Poster # 300

Novel Assay to Measure Integrated HIV DNA in PBMC from ART-Suppressed Persons

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Background

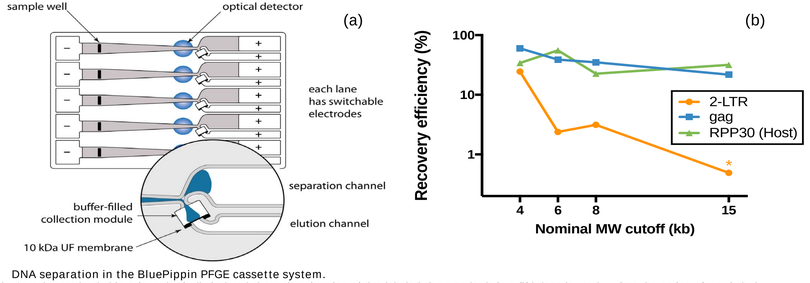
- Droplet digital PCR (ddPCR) allows precise and sensitive measurement of HIV DNA but does not distinguish between integrated and unintegrated molecular forms.[1]
- Alu-gag qPCR[2] assay combined with an efficiency assumption and correction is the current standard to assay to measure integrated HIV DNA.
- Utilizing Pulse-field gel electrophoresis (PFGE) we have shown a 98% clearance of circular forms of HIV DNA and a 43% recovery of high molecular weight host genome RPP30 DNA.[3]

Objective

- Develop and cross validate a new method, utilizing a combination of PFGE and ddPCR, to easily measure integrated HIV DNA in PBMCs from ART suppressed persons.

Methods

- DNA was extracted from PBMCs collected from 10 ART-suppressed persons.
- Integrated HIV DNA was quantified from 2-63µg of extracted DNA, by a standard Alu-gag qPCR assay.
- 1µg of DNA was used to measure HIV 2-LTR, HIV gag, and RPP30 without any prior separation by ddPCR (Before PFGE gag).
- 5µg of DNA from each sample was loaded per well of BluePippin gel 0.75% DF Cassettes and separated using a PFGE 15kb High-Pass protocol (After PFGE gag), and levels of HIV 2-LTR, HIV gag, and RPP30 were then measured by ddPCR. (a,b)

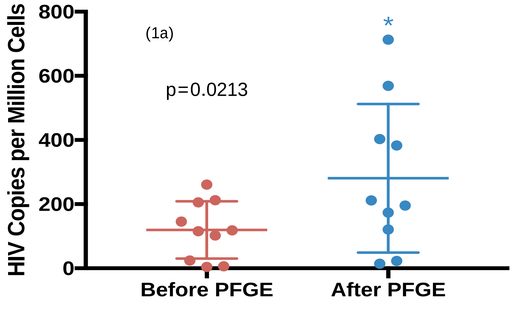


➢ DNA separation in the BluePippin PFGE cassette system.
(a) Samples are loaded into four physically-isolated channels. Migration of dye-labeled size standards in a fifth lane is used to time the gating of a switch that redirects flow into the elution channel. Electrical pulse sequences defined by manufacturer protocols use a series of voltage ramps to maximize resolution near a target molecular-weight cutoff.
(b) Optimal resolution of high molecular weight DNA versus episomal DNA was obtained with the High-Pass 15-20kb sequence, using a nominal collection cutoff of 15kb. *Below limit of detection.

References
1. Strain MC, Lada SM, Luong T, et al. Highly Precise Measurement of HIV DNA by Droplet Digital PCR. *PLoS ONE*. 2013
2. Liszewski MK, Yu JJ, O'Doherty U. Detecting HIV-1 integration by repetitive-sampling *Alu-gag* PCR. *Methods*. 2009
3. Steven M Lada, Caroline C Ignacio, Douglas D Richman, Matthew C Strain. Measurement of Integrated HIV DNA by Pulse-Field Gel Electrophoresis and ddPCR. Poster presented at: Conference of Retroviruses and Opportunistic Infections; 2016 Feb 22-26; Boston, MA

Results

Figure 1 a,b – Integrated Proviral HIV DNA Detected in ART-Suppressed Persons by PFGE+ddPCR



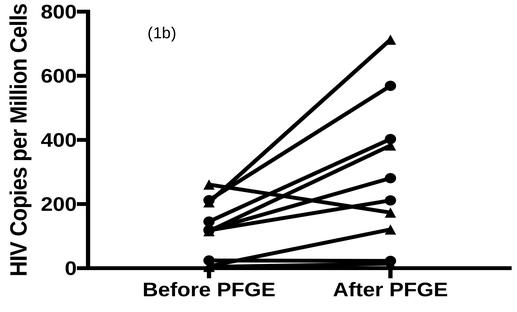


Figure 2 – HIV DNA Detection by Alu-gag qPCR

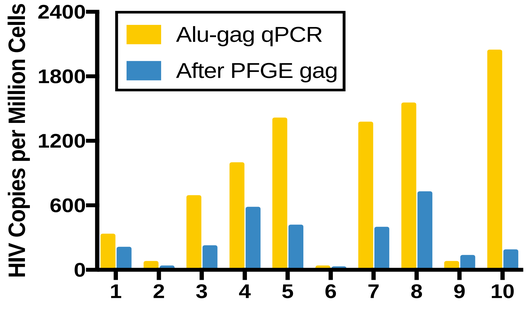
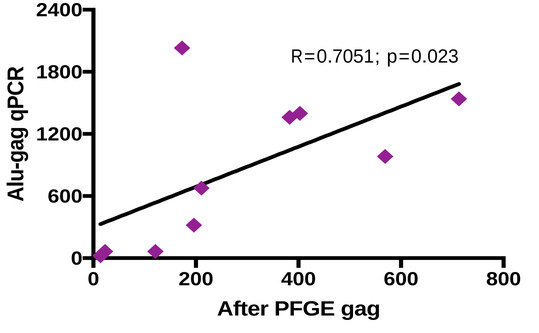


Figure 3 – Correlation of Alu-gag qPCR vs. After PFGE gag



- Before PFGE, ddPCR detected episomal HIV 2-LTR in 6/10 samples and HIV gag in all 10 ART-suppressed persons. (1a) After PFGE, HIV 2-LTR was undetectable while HIV gag was still detected in all 10 samples. (1a)
- Overall, After PFGE has increased sensitivity of detection of HIV gag with statistical significance as determined by paired t test. [p=0.0213] (1a) 2 samples showed decreased detection of HIV gag with After PFGE protocol. (1b)
- Cellular DNA recovery after PFGE as measured by RPP30, varied between 9-42% with an average of 21% across samples tested.
- Levels of integrated HIV DNA measured by Alu-gag qPCR (2) and After PFGE gag proviral assays after unblinding were highly correlated by Spearman's rho. [R=0.7051; p=0.023] (3)

Conclusions

- The After PFGE gag Integrated HIV DNA Assay removed episomal and linear species of HIV DNA to enable detection of provirus efficiently with as little as 5µg (~760,000 cells) from HIV-infected persons receiving suppressive ART.
- These results were comparable to the standard Alu-gag qPCR proviral assay, but the After PFGE gag assay required fewer cells (over 10 times less cells) and was less technically difficult to perform.
- Thus, the combination PFGE and ddPCR assay provides a sensitive and precise approach to the measurement of integrated HIV DNA with sufficient throughput for translational research cure studies measuring the circulating HIV reservoir.
- More clinical validation studies are in progress.

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