACCAGC	(PLoS One. 2015;10(2):e0117852)
	AceIN-B3b	AACATACATATGRTGYTTTACTA	
	AceIN-B3a	TCTTTGAAAYATACATATGRTG	
	AceIN-FIPf	CTTGGCACTACYTTTATGTCACTAAARCTYCTCTGGAAAGGTG	
	AceIN-FIPe	CTTGGTACTACYTTTATGTCACTAAARCTACTCTGGAAAGGTG	
	AceIN-BIP	GGAYTATGGAAAACAGATGGCAGCCATGTTCTAATCYTCATCCTG	
	AceIN-LF	TCTTGTATTACTACTGCCCCTT	
	AceIN-LB	GTGMTGATTGTGTGGCARGTAG	

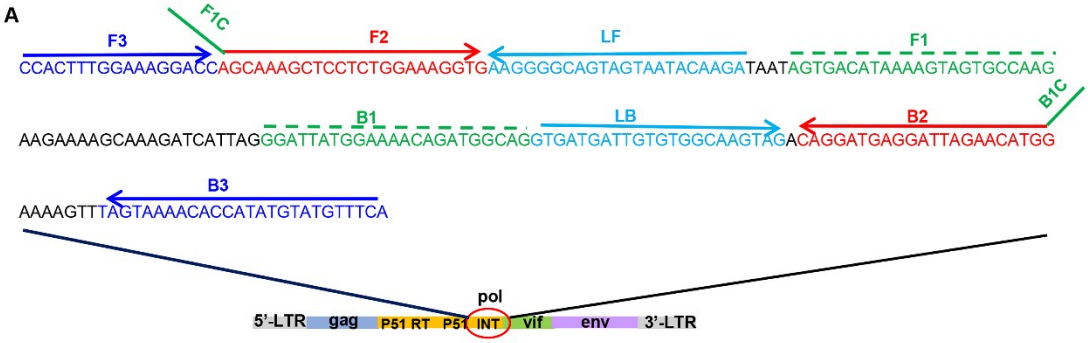
	AceIN-F3	CCMMTTTGGAAAGGACCAGC	
RT-qPCR assay of HIV-1	HIV-1 F	CAATTTTAAAAGAAAAGGGGGGATTG	This study
	HIV-1 R	TAAACCCGAAAATTTTGAATT	
	HIV-1 P	FAM-ACAGTGCAGGGGAAAG-MGB	

The artificially introduced mutations are highlighted in red.

Table S2. Detection rates of different HIV-1 serotypes by the conventional and the novel mismatch-tolerant RT-LAMP assays.

HIV-1 subtype	The conventional RT-LAMP			The mismatch-tolerant RT-LAMP			total
	Positive	Negative	Detection rate (%)	Positive	Negative	Detection rate (%)	
B	3	1	75	3	1	75	4
C	1	1	50	1	1	50	2
01_AE	2	4	33.3	4	2	66.7	6
07_BC	0	1	0	0	1	0	1
08_BC	2	1	66.7	3	0	100	3
57_BC	1	1	50	2	0	100	2
87_cpx	0	2	0	0	2	0	2
96_cpx	0	1	0	1	0	100	1
62_BC	0	1	0	0	1	0	1
65_cpx	1	0	100	1	0	100	1
URF	7	14	33.3	14	7	66.7	21
Total	17	27	38.6	29	15	65.9	44

Note: The detection rate was calculated using the formula: (number of positive results/total number) ×100%.


