

Measuring the inducible, replication-competent HIV reservoir using an ultra-sensitive p24 readout, the digital ELISA viral outgrowth (DEVO) assay.

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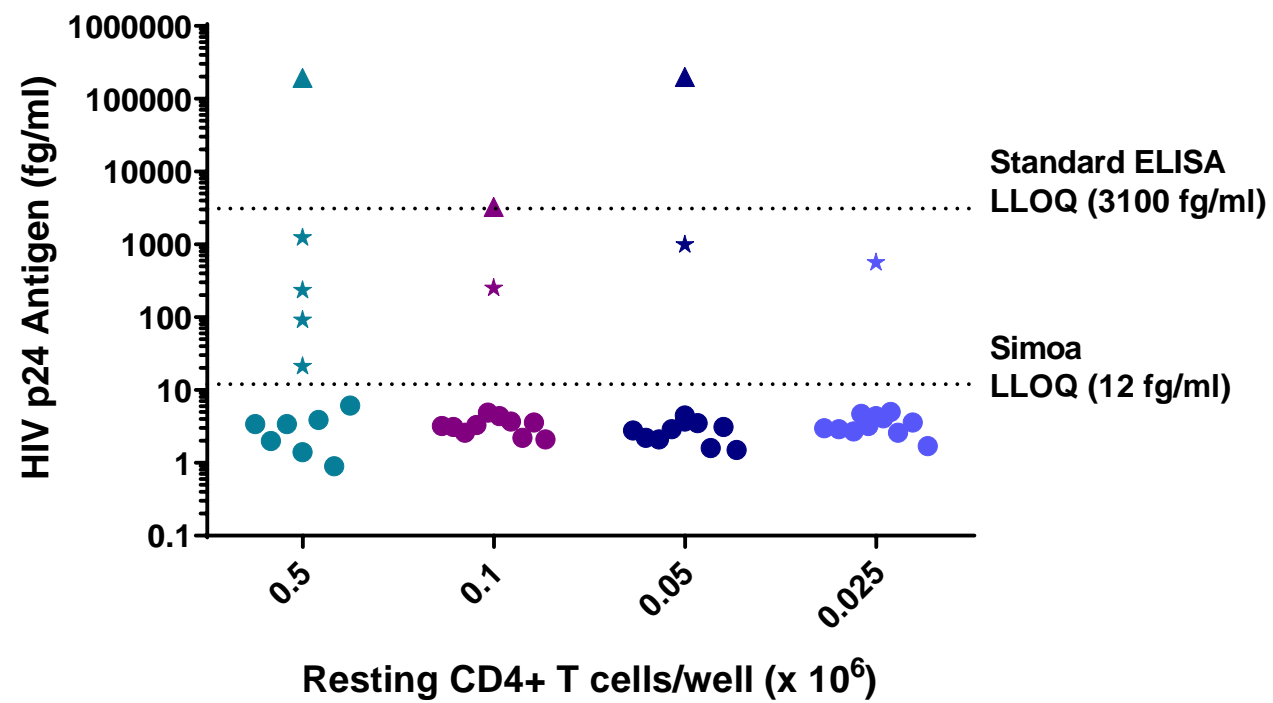


Figure S1. Ultra-sensitive p24 detection by Simoa versus standard ELISA in a viral outgrowth assay at day 8 post-stimulation. Each dot represents a well at the indicated cell dilution. Stars indicate p24 positive wells detected by Simoa only. Triangles indicate wells detected by both Simoa and standard p24 ELISA. LLOQ, lower limit of quantitation.

