Supplemental Material Table 1. Reproducibility of RT-PCR amplification of the 3'end of Gag (p2/p7/p1/p6)- and Pol (PR/RT/INT)-coding sequences as a single large fragment (3,428 nt) or two overlapping shorter fragments (1,657 and 2,002 nt)

(A) Viral load <1,000 copies/ml

Large fragment

Replicate 2 \ Replicate 1	+	-
+	3	1
-	1	0

(n = 5)

Two fragments

Replicate 2 \ Replicate 1	+	-
+	4	0
-	0	1

(n = 5)

(B) Viral load >1,000 copies/ml

Large fragment

Replicate 2 \ Replicate 1	+	-
+	15	0
-	0	0

(n = 15)

Two fragments

Replicate 2 \ Replicate 1	+	-
+	15	0
-	0	0

(n = 15)

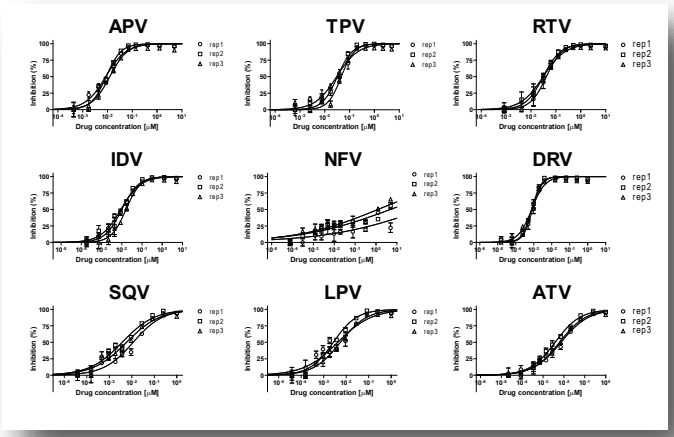
RT-PCR amplification reproducibility involved the analysis of 20 plasma samples from HIV-infected individuals with different viral loads: (A) 1,000 copies/ml (n = 5) and (B) >1,000 copies/ml (i.e., 1,001 – 5,000 copies/ml, n = 5; 5,001 – 10,000 copies/ml, n = 5; and >10,000 copies/ml, n = 5). RT-PCR amplification of a single (p2/p7/p1/p6/PR/RT/INT) or two (p2/p7/p1/p6/PR/5'RT + 3'RT/INT) fragments was performed by two different operators, using different lots of critical reagents over a seven-day period. A perfect (100%) RT-PCR amplification reproducibility was observed using plasma samples with viral loads >1,000 copies/ml. Interestingly, a 100% reproducibility was also obtained when RT-PCR amplifying the two overlapping fragments from plasma samples with <1,000 copies/ml.

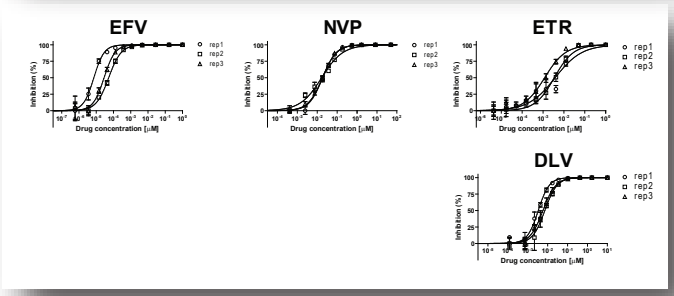
Supplemental Material Table 2. Different methods used to define biological cutoffs.