A
F1C
F3
F2
LF
F1
CCACTTTGGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAG
B1
LB
B2
B1C
AAGAAAAGCAAAGATCATTAGGGATTATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAGAACATGG
B3
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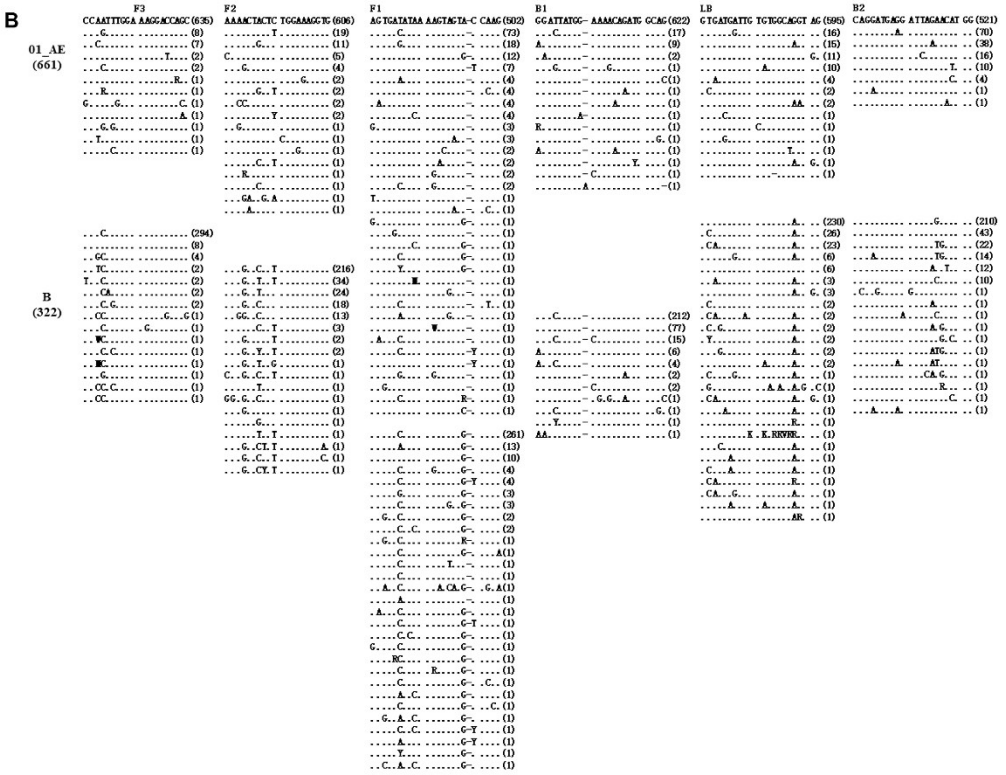
pol
5'-LTR
gag
P51 RT
P51
INT
vif
env
3'-LTR

B


F3
F2
F1
B1
LB
B2
01_AE (661)
B (322)

