A Scalable RNA RT-PCR Based Assay to Quantify Latency Disruption in HIV-1 Infected Cells

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Background. Quantifying the HIV reservoir in treated patients with viral suppression:

- HIV Outgrowth Culture: 1 in 1,000,000
  - Resource intensive
  - Original infected cells lost
  - Underestimate
- HIV DNA PCR: 1 in 1,000
  - Fast
  - Original infected cells lost
  - Overestimate
- HIV RNA RT-PCR
  - Fast
  - Original infected cells retained
  - Some virologic realism

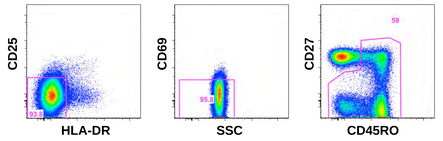
Purpose:

Establish a scalable RT-PCR based method for quantifying the frequency of HIV producing cells in the blood and tissues of patients with viral suppression.

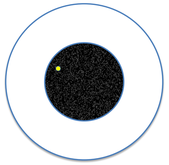
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Method Sequence:

1. Purify resting memory CD4+ T cells from PBMC of suppressed patient.



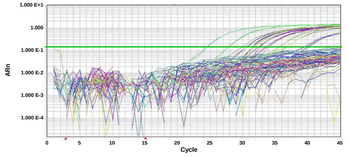
2. Culture with infected cells in limiting dilution with:
1) Reverse-transcriptase inhibitor efavirenz
2) Bead-bound antibodies to CD2, CD3, and CD28



3. Purify RNA from culture media on magnetic beads with robot & DNAase treat.



4. RT-PCR for HIV *gag* RNA

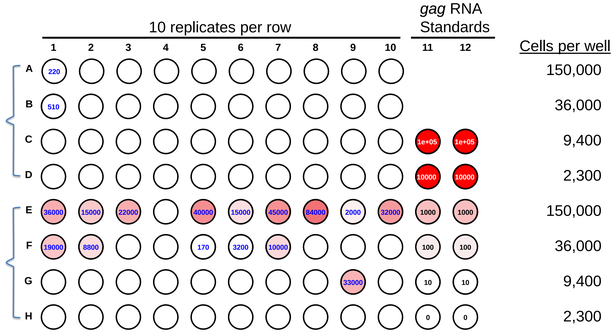


$$\frac{1}{\text{Fraction Sampled Culture Media}} \times \frac{1}{\text{Fraction Spiked RNA Recovered}} \times \frac{1}{\text{Fraction Sampled RT-PCR Reaction}} \times \text{(}gag\text{ RNA copies in RT-PCR well)} = gag \text{ RNA copies in culture well}$$

Data Analysis and Results:

5. No stimulation

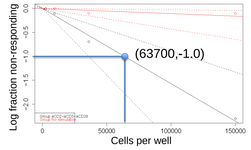
Stimulate Bead-Anti CD2 CD3 CD28



Red shading intensity proportional to HIV *gag* RNA detected by RT-PCR.
Black numbers in circles, measured HIV *gag* RNA copies by RT-PCR.
Blue numbers in circles, estimated HIV *gag* RNA copies in culture well.

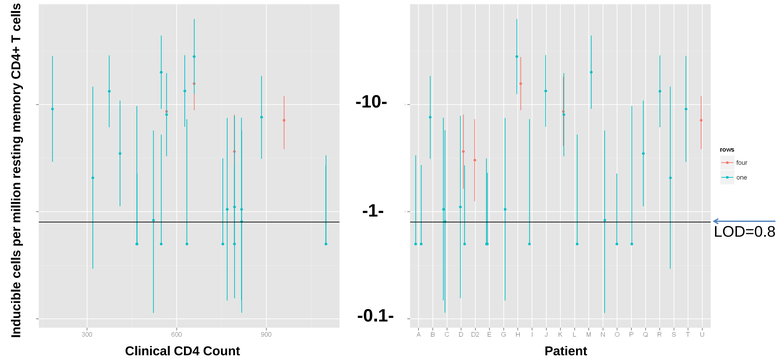
Data Analysis and Results (continued):

6. Statistics with R package
Extreme Limiting Dilution Analysis
(Hu and Smyth, J Immunol Methods, 2009)



7. Single ACH-2 sorted into each well (A-F) with PMA and 200,000 CD4+ cells from HIV negative donor (A-G). HIV RNA detected in 63% of wells.
47 copies of *gag* RNA in well F8.

8. HIV producing cells per million resting memory CD4+ T cells



Negative assays plotted at 0.5 (1 of 10 positive at 150,000 cells per well with 3 negative rows).
Limit of detection (LOD) is 0.5/0.6 = 0.8 cells per million, assuming production of at least 24 virions.
Experiments done as 4 rows of 10 replicates in dilution series (red) or 1 row of 10 replicates (blue-green).
Length of colored lines show 95% confidence interval.

Conclusion:

This assay represents a scalable bench-to-bedside method for quantifying the frequency of HIV producing cells in the blood and tissues of patients with viral suppression and for evaluating reservoir elimination strategies for such patients.