

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)	TTTGTATTACTACTGCCCTTCACCTTTCC			
	F1-Mu-C(F)	GGGGCAGTAGTAATACAAATAATAGTGAC			
	F1-Mu-C(R)	CTTGTATTACTACTGCCCTTCACCTTTCC			
	LB-Mu-A(F)	ATGATTGTGTGGCAAGTAACAGGATGAGG			
	LB-Mu-A(R)	TTACTTGCCACACAATCATCACCTGCCATC			
	LB-Mu-C(F)	ATGATTGTGTGGCAAGTACACAGGATGAGG			
	LB-Mu-C(R)	GTACTIONGCCACACAATCATCACCTGCCATC			
	FIP-Mu-A(F)	AAGCTCCTCTGGAAAGGTAAGGGGGCAGTA			
	FIP-Mu-A(R)	CCTTTCCAGAGGAGCTTTGCTGGTCCTTTT			
	FIP-Mu-C(F)	AAGCTCCTCTGGAAAGGCAAGGGGGCAGTA			
	FIP-Mu-C(R)	CCTTTCCAGAGGAGCTTTGCTGGTCCTTTG			
	FIP-Mu-2G(F)	AAAGCTCCTCTGGAAAGGGGAAGGGGGCAGT			
	FIP-Mu-2G(R)	CCCTTTCCAGAGGAGCTTTGCTGGTCCTTT			
	FIP-Mu-2C(F)	AAAGCTCCTCTGGAAAGGCGAAGGGGGCAGT			
	FIP-Mu-2C(R)	GCCTTTCCAGAGGAGCTTTGCTGGTCCTTT			
	FIP-Mu-3A(F)	CAAAGCTCCTCTGGAAAGATGAAGGGGGCAG			
	FIP-Mu-3A(R)	TCTTTCCAGAGGAGCTTTGCTGGTCCTTTT			
	FIP-Mu-3C(F)	CAAAGCTCCTCTGGAAAGCTGAAGGGGGCAG			
	FIP-Mu-3C(R)	GCTTTCCAGAGGAGCTTTGCTGGTCCTTTT			
	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		CTTGGCACTACYTTTATGTCACTAAARCTYCTCT GGAAAGGTG			
AceIN-FIPe		CTTGGTACTACYTTTATGTCACTAAARCTACTCT GGAAAGGTG			
AceIN-BIP		GGAYTATGGAAAACAGATGGCAGCCATGTTCT AATCYTCATCCTG			
AceIN-LF		TCTTGTATTACTACTGCCCTT			
AceIN-LB		GTGMTGATTGTGTGGCARGTAG			

	AceIN-F3	CCMMTTTGGAAAGGACCAGC	
RT-qPCR assay of HIV-1	HIV-1 F	CAATTTTAAAAGAAAAGGGGGGATTG	
	HIV-1 R	TAAACCCGAAAATTTTGAATT	This study
	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%.

