SUPPLEMENTARY MATERIAL

Title

Duplex digital PCR assay on microfluidic chamber arrays for total HIV DNA reservoir quantification in persons with HIV

Authors

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Supplementary Table S1. Conditions used in the duplex pdPCR reaction for optimization.

Condition	Denaturing Temp (°C)[a]	Denaturing time (s)	Annealing and extension Temp (°C)[a]	Annealing and extension time (s)	Nº cycles	DNA. (ng)	Primer/probe conc. (nM)[a]
1	96	30	60	20	40	500	900/250
2	96	5	60	30	40	500	900/250
3	96	5	60	40	40	500	900/250
4	96	5	60	40	45	500	900/250
5	96	10	60	50	35	500	900/250
6	96	10	60	50	40	500	900/250
7[b]	96	10	60	50	40	340	900/250

[a] temperature and concentration conditions recommended by the manufacturer.
[b] condition selected as optimal for the rest of experiments.

Supplementary Table S2. Comparison of LTR-RU5 HIV and *RPP30* amplification in singleplex and duplex pdPCR assays.

	LTR-RU5 HIV (cp/ul)	*RPP30* (cp/ul)	Total HIV copies/10^6 cells
Singleplex	2562.3	6364.8	8.05×10^5
Duplex	2546.4	6252.7	8.14×10^5

Supplementary Table S3. Characteristics of participants with HIV.

Patient ID	Age	Gender	Time with HIV infection (years)	HIV-1 RNA (copies/mL)	Nadir CD4 (cells/µl)	CD4 counts (cells/µl)	CD8 counts (cells/µl)	CD4/CD8 ratio
1	51	Male	23.1	<20	250	1320	1350	0.97
2	62	Female	34.0	<20	289	940	770	1.23
3	53	Female	25.8	<20	309	560	1230	0.45
4	56	Female	15.9	<20	298	1070	660	1.62
5	67	Male	25.1	<20	30	810	1030	0.79
6	49	Male	22.4	<20	241	660	550	1.19
7	61	Female	19.7	<20	238	1590	720	2.22
8	47	Male	13.3	<20	230	1050	750	1.41
9	57	Male	25.4	<20	18	1430	990	1.44
10	46	Male	17.5	<20	16	550	770	0.71
11	68	Male	20.5	<20	171	420	280	1.50
12	57	Male	32.3	<20	205	790	1150	0.69
13	43	Male	35.3	<20	35	440	460	0.95
14	48	Male	15.8	<20	79	NA	NA	NA
15	52	Female	20.6	<20	59	592	625	0.95
16	50	Male	9.0	<20	371	803	720	1.12
17	54	Male	33.8	<20	302	729	508	1.44
18	61	Female	23.2	<20	133	882	626	1.41
19	53	Male	23.9	<20	350	923	390	2.37
20	55	Male	15.0	<20	311	1095	476	2.30
21	64	Female	30.8	<20	190	366	970	0.38
22	63	Male	27.3	<20	480	977	838	1.17
23	62	Male	22.4	<20	350	1332	710	1.88
24	59	Male	32.4	<20	200	902	815	1.11
25	52	Male	26.6	<20	246	1208	410	2.95
26	61	Male	31.0	<20	382	1132	858	1.32
27	60	Female	31.9	<20	164	391	567	0.69