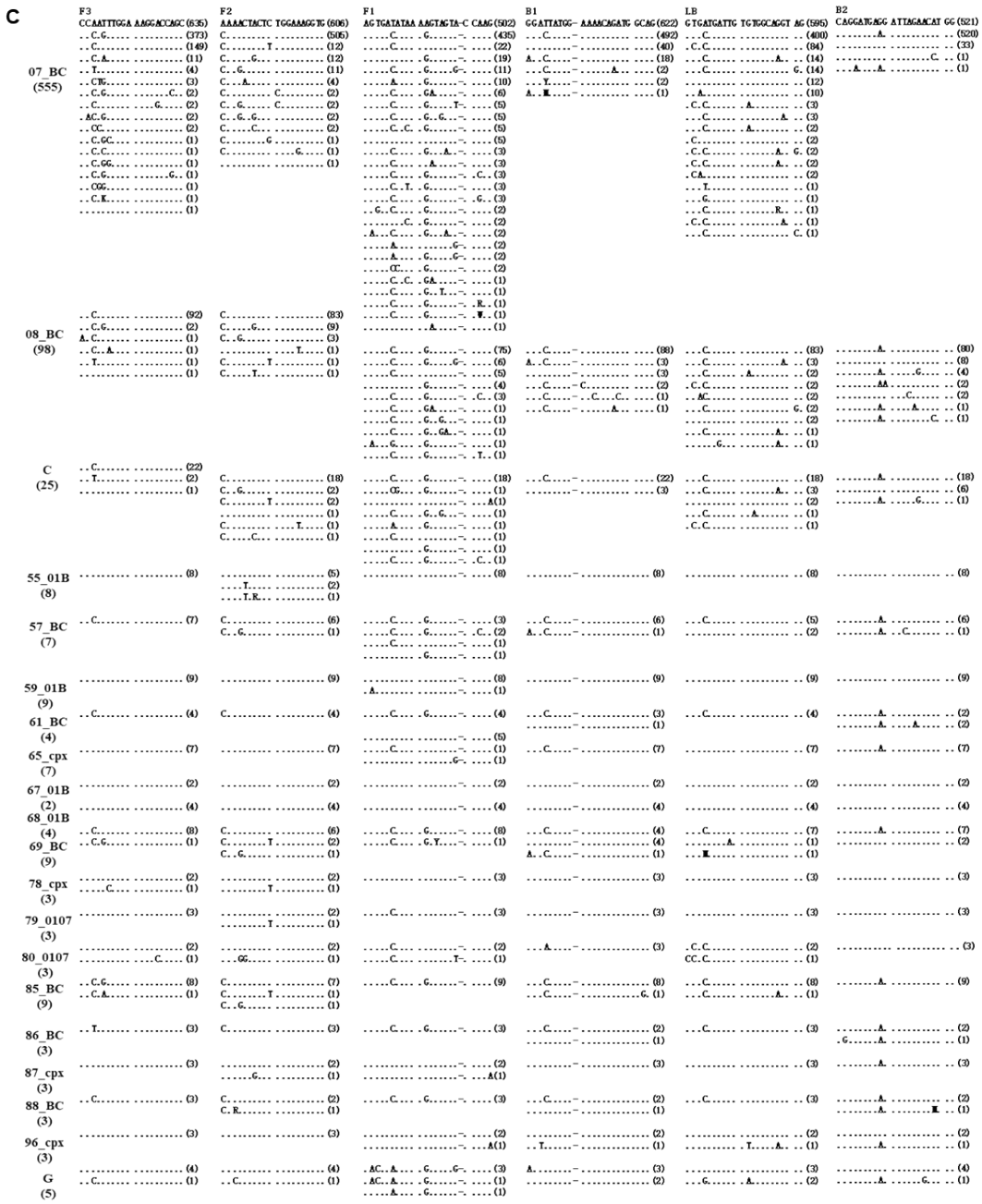


Figure S1. Locations (A) and sequence alignments (B and C) of all available HIV-1 strains. A total of 661 01_AE, 322 B, 555 07_BC, 98 08_BC, 25 C, 8 55_01B, 7 57_BC, 9 59_01B, 4 61_BC, 7 65_cpx, 2 67_01B, 4 68_01B, 9 69_BC, 3 78_cpx, 3 79_0107, 3 80_0107, 9 85_BC, 3 86_BC, 3 87_cpx, 3 88_BC, 3 96_cpx, and 5 G HIV-1 sequences were downloaded from HIV Database (https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html) on April 10, 2019. The number of identical sequences is shown in parenthesis following each unique sequence. Dot, identity with the topmost sequence; Dash, deletion.

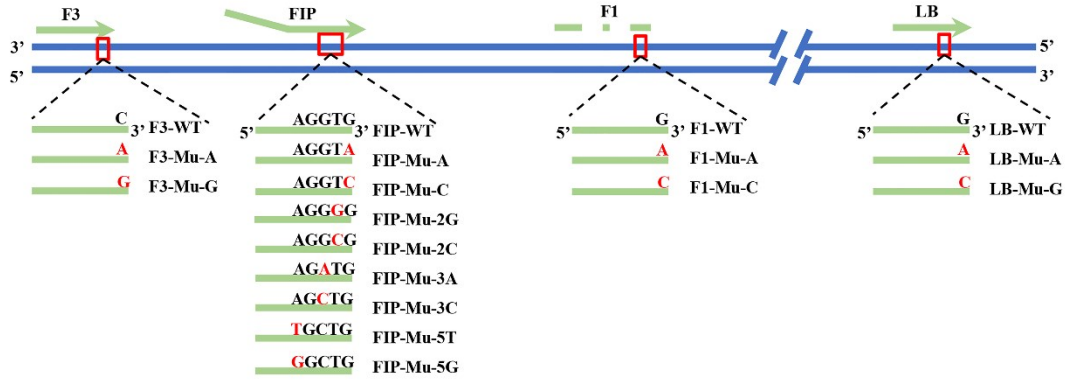


Figure S2. Schematic diagram of different HIV mutation templates. WT, wild-type; Mu, mutant.

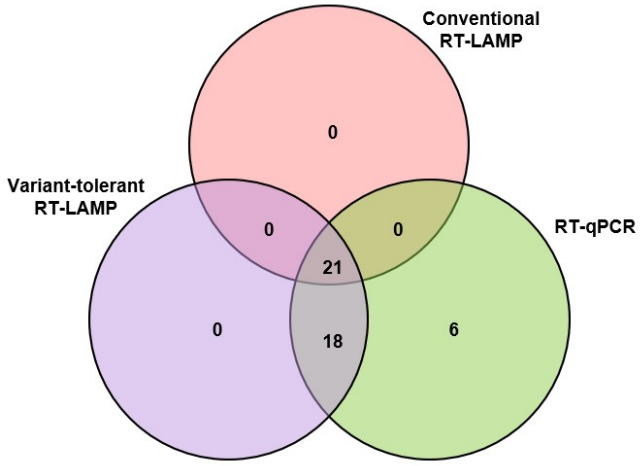


Figure S3. Numbers of HIV-1 positive samples detected by three different assays.