	F3	F2	F1	B1	LB	B2
	CCAATTGGA AAGGACAGC (636)	AAACTACTC TGGAAAGTC (606)	AGTGATATA AAGTAGTA-C CAAG (502)	GGATTATGG- AAACAGATG GCAG (622)	GTGATGATG TGTGGCAGT AG (595)	CAGGATAGG ATTAGACAT GG (821)
	..C.G..... (373)	C..... (505)	..C..... (435)	..C..... (492)	..C..... (400)A..... (520)
	..C..... (149)	C.....T..... (12)	..C..... (22)	..C..... (40)	..C..... (94)C..... (3)
	..C.A..... (11)	C.....G..... (12)	..C.....G..... (19)	A..C..... (18)	..C.....A..... (14)C..... (1)
07_BC	..T..... (4)	C.....G..... (11)	..C.....G..... (11)	..C.....A..... (2)	..C.....G..... (14)A..... (1)
(555)	..CW..... (3)	C.....A..... (4)	..A.....G..... (10)	..Y..... (2) (12) (1)
	..C.G.....C..... (2)	C.....C..... (2)	..C.....G..... (6)	A..H..... (1)	..C.....A..... (10) (1)
	..C.....G..... (2)	C.....C..... (2)	..C.....G.....T..... (5)	..C.....A..... (3)	..C.....A..... (3) (1)
	..AC.G..... (2)	C.....G.....G..... (2)	..C.....C.....G..... (5)	..C.....A..... (3)	..C.....A..... (3) (1)
	..OC..... (2)	C.....C..... (2)	..C.....C.....C..... (5)	..C.....A..... (2)	..C.....A..... (2) (1)
	..C.GC..... (1)	C.....G..... (1)	..A.....G..... (2)	..C..... (2)	..C..... (2) (1)
	..C.G.....C..... (1)	C.....G.....C..... (1)	..C.....G.....A..... (2)	..C.....A..... (2)	..C.....A..... (2) (1)
	..C.GG..... (1)	C.....G.....C..... (1)	..C.....A..... (3)	..C.....A..... (2)	..C.....A..... (2) (1)
	..C.G.....G..... (1) (1)	..C.....G.....C..... (3)	..C.....A..... (2)	..C.....A..... (2) (1)
	..GG..... (1) (1)	..C.....T.....G..... (3)	..T..... (1)	..T..... (1) (1)
	..C.K..... (1) (1)	..C.....G.....G..... (3)	..C..... (1)	..C..... (1) (1)
 (1) (1)	..G.....C.....G..... (2)	..C.....R..... (1)	..C.....R..... (1) (1)
 (1) (1)	..A.....C.....G.....A..... (2)	..C.....A..... (1)	..C.....A..... (1) (1)
 (1) (1)	..A.....A.....G..... (2) (2) (2) (1)
 (1) (1)	..A.....G.....G..... (2)	..C.....G..... (1)	..C.....G..... (1) (1)
 (1) (1)	..OC.....G..... (2)	..C.....G.....A..... (1)	..C.....G.....A..... (1) (1)
 (1) (1)	..C.....C.....GA..... (1)	..C.....G.....T..... (1)	..C.....G.....T..... (1) (1)
 (1) (1)	..C.....G.....K..... (1) (1) (1) (1)
 (1) (1)	..C.....G.....H..... (1) (1) (1) (1)
 (1) (1)A..... (1) (1) (1) (1)
08_BC (92)	C..... (83)C.....G..... (75)	..C..... (88)	..C..... (83)A..... (80)
(98)	..C.G..... (2)	C.....G..... (9)	..C.....C.....G..... (6)	A..C..... (3)	..C.....A..... (3) (3)
	..C.A..... (1)	C.....G..... (3)	..C.....G..... (5)	..C..... (2)	..C.....A..... (4)G..... (4)
	..T..... (1)	C.....T..... (1)	..C.....G..... (4)	..C.....C..... (2)	..C.....A..... (2)A..... (2)
 (1)	C.....T..... (1)	..C.....G.....C..... (3)	..C.....C.....C..... (1)	..C..... (2)C..... (2)
 (1)	C.....T..... (1)	..C.....GA..... (1)	..C.....A..... (1)	..C.....G..... (2)A.....A..... (1)
 (1) (1)	..C.....G.....G..... (1)	..C..... (2)	..C.....A..... (2)C..... (1)
 (1) (1)	..C.....G.....G..... (1)	..C.....A..... (1)	..C.....A..... (1) (1)
 (1) (1)	..A.....G.....G..... (1)	..G.....A..... (1)	..G.....A..... (1) (1)
 (1) (1)	..C.....G.....T..... (1) (1) (1) (1)
C	..C..... (22)	C..... (18)C.....G..... (18)	..C..... (22)	..C..... (18)A..... (18)
(25)	..T..... (1)	C.....G..... (2)	..G.....G..... (1) (3)	..C.....A..... (3) (6)
 (1)	C.....T..... (2)	..C.....G.....A..... (1) (2)	..C.....A..... (2)G..... (1)
 (1)	C.....T..... (1)	..C.....G.....G..... (1) (1)	..C.....A..... (1) (1)
 (1)	C.....T..... (1)	..A.....G..... (1) (1)	..C.....A..... (1) (1)
 (1)	C.....G.....C..... (1)	..C.....G..... (1) (1)	..C.....A..... (1) (1)
 (1) (1)	..C.....G.....C..... (1) (1) (1) (1)
55_01B (8) (5)C.....G..... (8) (8) (8) (8)
(8) (2)	..T..... (2) (2) (2) (2) (2)
	..T.R..... (1) (1) (1) (1) (1) (1)
57_BC (7)	C..... (6)C.....G..... (3)	..C..... (6)	..C..... (5)A..... (6)
(7) (1)	C.....G..... (1)	..C.....G.....C..... (2)	A..C..... (1) (2)A.....C..... (1)
 (1) (1)	..C..... (1) (1) (1) (1)
 (1) (1)	..G..... (1) (1) (1) (1)
59_01B (9) (9) (8) (9) (9) (9)
(9) (1) (1)	A..... (1) (1) (1) (1)
61_BC (4)	C..... (4)C.....G..... (4) (3)	..C..... (4)A..... (2)
(4) (1) (1) (5) (1) (1)A.....A..... (2)
65_cpx (7) (7)C..... (1) (7) (7)A..... (7)
(7) (1) (1)	..G..... (1) (1) (1) (1)
67_01B (2) (2) (2) (2) (2) (2)
(2) (4) (4) (4) (4) (4) (4)
68_01B (4) (4) (4) (4) (4) (4)
(4)	..C..... (8)	C..... (6)C.....G..... (8)	..C..... (4)	..C..... (7)A..... (7)
69_BC	..C.G..... (1)	C.....T..... (2)	..C.....G.....Y..... (1)	..C..... (4)	..C.....A..... (2)A..... (2)
(9) (1)	C.....G..... (1) (1)	A..C..... (1)	..H..... (1) (1)
78_cpx (2) (2) (3) (3) (3) (3)
(3)	..C..... (1)T..... (1) (1) (1) (1) (1)
79_0107 (3) (2)C..... (3) (3) (3) (3)
(3) (1)T..... (1) (1) (1) (1) (1)
80_0107 (2) (2)	..C..... (2)	..A..... (3)	..C..... (2) (3)
(3)	..C..... (1)	..G..... (1)	..C.....T..... (1) (1)	CC.C..... (1) (1)
85_BC (8)	C..... (7)C.....G..... (9) (8)	..C..... (8)A..... (8)
(9)	..C.A..... (1)	C.....T..... (1) (1)	..C.....G..... (1)	..C.....A..... (1) (1)
 (1)	C.....G..... (1) (1) (1) (1) (1)
86_BC	..T..... (3)	C..... (3)C.....G..... (3) (2)	..C..... (3)A..... (2)
(3) (2) (1) (2) (1)	..G.....A..... (1) (1)
87_cpx (3) (2) (2) (3) (3)A..... (3)
(3)	..G..... (1)A..... (1)A..... (1) (1) (1) (1)
88_BC (3)	C..... (2)C.....G..... (3) (2)	..C..... (3)A..... (2)
(3)	..C..... (1)	C.K..... (1) (1) (1)A.....H..... (1) (1)
96_cpx (3) (3) (2) (2) (2) (2)
(3) (4) (4)	..AC.A.....G.....G..... (3)	..T..... (1)T.....A..... (1)A..... (1)
G	..C..... (1)	..C..... (1)	..AC.A.....G..... (1) (2)	..G.....A..... (2)A.....G..... (1)
(5) (1) (1)	..AC.A.....G..... (1) (1) (1) (1)

