

Supplementary Information

Chemical and thermal treatments for rapid HIV-1 lysis in whole blood and saliva for point-of-care nucleic acid detection

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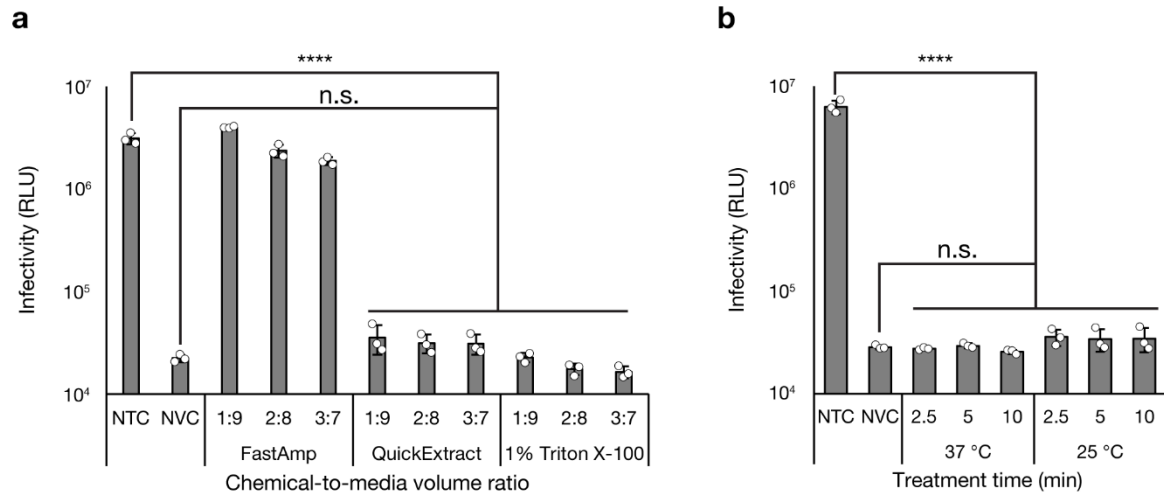
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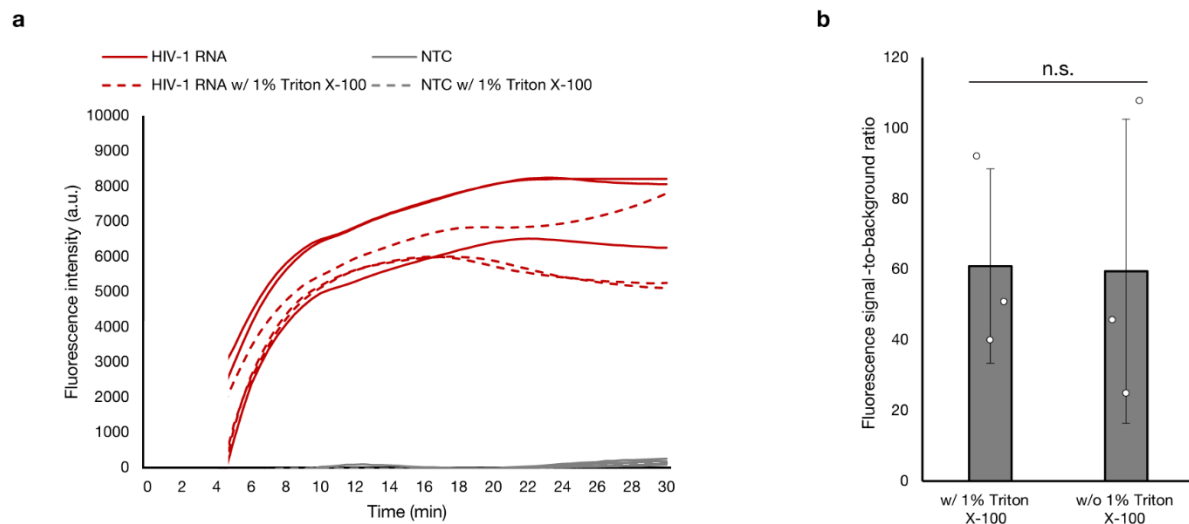
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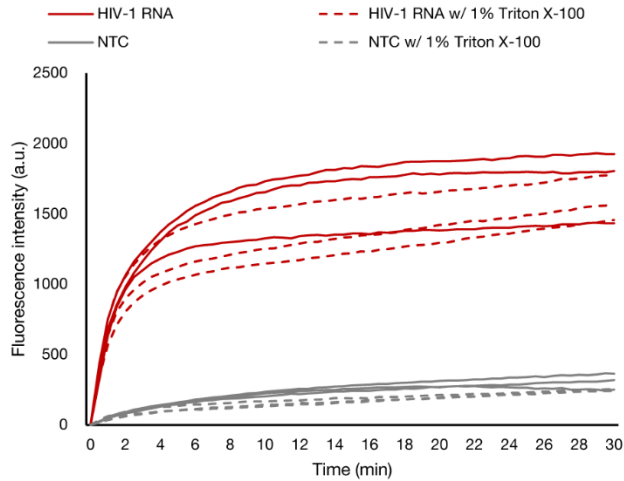
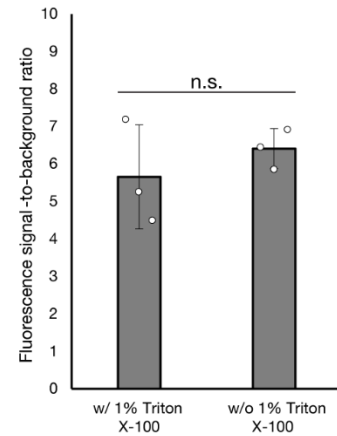
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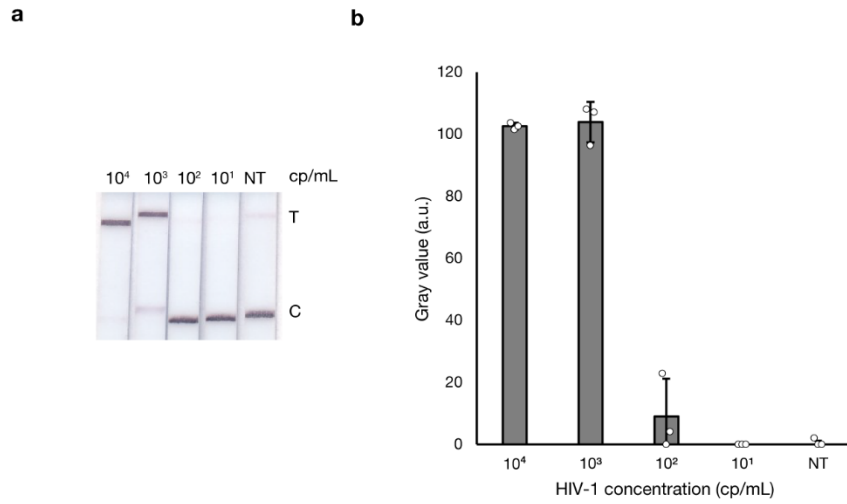
Supplementary Figure 1. Chemical treatment of HIV-1-spiked culture media samples. **(A)** Infectivity as measured in RLU for TZM.bl cells infected by HIV-1 AD8 cultured with media samples treated with FastAmp solution, QuickExtract solution or 1% Triton X-100 solution using different chemical-to-media volume ratios. Treatment of samples with 1% Triton X-100 was performed at 65 °C for 10 min. Statistical significance was assessed using one-way ANOVA with post-test Bonferroni comparison. **** indicates $P < 0.0001$ and n.s. indicates not significant. **(B)** Infectivity as measured in RLU for TZM.bl cells infected by HIV-1 AD8 cultured with media samples treated with 1% Triton X-100 solutions (1:9 v/v) at 37 °C or 25 °C. Statistical significance was assessed using one-way ANOVA with post-test Bonferroni comparison. **** indicates $P < 0.0001$ and n.s. indicates not significant. NTC: No treatment control. NVC: No virus control. Each bar represents the mean $\pm$ SD obtained from 3 technical replicates.