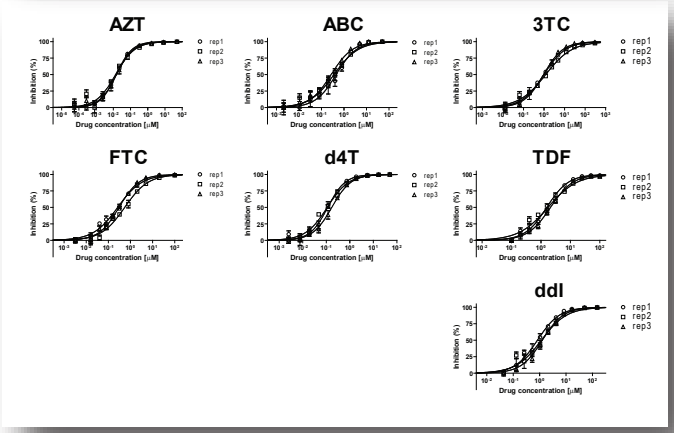
Drug	IC_{50} - based [a]	Mean + 2SD [b]	97.5th percentile [c]	99th percentile [d]
APV	1.67	1.27	1.40	1.55
TPV	1.95	1.67	1.85	2.20
RTV	1.78	1.68	2.09	2.32
IDV	1.61	1.50	1.61	1.62
NFV	2.16	3.15	3.62	3.81
DRV	1.72	1.28	1.29	1.38
SQV	2.41	2.07	2.52	2.85
LPV	2.04	1.45	1.88	2.14
ATV	1.98	1.63	1.52	2.01
EFV	2.09	2.49	2.96	3.09
NVP	2.01	2.22	2.35	2.89
ETR	2.06	1.57	2.14	2.25
DLV	2.25	3.94	4.04	6.89
AZT	1.65	1.82	1.97	2.37
ABC	1.51	1.22	1.33	1.36
3TC	1.64	1.16	1.19	1.22
FTC	1.57	1.32	1.40	1.45
d4T	1.66	1.23	1.24	1.31
TDF	1.45	1.40	1.39	1.62
ddI	1.53	1.22	1.28	1.39
RAL	1.63	1.44	1.34	1.34

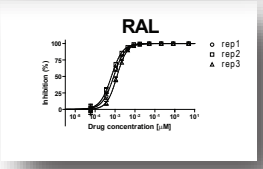
Upper BCO values for each antiretroviral drug were calculated using four different criteria, i.e., twice the coefficient of variation of the IC_{50} values plus one [a], the mean FC plus two standard deviations [b], the 97.5th percentile [c] or the 99th percentile [d] of the FC distribution.

A









B

Fold change in susceptibility to **PIs**

Drug	rep-1	rep-2	rep-3
APV	0.49	0.51	0.72
TPV	0.51	0.55	0.79
RTV	2.30	1.69	1.68
IDV	1.33	1.49	2.40
NFV	>max	>max	829
DRV	0.71	0.63	0.63
SQV	8.60	2.80	4.40
LPV	1.48	0.94	1.93
ATV	14.0	7.30	12.0

Fold change in susceptibility to **NNRTIs**

Drug	rep-1	rep-2	rep-3
EFV	0.24	1.73	0.94
NVP	1.63	1.45	1.57
ETR	1.14	1.03	0.36
DLV	0.32	0.69	0.55

Fold change in susceptibility to **NRTIs**

Drug	rep-1	rep-2	rep-3
AZT	3.30	3.00	3.50
ABC	2.90	2.40	1.77
3TC	2.40	2.90	2.30
FTC	2.20	4.60	2.50
d4T	1.81	1.73	2.70
TDF	2.20	2.40	2.90
ddI	1.41	1.99	2.20

Fold change in susceptibility to **INSTIs**

Drug	rep-1	rep-2	rep-3
RAL	0.55	0.42	0.79

Supplemental Material Figure 1. Reproducibility of the entire HIV-1 phenotypic assay. Three aliquots of a plasma sample obtained from a treatment-experienced HIV-infected individual were processed and analyzed in parallel. Phenotypic drug susceptibility profiles (A) and fold-change in susceptibility to PIs, NNRTIs, NRTIs, and INSTI (B) are shown for each independent p2-INT-recombinant virus or replicate (rep). Mutations associated with reduction in drug susceptibility included: PR (L10FHLY, D30DN, A71AT, N88DN), RT (M41LM, T69ADNT, V75IMV, F77FL, T215NSTY), and INT (none).