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.

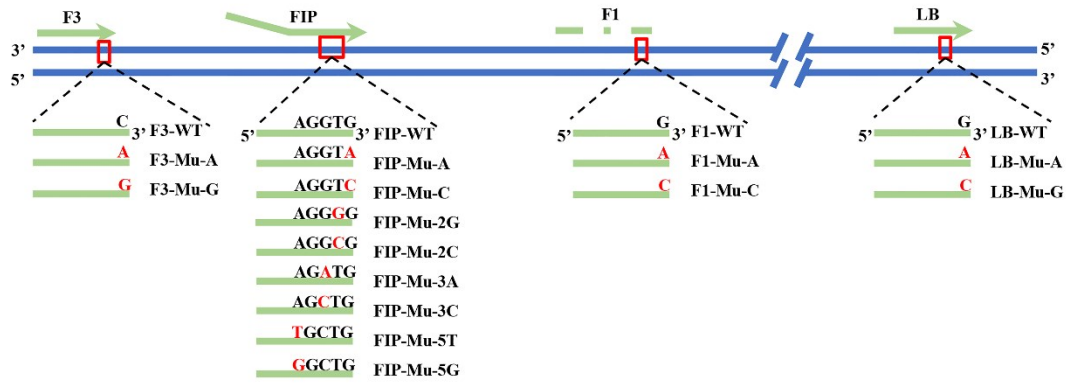


Figure S2. Schematic diagram of different HIV mutation templates. WT, wild-type; Mu, mutant.

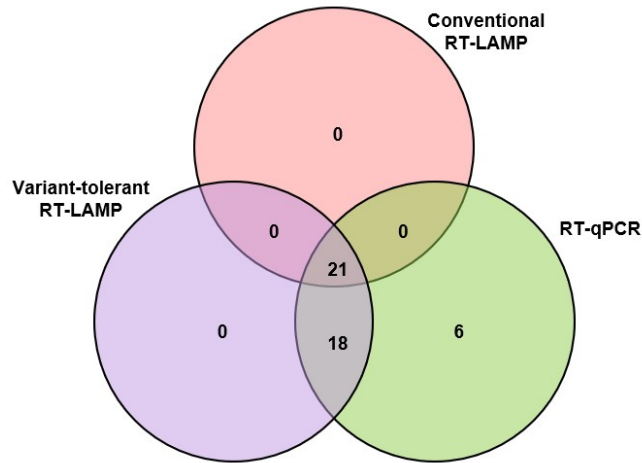


Figure S3. Numbers of HIV-1 positive samples detected by three different assays.