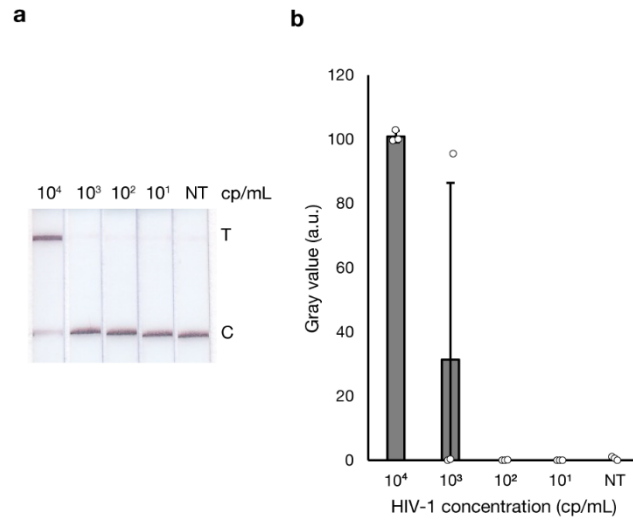
Supplementary Figure 2. Detection of purified HIV-1 RNA with and without 1% Triton X-100 using an exo probe-based RT-RPA assay. **(A)** Time course of raw fluorescence signal generated for purified HIV-1 RNA samples (10^8 copies/mL) with and without 1% Triton X-100. NTC: No target control. Each line represents the signal from an independent measurement, $n=3$. **(B)** Fluorescence signal-to-background ratio at 30 min obtained from panel A. Each bar represents the mean $\pm$ SD obtained from 3 technical replicates. Statistical significance was assessed using Welch's t-test. n.s. indicates not significant.

a**b**

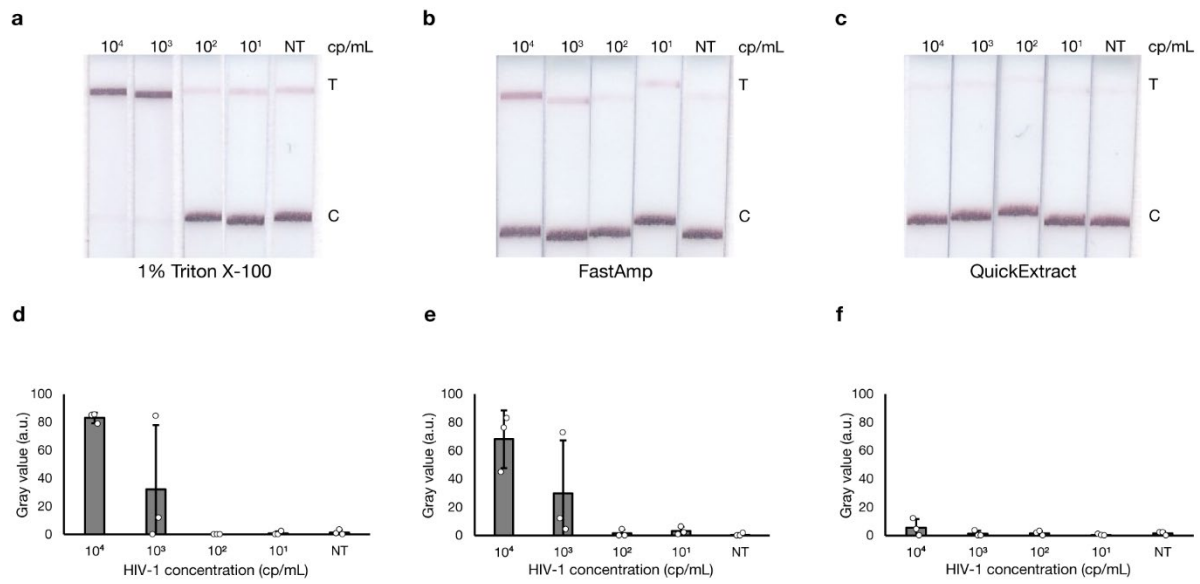
Supplementary Figure 3. Detection of purified HIV-1 RNA with and without 1% Triton X-100 using a fluorescence-based CRISPR-Cas13a assay. **(A)** Time course of raw fluorescence signal generated for purified HIV-1 RNA samples (10^{10} copies/mL) with and without 1% Triton X-100. NTC: No target control. Each line represents the signal from an independent measurement, $n=3$. **(B)** Fluorescence signal-to-background ratio at 30 min obtained from panel A. Each bar represents the mean $\pm$ SD obtained from 3 technical replicates. Statistical significance was assessed using Welch's t-test. n.s. indicates not significant.



