A novel assay to quantify replication-competent latent HIV-1 from rCD4+T cells

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Overview of Study

- A sensitive assay that can accurately quantify inducible replication-competent latent HIV-1 from resting CD4⁺ T cells is critical for HIV-1 eradication studies.
- Here, we describe a reporter cell-based assay (TZA) to quantify inducible replication-competent latent HIV-1 that is more sensitive, cost-efficient, and faster than the Q-VOA.
- Using this assay we show that the size of the inducible latent HIV-1 reservoir in aviremic participants on therapy is approximately 70-fold larger than previous estimates.

Assay Development

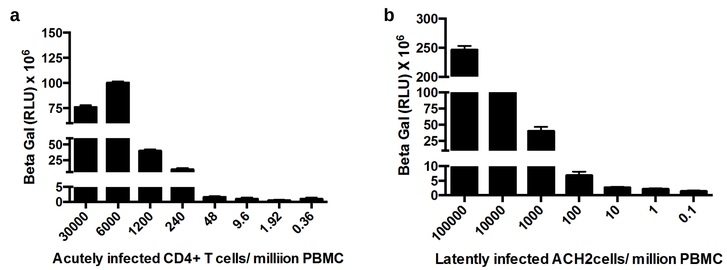


Figure 1: Sensitivity of TZM-bl cells to HIV-1 infection.

a) Serially diluted acutely infected HIV-1-positive CD4⁺ T lymphocytes (30% infection frequency as determined by flow cytometry of intracellular p24) were serially diluted with uninfected CD8⁺ T cell-depleted PBMC. 1×10^5 cells from each serial dilution was added to 5×10^4 TZM-bl cells in a 96-well plate. β-gal activity was measured by chemoluminesence 48 hours later. The relative light units (RLU) for the control were subtracted from each assay sample.

b) Latently infected HIV-1 infected ACH2 cells were treated with 100nM phorbol 12-myristate 13-acetate and then serially diluted with uninfected CD8⁺ T cell-depleted PBMC, and added them to TZM-bl cells, as described above.

Assay to Quantify Inducible Replication-Competent HIV-1 from rCD4⁺ T Cells

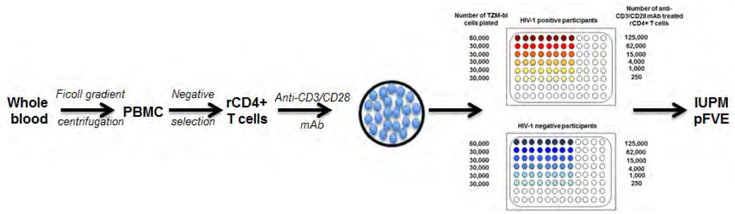


Table 1: Characteristics of study participants

Patient ID	Age	Current CD4+ T cell count (cells/mm³)	Current ART regimen	Years on cART	Current viral load (copies/mL)
30	63	1,264.0	TDF+FTC+ATV/r	15	<15
871	71	509.2	TDF+FTC+NVP	12	<15
119	40	850.1	TDF+FTC+RAL	5	<15
204	51	859.2	TDF+FTC+ATV/r	18	<15
17	52	636.4	RAL+DRV/r	14	<15
8	57	859.7	AZT+3TC+NFV	14	<15
184	57	389.6	TDF+FTC+EFV	17	<15
634	56	510.5	d4T+ddI+NFV	17	<15
144	34	797.1	TDF+FTC+EVG/c	1	<15
25	57	700.6	d4T+3TC+EFV	14	<15
183	66	615.1	AZT+3TC+EFV	19	<15
531	52	374.4	TDF+FTC+ATV/r	13	<15
258	59	1,586.7	TDF+RAL+ATV/r	12	<15
286	53	469.3	ABC+3TC+RAL	12	<15
29	54	612.7	TDF+FTC+EFV	13	<15
Mean ± S.D.	54.8 ± 9.2	735.6 ± 329.0		13.1 ± 4.7	<15