Primer	F3	F2	LF	F1	B1	LB	B2	B3
4838 (01_AE)	CCMMTTTGGGA AAGGACCAGCAAARCTYCTC	TGGAAAGGAG AAGGGGCAGT AGTAATACAA GA	AGTGACATAA ARGTAGTGCC AAG	GGATTATGGA AACAGATGG CAG	GTGTMGATTG TGTGGCARGT AG	CAGGATGARG ATTAGAACAT GG	TAGTAAARCA YCATATGTAT RTCTCAAAGA	
4780 (01_AE)	A. AA..... C. A. A. G. . T. G. . T. T. A. C. G. A. . G. G. G. AT. T. G.	
4774 (57_BC)	.. AC..... . G. C. T . . G. . T. G. G. G. G. A. C. G. G. A. AG. T. G. T. A. G. .	
4799 (96_cpx)	.. TA. C. C. A. A. G. . T. G. G. G. A. G. G. A. C. G. A. AG. T. A. C. G. T.	
4663 (B)	A. AA..... C. A. A. G. A. T. G. G. G. A. G. G. A. T. G.	
4635 (08_BC)	.. CA..... C. A. A. G. A. T. G. G. G. C. A. A. A. A. C. G.	
4624 (URF)	.. CA..... C. A. A. G. A. T. G. G. G. C. G. A. A. A. C. A. T.	
4651 (URF)	.. AA..... A. A. G. . T. G. G. G. A. G. G. A. T. G.	
4662 (URF)	.. AA..... A. A. G. . T. G. T. A. A. G. G. A. T. G.	
4665 (URF)	A. AA..... AT. G. G. . T. G. T. A. C. T. G. G. A. T. G. G. .	
4808 (URF)	A. AA..... C. A. A. G. A. T. G. T. A. A. G. G. A. T. C. . C. A.	
4825 (URF)	.. CA..... C. A. A. G. A. T. G. T. A. A. G. G. G. A. C. G. T.	
4867 (URF)	A. AA..... C. A. A. G. A. T. G. G. A. C. G. A. A. C. A. T.	
	▲ ▲	▲ ▲ ▲ ▲	▲ ▲	▲ ▲ ▲	▲	▲	▲ ▲ ▲	▲ ▲ ▲ ▲ ▲

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.

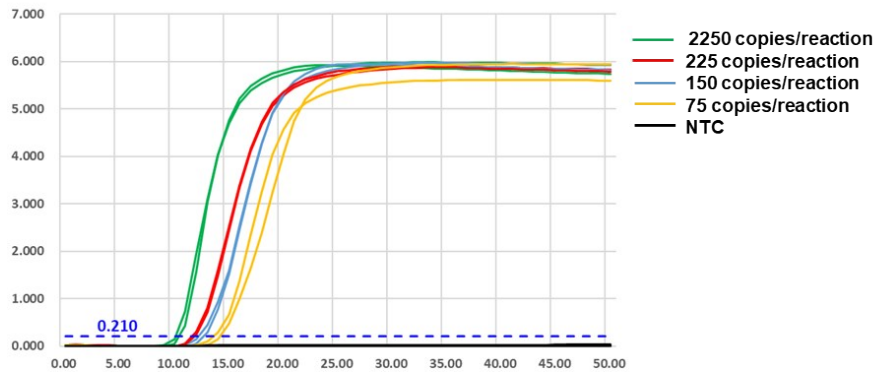


Figure S5. Mismatch-tolerant RT-LAMP detection by rapidly magnetic bead-based RNA extraction. The threshold is determined as 3 standard deviation from the background (0.210, dotted blue line).

NTC: no template control.