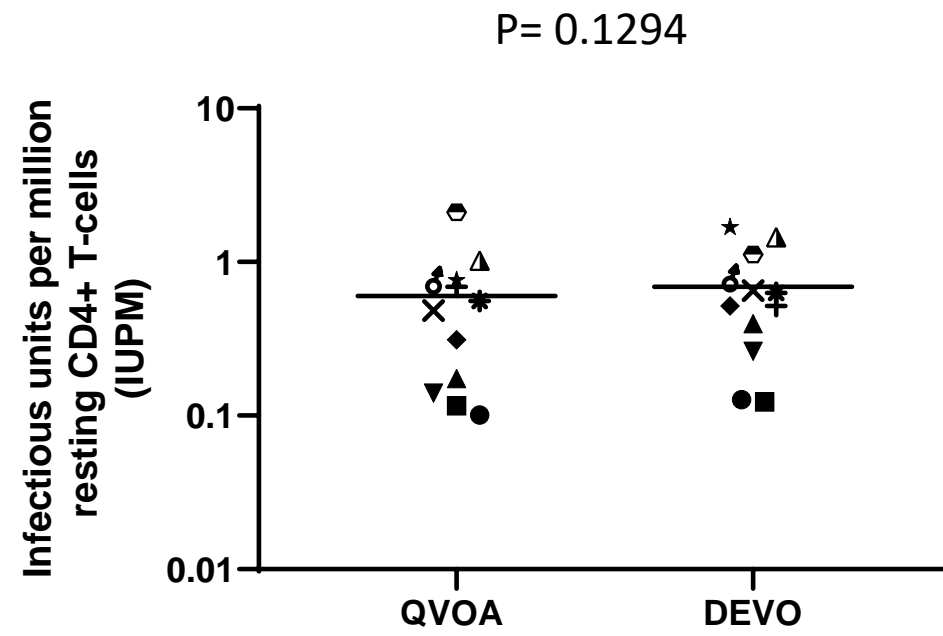


Figure S2. Comparison of IUPM values obtained in the DEVO assay at day 8 post-stimulation versus IUPM values obtained from the QVOA at day 15 post-stimulation. Wilcoxon matched-pairs signed rank test was used to compare differences.

Table S1. Clinical characteristics of HIV+ donors[‡]

Donor ID	Race	Sex	Age	CD4 Count	Years on ART	Years Suppressed*	ART Regimen	Treatment Status
DV-01	AA	M	26	535	0.95	0.7	DTG/ABC/3TC	AHI
DV-02	W	M	57	847	32	9.4	EFV/TDF/FTC	CHI
DV-03	W	M	31	278	6.69	6.45	TVD/ETR	CHI
DV-04	AA	F	37	873	16	12	TVD/ATV	AHI
DV-05	W	F	55	893	6.7	6	FTC/RPV/TAF	CHI
DV-06	W	M	60	902	7.28	6.84	ATV/FTC/TDF/RTV	CHI
DV-07	W	M	23	668	1.95	1.86	DTG/ABC/3TC	CHI
DV-08	W/H	M	28	1128	0.97	0.91	DTG/ABC/3TC	AHI
DV-09	AA	F	46	835	6.9	6.6	EFV/TDF/FTC	CHI
DV-10	W	M	50	574	4.04	4	EVG/COBI/FTC/TDF	AHI
DV-11	W	M	47	516	2.33	2.26	EVG/COBI/FTC/TDF	AHI
DV-12	AA	M	62	834	2.03	1.89	DTG/ABC/3TC	CHI
Mean	--	--	43.5	740.25	7.32	4.91	--	--

* (<50 copies HIV RNA/ml)

[‡]Clinical characteristics at first donation

Table S2: Evolution of HIV p24 in wells in the DEVO assay. Wells are scored positive on day 8 if confirmed on day 12 (confirmed wells are shown in blue). Representative assay using resting CD4+ T cells from participant DV-09 is shown. High, above the upper limit of quantitation. Low, below the limit of detection.

