

Electronic Supplementary Information (ESI)

**Performance of Point-of-Care Diagnosis of AIDS:
Label-Free One-Step-Immunoassay vs. Lateral Flow Assay**












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Concentration of anti-gp41 IgG standard (µg/mL)											
0		20		50		100		200		500	
Strip	RPI	Strip	RPI	Strip	RPI	Strip	RPI	Strip	RPI	Strip	RPI
	1.0		1.0		1.0		1.0		1.0		1.0
Control											
AIDS											
		0 ± 0 (Avg \pm S.D)		0.06 ± 0.01		0.08 ± 0.01		0.06 ± 0.01		0.15 ± 0.02	

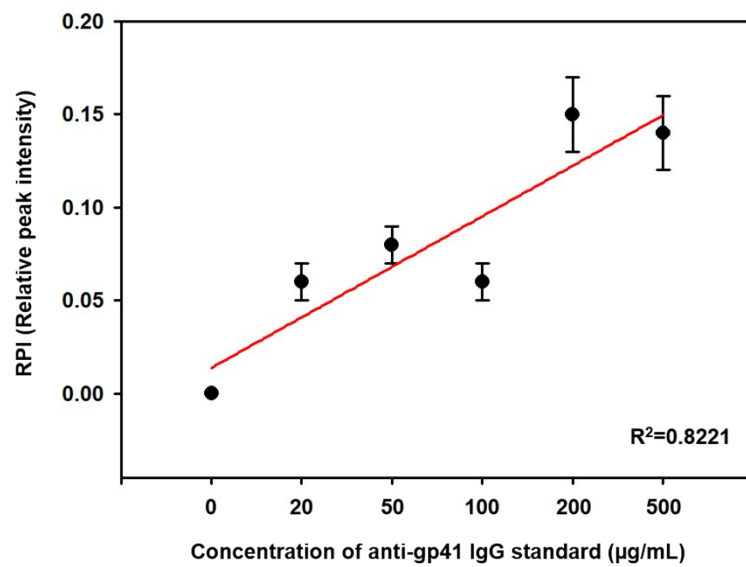








Fig. S11 Numerical relationships between test line intensity (RPI) and concentration of standard target (anti-gp41 IgG) in LFA.

Concentration of anti-gp41 IgG standard (ng/mL)											
0		0.001		0.01		0.1		1		10	
Well	O.D. _{.555nm}	Well	O.D. _{.555nm}	Well	O.D. _{.555nm}	Well	O.D. _{.555nm}	Well	O.D. _{.555nm}	Well	O.D. _{.555nm}
	0.44 ± 0.06 (Avg ± S.D)		0.53 ± 0.08		0.60 ± 0.05		0.71 ± 0.06		1.02 ± 0.12		1.19 ± 0.07

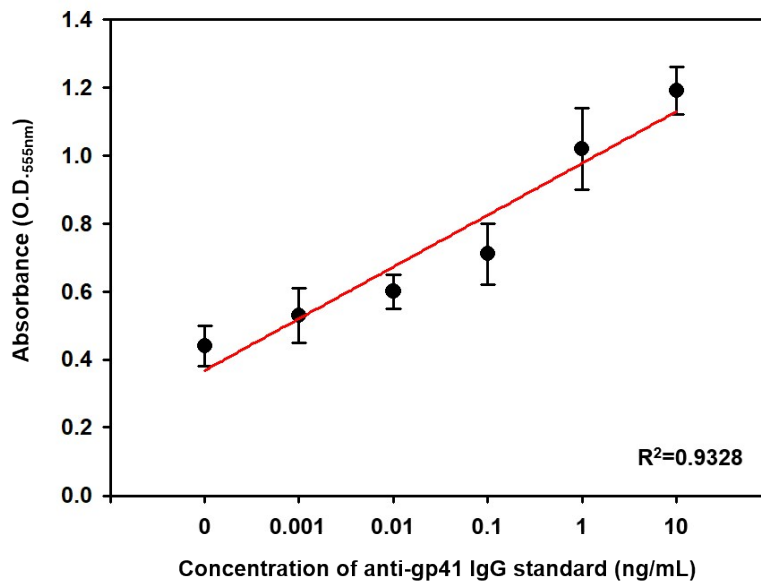
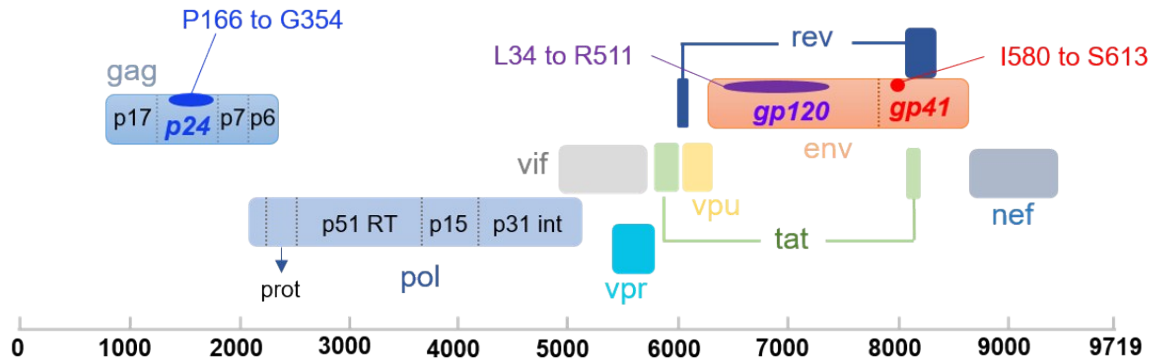