28	58	Female	23.5	<20	80	644	531	1.21
29	54	Male	15.7	<20	286	558	409	1.36
30	51	Female	17.9	<20	344	1019	499	2.04
31	72	Female	23.5	<20	391	827	297	2.79
32	50	Male	29.0	<20	412	886	802	1.11
33	60	Male	14.0	<20	428	810	669	1.21
34	57	Male	24.7	<20	349	714	489	1.46
35	53	Male	26.6	<20	611	887	418	2.12
36	66	Male	18.6	<20	350	518	925	0.56
37	53	Male	33.1	<20	183	662	567	1.17
38	56	Male	22.5	<20	8	775	1205	0.64
39	55	Male	28.1	<20	300	867	867	1.00
40	55	Male	22.7	<20	NA	649	325	2.00
41	56	Male	21.8	<20	304	1063	784	1.36
42	66	Male	8.7	<20	82	511	569	0.90
43	58	Male	22.3	<20	80	714	847	0.84
44	61	Female	31.8	<20	400	518	370	1.40
45	62	Male	26.4	<20	80	756	830	0.91
46	58	Female	29.4	<20	267	577	803	0.72
47	68	Female	25.4	<20	100	577	930	0.62
48	53	Male	20.0	<20	420	688	478	1.44
49	51	Male	10.9	<20	465	724	706	1.03
50	50	Male	12.7	<20	366	669	669	1.00
51	55	Male	2	146659	459	526	353	1.49
52	37	Female	1	45995	264	375	NA	NA
53	33	Male	3	36619	NA	737	1534	0.48
54	35	Male	5	31960	297	297	914	0.32
55	34	Female	2	85435	NA	807	1096	0.73
56	42	Male	22	4884	299	417	914	0.45

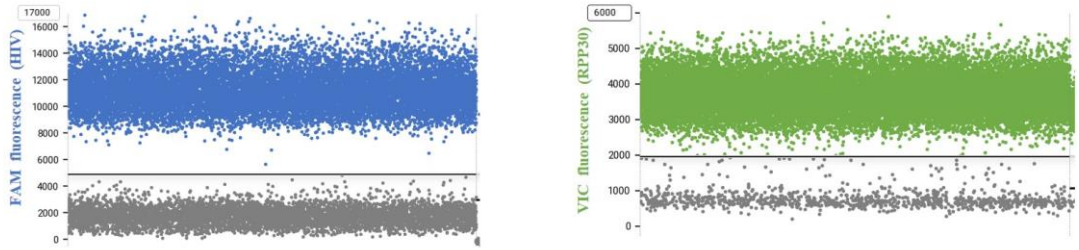
NA: not available

Supplementary Table S4. Primer and probe sequences and characteristics.

Gene	Primer/probe name	Primer/probe sequence (5 → 3')	strand	%GC	Tm (ºC)	Product (bp)	Fluorophore/Quencher (5'/3')
HIV LTR-RU5	RU5-for	TTAAGCCTCAATAAAGCTTGCC	forward	40.91	57.28	130	
	RU5-rev	GTTCGGGCGCCACTGCTAGA	reverse	65.00	64.94		
	RU5-probe	CCAGAGTCACACAACAGACGGGCACA	reverse	57.69	68.68		6-FAM/MGB
RPP30	RPP30-for	GATTTGGACCTGCGAGCG	forward	61.11	58.91	62	
	RPP30-rev	GCGGCTGTCTCCACAAGT	reverse	61.11	59.97		
	RPP30-probe	CTGACCTGAAGGCTCT	forward	56.25	51.42		VIC/MGB

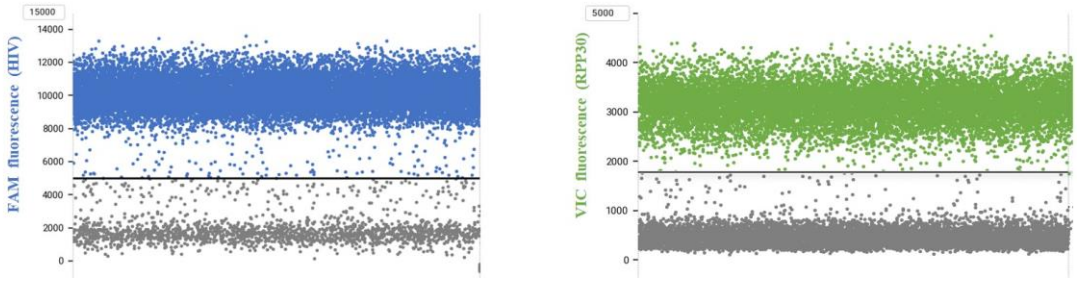
a)