Cell Dilution (x 10 ⁶)	Well Number	HIV p24 Day 8 (fg/ml)	HIV p24 Day 12 (fg/ml)
0.5	A1	High	High
0.5	A2	313.85	3285.80
0.5	A3	1.75	5.79
0.5	A4	33.16	5.37
0.5	A5	0.76	0.05
0.5	A6	3.10	6.19
0.5	A7	2694.202	2722.05
0.5	A8	34.14	9.04
0.5	A9	Low	Low
0.5	A10	6.87	7515.01
0.5	A11	37.88	6935.88
0.5	A12	11671.77	13328.83
0.1	B1	0.91	57.00
0.1	B2	4000.04	High
0.1	B3	3.14	3.23
0.1	B4	16.37	1.42
0.1	B5	Low	0.96
0.1	B6	0.59	7633.96
0.1	B7	1.95	Low
0.1	B8	Low	1.40
0.1	B9	0.98	Low
0.1	B10	3.28	12.29
0.1	B11	2.82	5.38
0.1	B12	Low	14291.94
0.05	C1	1.78	79.11
0.05	C2	1.88	18.41
0.05	C3	3.10	6.61
0.05	C4	8.50	231.36
0.05	C5	Low	1.80
0.05	C6	3.74	3.04
0.05	C7	Low	1.37
0.05	C8	Low	1.77
0.05	C9	0.20	0.19
0.05	C10	5.42	Low
0.05	C11	Low	2.71
0.05	C12	Low	16.25
0.025	D1	7.526	0.54
0.025	D2	1.724	35.01
0.025	D3	High	High
0.025	D4	6.99	6.12
0.025	D5	5.03	1.54
0.025	D6	15.88	60.97
0.025	D7	1.80	1.25
0.025	D8	2.83	2.57
0.025	D9	2.39	3529.38
0.025	D10	1.45	3.42
0.025	D11	0.07	4.84
0.025	D12	1.84	4.20

Table S3: Evolution of HIV p24 in wells in the DEVO assay. Wells are scored positive on day 8 if confirmed on day 12 (confirmed wells are shown in blue). Representative assay using resting CD4+ T cells from participant DV-02 is shown. High, above the upper limit of quantitation. Low, below the limit of detection.

Cell Dilution (x 10 ⁶)	Well Number	p24 Day 8 (fg/ml)	HIV p24 Day 12 (fg/ml)
0.5	A1	1.45	Low
0.5	A2	443.00	347.24
0.5	A3	1.64	248.54
0.5	A4	Low	41.04
0.5	A5	131.66	133.06
0.5	A6	237.58	85.85
0.5	A7	Low	33.29
0.5	A8	Low	19.61
0.5	A9	Low	24.37
0.5	A10	Low	49.26
0.5	A11	Low	20.84
0.5	A12	1471.49	129519.94
0.25	B1	Low	Low
0.25	B2	34537.62	High
0.25	B3	Low	High
0.25	B4	Low	Low
0.25	B5	50.91	25.49
0.25	B6	3047.45	High
0.25	B7	307.82	279.21
0.25	B8	Low	20.55
0.25	B9	1.73	15.56
0.25	B10	1.91	43.95
0.25	B11	16324.21	High
0.25	B12	Low	13.12
0.1	C1	1.50	Low
0.1	C2	0.41	Low
0.1	C3	4480.21	1872.05
0.1	C4	8.85	2.07
0.1	C5	309.07	163.33
0.1	C6	Low	30.61
0.1	C7	58843.07	High
0.1	C8	0.01	16.11
0.1	C9	Low	22.22
0.1	C10	Low	967.62
0.1	C11	2.02	22.93
0.1	C12	Low	19.25
0.05	D1	Low	Low
0.05	D2	4.13	Low
0.05	D3	106.98	235.21
0.05	D4	Low	Low
0.05	D5	38.61	26.43
0.05	D6	0.14	18.65
0.05	D7	Low	421.03
0.05	D8	Low	21.48
0.05	D9	Low	18.94
0.05	D10	Low	17.72
0.05	D11	Low	17.37
0.05	D12	Low	26.13
0.025	E1	Low	Low
0.025	E2	Low	Low
0.025	E3	Low	Low
0.025	E4	5267.67	41626.69
0.025	E5	85.82	218.06
0.025	E6	Low	32.45
0.025	E7	Low	23.47
0.025	E8	0.56	14.45
0.025	E9	Low	27.67
0.025	E10	Low	16.49
0.025	E11	Low	19.60
0.025	E12	Low	21.52