Fig. SI2 Numerical relationships between O.D._{.555nm} (measured at 25 min after assay begins) and concentration of standard target (anti-gp41 IgG) in one-step-immunoassay.



HIV gag protein

MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL LETSEGCRQI LGQLQPSLQT
 GSEELRSLYN TVATLYCVHQ RIEIKDTKEA 100 LDKIEEEQNK SKKKAQAAAA DTGHSNQVSQ NYPVQNIQ
 QMVHQAIQPR TLNAWVKVVE EKAFS **P166 EVIPMFSA** **SEGAT PQDLNTMLNT VGGHQAAMQM LKETINEEAA**
EWDRVHPVHA GPIAPQMRE PRGSDIAGTT STLQEQIGWM TNNPPIPVGE IYKRWIILGL NKIVRMYSP
SILDIRQGP EPFRDYVDRF YKTLRAEQAS QEVKNWMTET LLVQANPDC KTILKALGPA ATLEEMMTACQGV
G354 GPGHKA RVLAEAMSQV TNSATIMMQR GNFRNQKIV KCFNCGKEGH 400 TARNCRAPRK KGCWKCGKEG
 HQMKDCTERQ ANFLGKIWPS YKGRPGNFLQ SRPEPTAPPE ESFRSGVETT TPPQKQEPID KELYPLTSLR
 SLFGNDPSSQ 500

HIV envelope glycoprotein [gp160 (gp120+gp41)]

MRVKEKYQHL WRWGWRWGTG LLGM **L34 MICA** **TEKLWVTVYY GVPVWKEATT TLFCASDAKA**
YDTEVHNWVA THACVPTDPN PQEVVLNVNT ENFMWKNM VEQMHEDIIS LWDQSLKPCV KLTPLCVSLK
CTDLKNDTNT NSSSGRMIME KGEIKNCSFN ISTSIRGKVQ KEYAFFYKLD IIPIDNTTS YKLTSCNTSV
ITQACPKVSF EPIPIHYCAP AGFAILKCNN KTFNGTGPC T NVSTVQCTHG IRPVVSTQLL LNGSLAEEVV
VIRSVNFTDN AKTIIVQLNT SVEINCTRPN NNTRKRIRIQ RGPGRAFTI GKIGNMRQAH CNISRAKWN
TLKQIASKLR EQFGNNKTII FKQSSGGDPE IVTHSFNCGG EFFYCNSTQL FNSTWFNSTW STEGSNNTEG
SDTITLPCR KQIINMWQKV GKAMYAPPIS GQIRCSSNIT GLLLTRDGGN SNESEIFRP GGGDMRDNWR
SELYKYKVVK IEPLGVAPTK AKRRVVQREK R511 AVGIGALFL GFLGAAGSTM GAASMTLTVQ ARQLLSGIVQ
 QQNNLLRAIE AQHLLQLTV WGIKQLQAR **I580 LAVERYLKDQQLGIWGCSGKLICTTAVP** **WNA S613** WSNKSLE
 QIWNHTTWME WDREINNYTS LIHSLIEESQ NQEQKNEQEL LECDKWASLW NWFNITNWLW YIKLFIMIVG
 GLVGLRIVFA 700 VLSIVNRVRQ GYSPLSFQTH LPTPRGPDRP EGIEEEGGER DRDRSIRLVN GSLALIWDDL
 RSLCLFSYHR LRDLLIVTR IVELLGRRGW EALKYWWNLL 800 QYWSQELKNS AVSLLNATAI AVAEGTDRVI
 EVVQGACRAI RHIPRRIRQG LERILL

Fig. SI3 Sequences of antigenic epitopes (envelope glycoproteins, gp41 and gp120 and gag protein, p24) of HIV that are used to capture anti-HIV antibodies in patient sera.