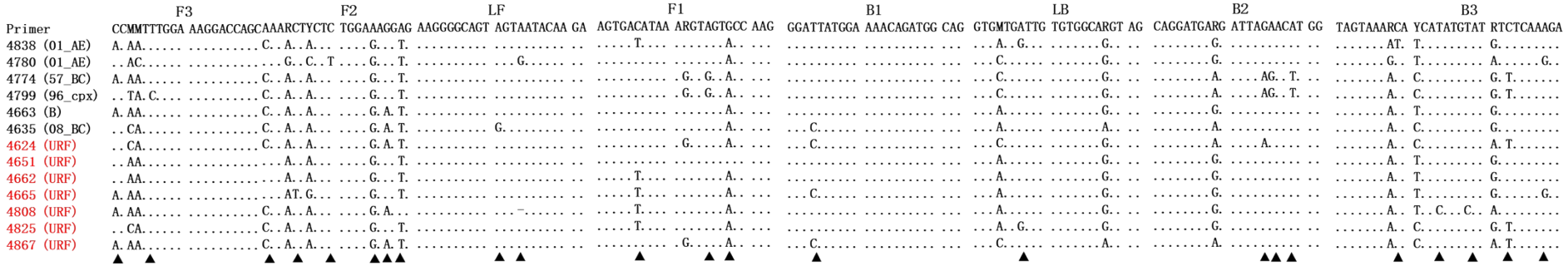


Figure S4. Alignment analysis of primer sequences for clinical samples. A total of 13 samples were analyzed, including seven cases of URF (marked in red), two cases of 01_AE, one case each for 57_BC, 96_cpx, B, and 08_BC, and the black triangle indicates the mutation site. Dot, identity with the topmost sequence; Dash, deletion.

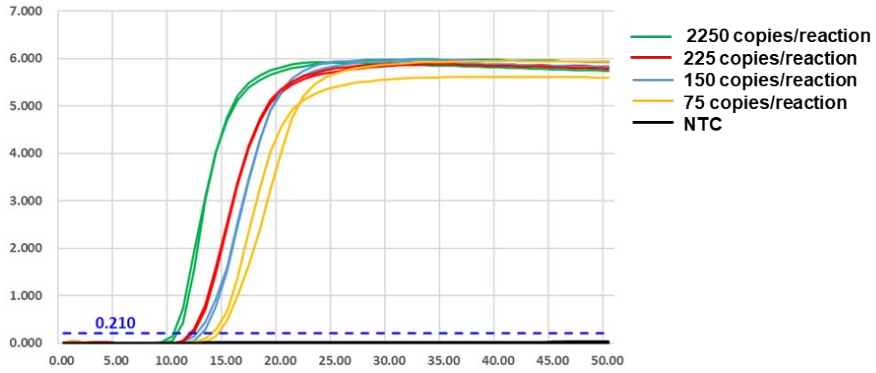


Figure S5. Mismatch-tolerant RT-LAMP detection by rapidly magnetic bead-based RNA extraction. The threshold is determined as 3 standard deviation from the background (0.210, dotted blue line).

NTC: no template control.