	Enzyme activation	Denaturation	Annealing and extension	Number of cycles
PCR Conditions	10 min- 96ºC	10 sec- 96ºC	50 sec- 60ºC	40

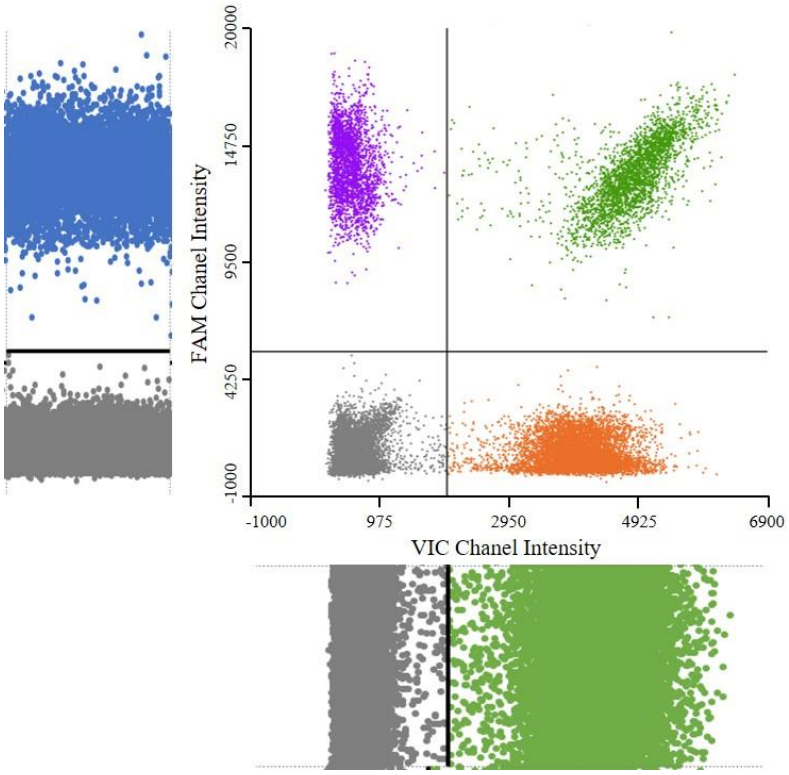


b)