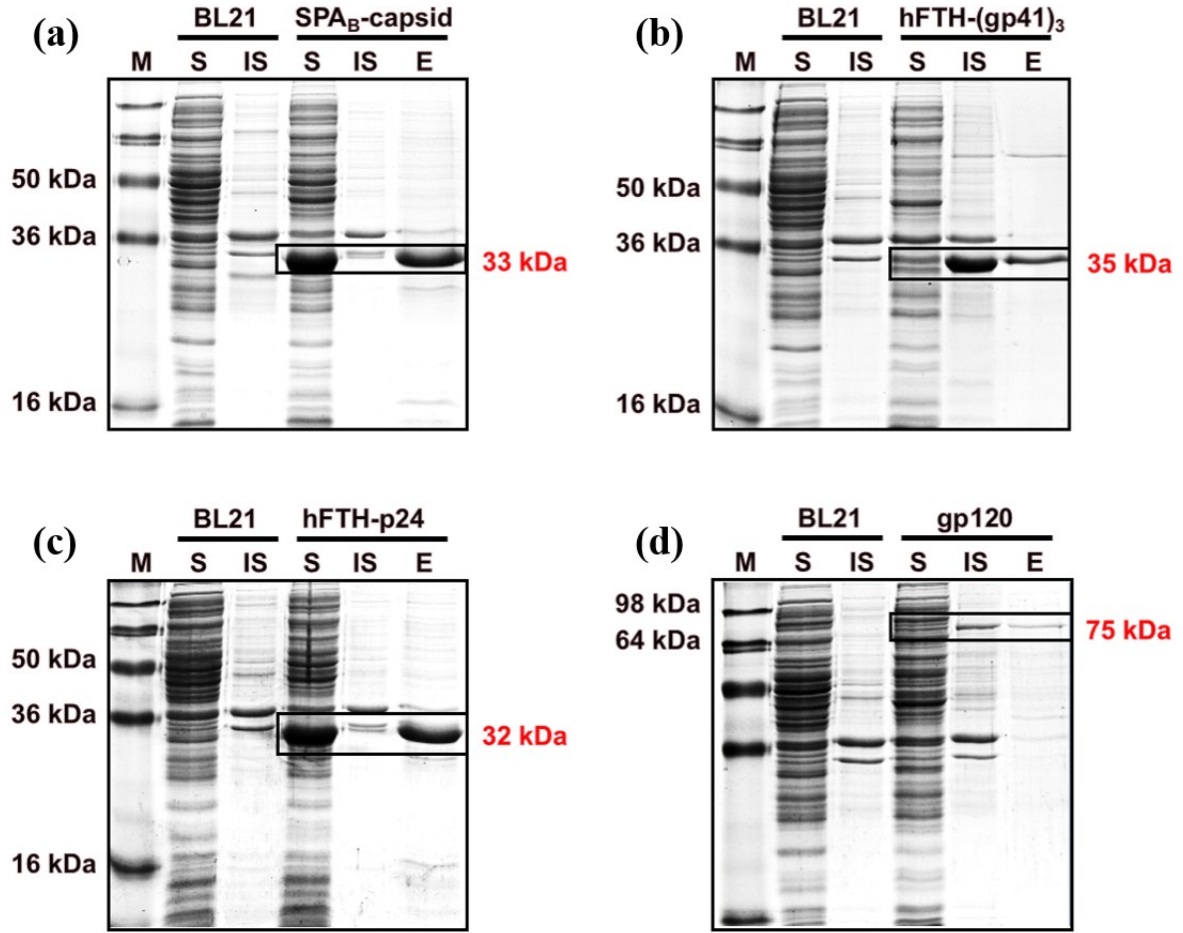


Fig. S14. Results of SDS-PAGE analyses of three types of probes to capture anti-HIV antibodies. (a) H₆-SPA_B-capsid, (b) hFTH-(gp41)₃-H₆, (c) hFTH-p24-H₆, and (d) N-ePGK-gp120. (M: Seeblue protein marker, S and IS: soluble and insoluble fraction of recombinant *E. coli* lysates, E: purified and eluted protein fraction. BL21: wild-type *E. coli* (control).