Supplementary Figure 4. Detection of purified HIV-1 RNA using a CRISPR-Cas13a-based LFA. **(A)** Representative test results of blood samples spiked with HIV-1 AD8 processed using a commercial RNA purification kit. “T” and “C” indicate the positions of the test and control lines, respectively. **(B)** Quantification of test line intensity from the lateral flow strips in panel A. Each bar represents the mean $\pm$ SD obtained from 3 technical replicates using new test strips. NT: No target.



Supplementary Figure 5. Detection of HIV-1 in whole blood samples treated at 56 °C for 10 min using a CRISPR-Cas13a-based LFA. **(A)** Representative test results of whole blood samples spiked with varying concentrations of HIV-1 AD8 using the CRISPR-Cas13a-based LFA. “T” and “C” indicate the positions of the test and control lines, respectively. **(B)** Quantification of test line intensity from the lateral flow strips in panel A. Each bar represents the mean $\pm$ SD obtained from 3 technical replicates using new test strips. NT: No target.



Supplementary Figure 6. Detection of purified HIV-1 RNA containing different lysis chemicals using a CRISPR-Cas13a-based LFA. Representative test results of samples with varying concentrations of HIV-1 RNA containing (A) 1% Triton X-100, (B) FastAmp solution or (C) QuickExtract solution (1:9 v/v) using the CRISPR-Cas13a-based LFA. “T” and “C” indicate the positions of the test and control lines, respectively. NT: No target. Quantification of test line intensity from the lateral flow test strips in panels A-C corresponding to samples containing (D) 1% Triton X-100, (E) FastAmp solution, or (F) QuickExtract solution. Each bar represents the mean $\pm$ SD obtained from 3 technical replicates using new test strips.

Table S1: Sequences of RT-RPA primers, HIV-1 crRNA, and reporters.

Name	Sequence (5' -> 3')	Source	Note	Ref.
RT-RPA primer				
Forward	TAATACGACTCACTATAGGGTctctctggttagacca gatctgagcctggg	IDT	Upper-case letters represent T7 promotor sequence	-
Reverse	ggctctgagggatctctagttaccagagtcacac	IDT	-	-
crRNA				
HIV-1 crRNA	rGrGrGrGrArUrUrArArCrUrArCrCrCrArArAr ArArCrGrArArGrGrGrGrArCrUrArArArArCrUrA rGrUrUrArGrCrCrArGrArGrArGrCrUrCrCrCrAr GrGrCrUrCrArGrArU	IDT	-	-
Reporter				
Lateral flow assay reporter	/FAM/rUrUrU rUrUrU rUrUrU rUrUrU rUrUrU /Bio/	IDT	-	S1
Fluorescence assay reporter	/FAM/rUrUrU rUrUrU rUrUrU rUrUrU rUrUrU /IABkFQ/	IDT	-	S1
Exo probe- reporter	GCTTAAGCCTCAATAAAGCTTGCCTTGAG[T (FAM)]G-[dSpacer]T-[T(BHQ- 1)]CAAGTAGTGTGTGC-SpacerC3	Bio- search Techn- ologies	-	S2

References

- S1. Yang, J. *et al.* Engineered LwaCas13a with enhanced collateral activity for nucleic acid detection. *Nat. Chem. Biol.* **19**, 45–54 (2023).
- S2. Boyle, D. S. *et al.* Rapid Detection of HIV-1 Proviral DNA for Early Infant Diagnosis Using Recombinase Polymerase Amplification. *mBio* **4**, e00135-13 (2013).