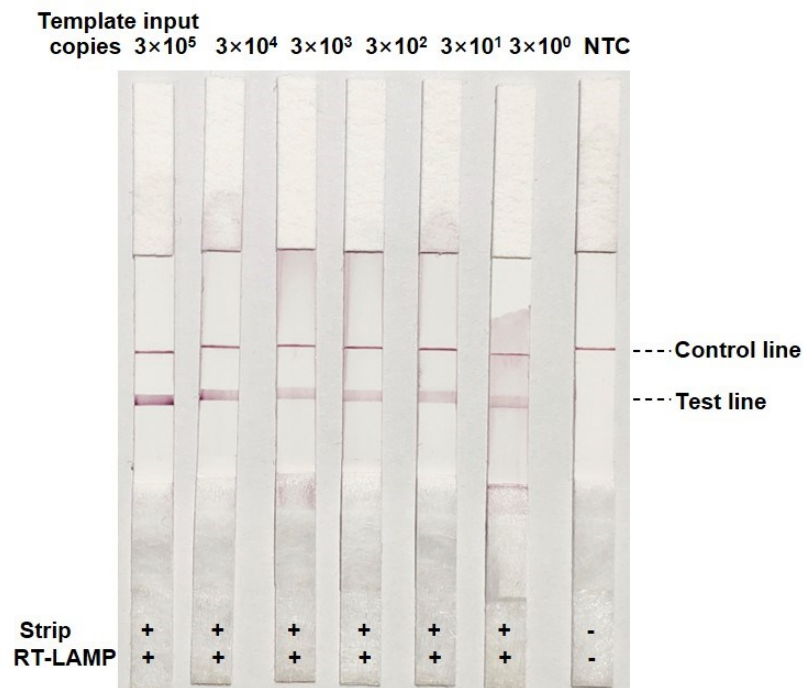


Figure S6. Lateral flow strip test of the sensitivity of RT-LAMP products. A single red line (control line) indicates a negative result, whilst two single red lines (control and test lines) indicate a positive result. ‘+’: positive results; ‘-’: negative results; Pos: positive control; NTC: no template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

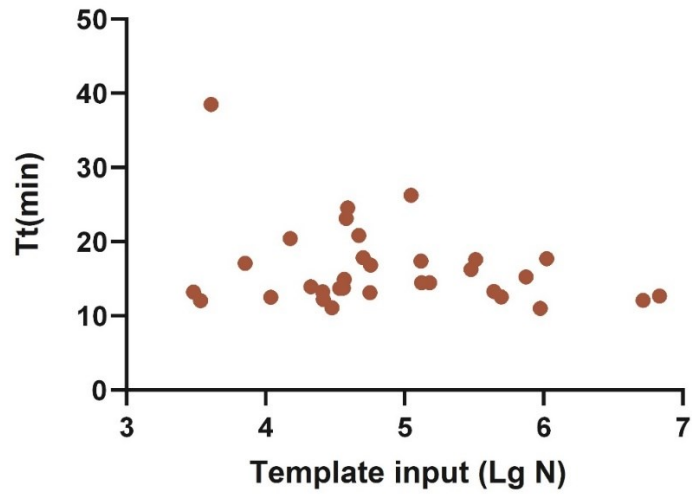


Figure S7. Correlation between the number of copies measured in 32 positive clinical samples and the novel RT-LAMP detection time. Tt: time threshold.

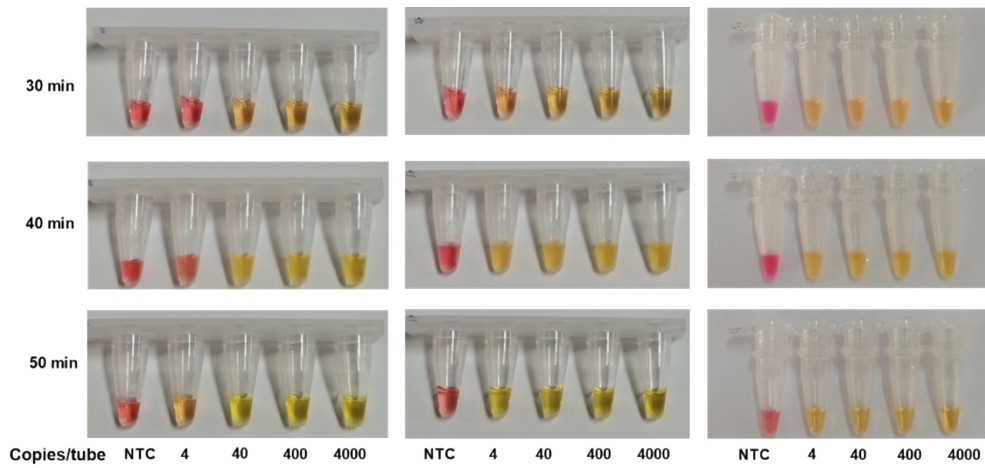


Figure S8. Visual detection of HIV-1 using the novel mismatch-tolerant RT-LAMP assays with

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