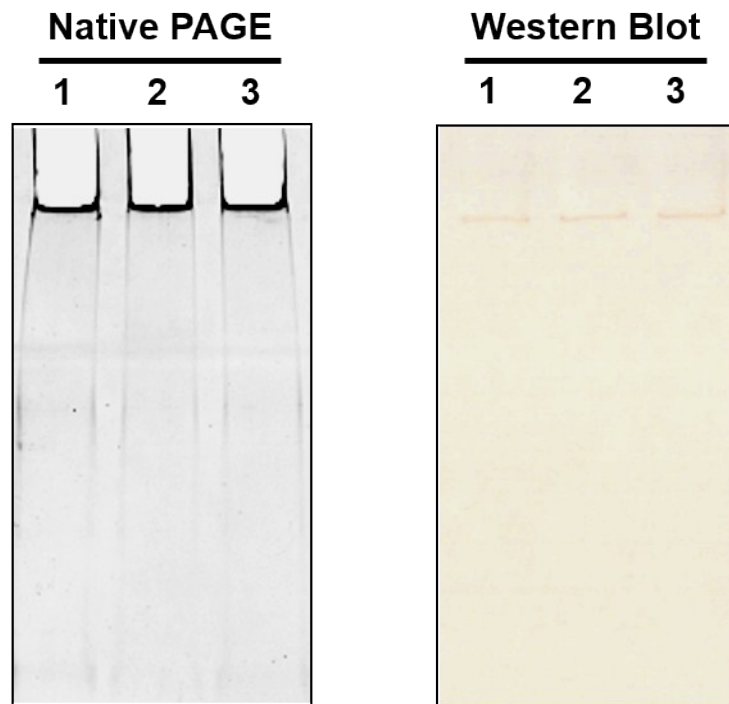


Fig. SI5 Results of native PAGE and Western blot analyses of 1) H₆-SPA_B-capsid, 2) hFTH-(gp41)₃-H₆, and 3) hFTH-p24-H₆, respectively. For Western blot, anti-His tag (H₆) antibody was used as primary antibody.


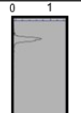

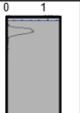







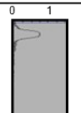

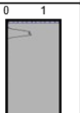







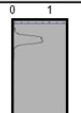





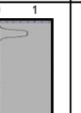



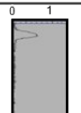

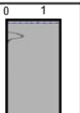






N1			N2			N3			N4			N5		
Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI
		1.0 0.0			1.0 0.0			1.0 0.0			1.0 0.0			1.0 0.0
N6			N7			N8			N9			N10		
Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI
		1.0 0.0			1.0 0.0			1.0 0.0			1.0 0.0			1.0 0.0
N11			N12			N13			N14			N15		
Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI
		1.0 0.0			1.0 0.0			1.0 0.0			1.0 0.0			1.0 0.0
N16			N17			N18			N19			N20		
Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI	Strip	Peak intensity	RPI
		1.0 0.0			1.0 0.0			1.0 0.0			1.0 0.0			1.0 0.0

Fig. SI6 Test results of 20 healthy controls in the type-2 LFA assay (LFA2 using three different probes, hFTH-(gp41)₃-H₆, hFTH-p24-H₆, and N-ePGK-gp120). (RPI represents relative peak intensity, i.e. intensity ratio of test to control line.)

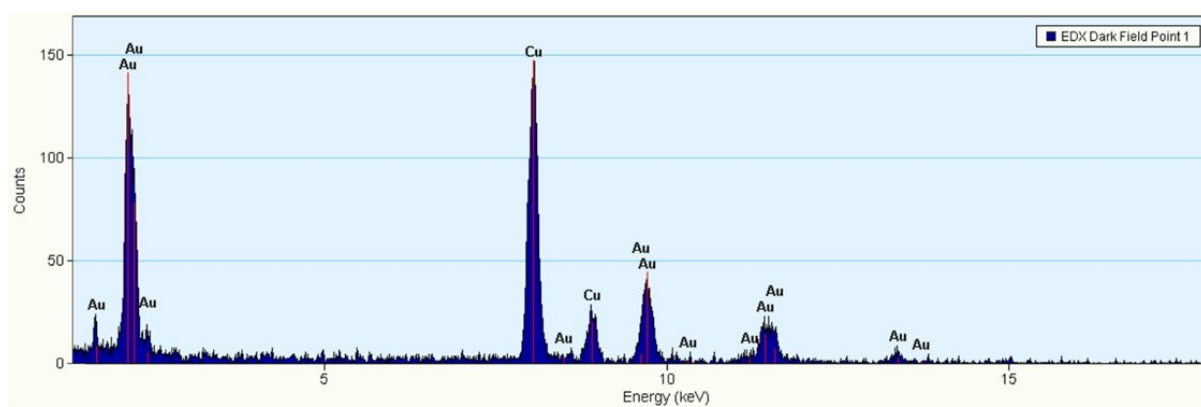


Fig. SI7 EDX spectroscopy analyses of assay solution of AIDS patient sera.

Table SI1. Recently reported assay performance of LFA-based diagnoses of AIDS

Method	Sensitivity / Specificity	Sample type	Ref.
The Aware HIV-1/2 U (LFA)	97.2% / 100%	Urine	Supplementary Ref. 1
Determine™ HIV-1/2 Ag/Ab Combo assay (LFA)	50, 86% / 62%	Serum	Supplementary Ref. 2,3
Lateral flow p24 assay (LFA)	95% / 99%	Plasma	Supplementary Ref. 4

Supplementary References

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