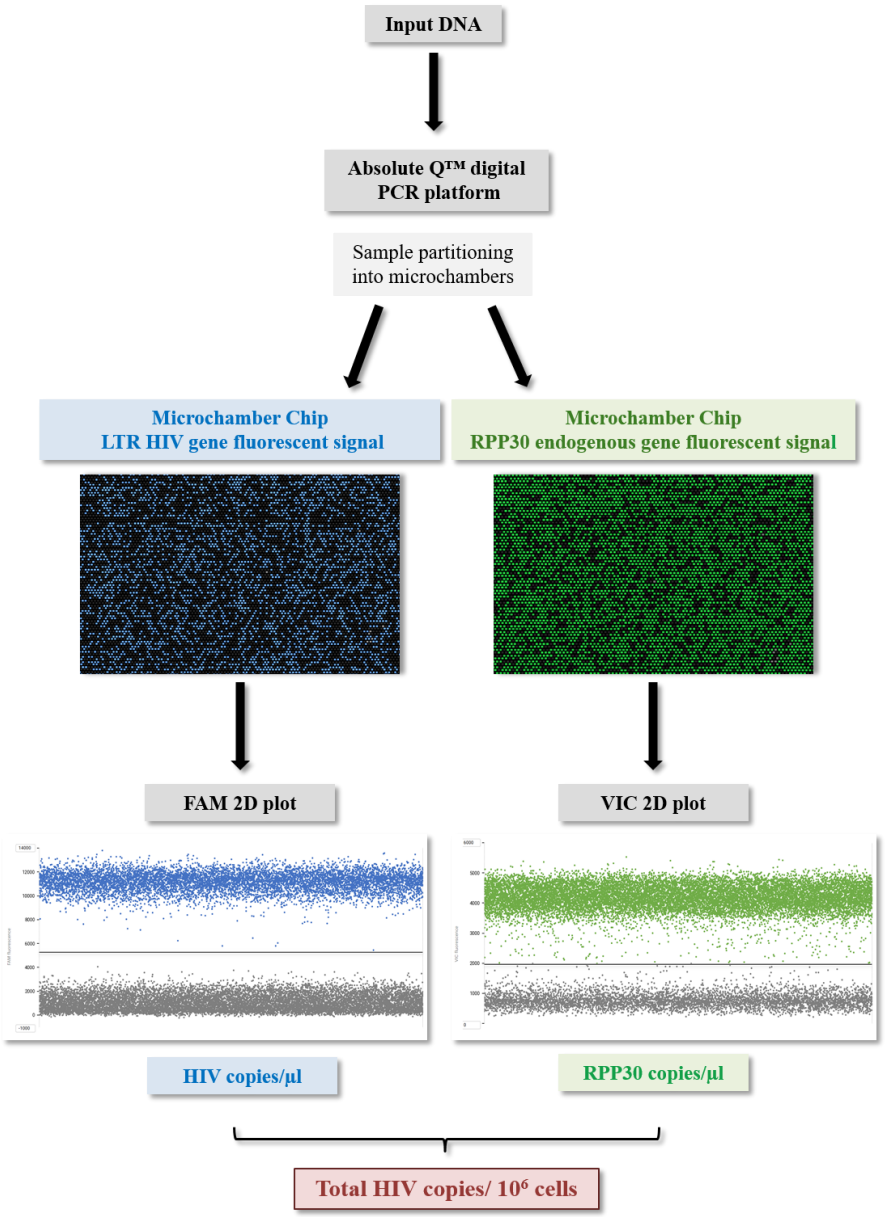
	Enzyme activation	Denaturation	Annealing and extension	Number of cycles
PCR Conditions	10 min- 96ºC	5 sec- 96ºC	30 sec- 60ºC	40



Supplementary Figure S1. 2D representative plots of FAM and VIC fluorescence under different PCR conditions. a) 2D representative plots of 8E5 DNA under the optimal PCR amplification conditions: 10 min at 96ºC followed by 40 cycles of 10 seconds at 96ºC and 50 seconds at 60ºC. No intermediate fluorescence is observed in the FAM channel for LTR-RU5 HIV amplification. b) 2D representative plots of 8E5 DNA under suboptimal PCR amplification conditions: 10 min at 96ºC followed by 40 cycles of 5 seconds at 96ºC and 30 seconds at 60ºC. Intermediate fluorescence is observed in the FAM channel for LTR-RU5 HIV amplification.



Supplementary Figure S2. Representative 2D plots from duplex pdPCR showing partition separation for the HIV target (FAM channel) and the *RPP30* gene (VIC channel). Clear cluster separation indicates minimal bleed-through between the FAM and VIC channels. The thresholds on both axes indicate the cut-off values used to distinguish positive and negative partitions.



Supplementary Figure S3. Digital PCR workflow for Total HIV reservoir quantification in Absolute Q™ digital PCR platform. Input DNA is loaded into MAP16 plates. Plates are introduced into the Absolute Q™ digital PCR platform for sample partitioning, PCR amplification and microchamber chip image acquisition. Chip positive fluorescent signals are obtained for LTR-RU5 HIV (blue FAM fluorescence) and *RPP30* gene (green VIC fluorescence). Positive and negative fluorescent chip signals are transformed into 2D plots. After thresholding, Poisson statistical analysis is applied to obtain the HIV and *RPP30* copies/µL. Finally, the number of total HIV copies /10^6 cells is calculated.