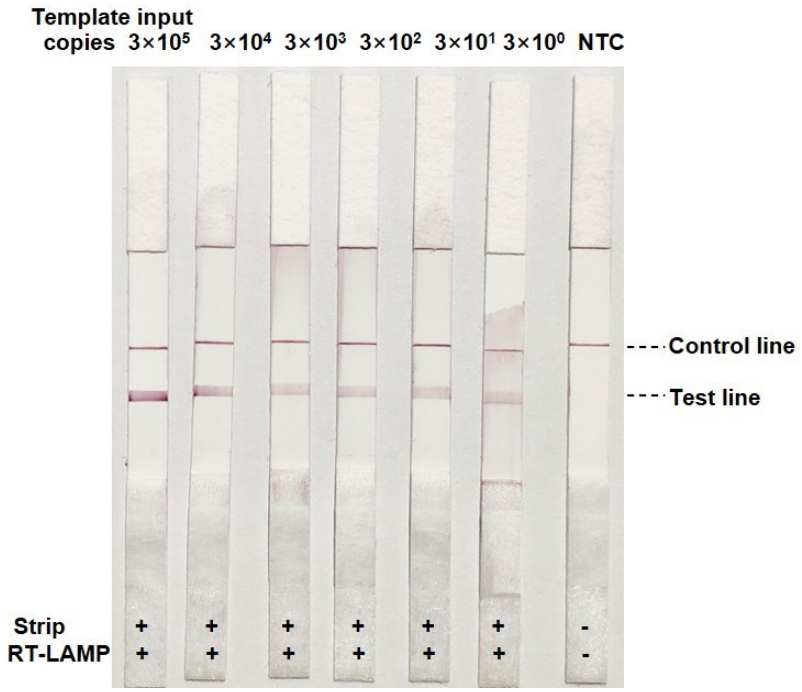


Figure S6. Lateral flow strip test of the sensitivity of RT-LAMP products. A single red line (control line) indicates a negative result, whilst two single red lines (control and test lines) indicate a positive result. '+': positive results; '-': negative results; Pos: positive control; NTC: no template control; Control line: represents the control result; Test line: represents the test result.

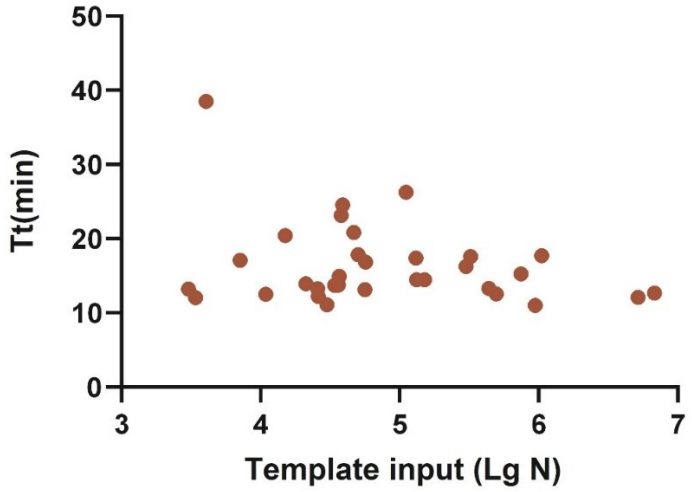


Figure S7. Correlation between the number of copies measured in 32 positive clinical samples and the novel RT-LAMP detection time. Tt: time threshold.

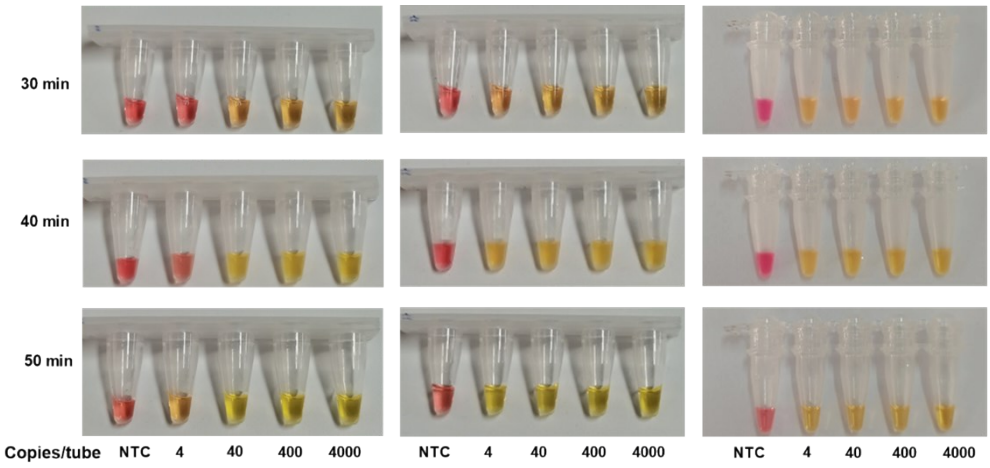


Figure S8. Visual detection of HIV-1 using the novel mismatch-tolerant RT-LAMP assays with

cresol red. The color change from burgundy to orange or yellow is considered as positive. NTC: no template control.