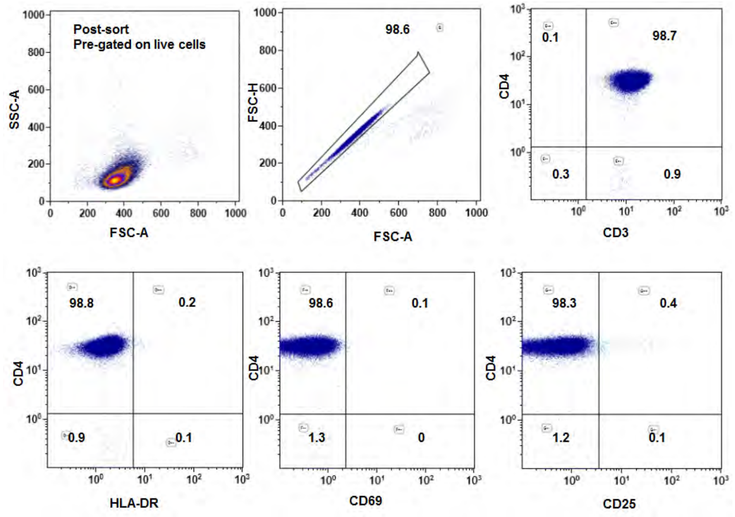


Figure 2: Flow cytometric analysis of the isolated rCD4⁺ T cell population. rCD4⁺ T cells purity was > 98% (n=16, 98.1 +/- 0.8%).

Comparison of TZA vs QVOA and Other Latency Assays

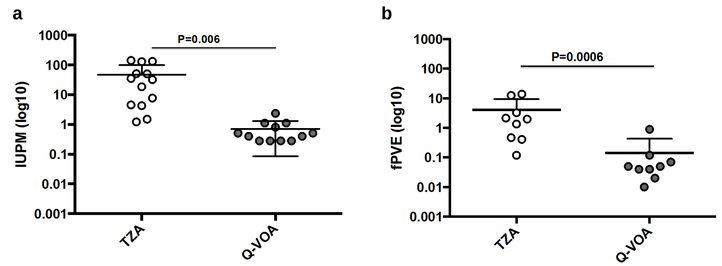


Figure 3: TZM-bl based assay (TZA) to quantify inducible replication-competent HIV-1 from rCD4⁺ T cells.

a) Statistical comparison of the infectious units per million cells (IUPM) determined by TZA (n=13) or Q-VOA (n=12) using a parametric unpaired T test (p=0061),

b) Statistical comparison of fractional HIV-1 provirus expression (fPVE) determined by TZA (n=9) or Q-VOA (n=9) using a parametric ratio paired T test (p=0.0006)

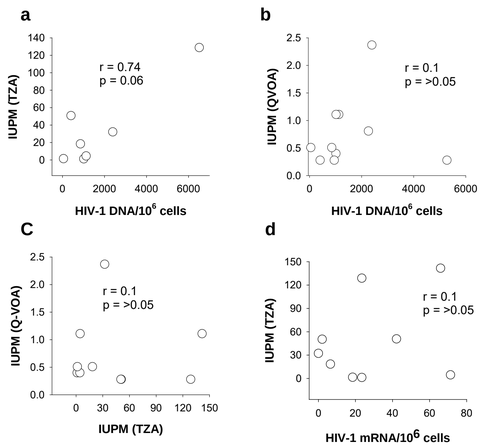


Figure 4: Correlations between TZA, Q-VOA, HIV-1 DNA and intracellular RNA.

a) Correlation between TZA and total HIV-1 DNA in rCD4⁺ T cells.

b) Correlation between Q-VOA and total HIV-1 DNA in rCD4⁺ T cells.

c) Correlation between TZA and Q-VOA.

d) Correlation between TZA and intracellular HIV-1 gag-pol mRNA. P values were obtained from the Pearson test.

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