Quantitative HIV-2 DNA Droplet Digital PCR: A new tool to study HIV-2 infection dynamics and treatment response

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Introduction

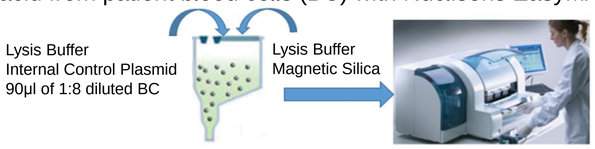
- Human Immunodeficiency Virus Type 2 (HIV-2) originated in West Africa and has spread to the US, particularly NYC.
- Like HIV-1, HIV-2 can progress to AIDS.
- Since HIV-2 RNA levels in patients are generally low, a negative HIV-2 RNA result does not exclude HIV-2 infection.
- HIV-2, a retrovirus, uses reverse transcriptase to reverse transcribe RNA into double stranded DNA. Some DNA enters the nucleus and integrates into the host's genome. This DNA could be used as a marker to improve diagnosis and monitor a patient's response to treatment.
- Droplet digital PCR (ddPCR) is a powerful tool used to achieve absolute quantification with a high level of precision and single-molecule sensitivity. It does not require a standard curve.

Objective

Develop a quantitative HIV-2 DNA droplet digital PCR (ddPCR) assay and determine if HIV-2 RNA & DNA levels are correlated in HIV-2 infected patients.

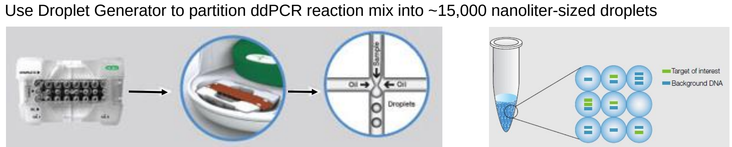
Methods

1. Extract nucleic acid from patient blood cells (BC) with Nuclisens EasyMAG

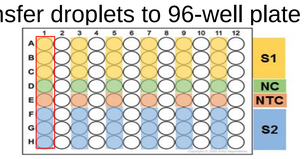


2. Prepare mastermix and make droplets
   - Bio-Rad ddPCR Supermix for Probes
   - Primers for HIV-2 and Internal Control (IC)
   - TaqMan probes (FAM for HIV-2, HEX for IC)
   - Sample DNA Extract

Use Droplet Generator to partition ddPCR reaction mix into ~15,000 nanoliter-sized droplets

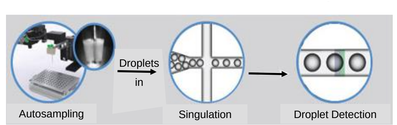


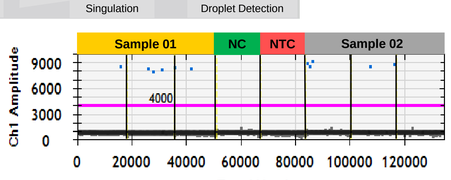
3. Transfer droplets to 96-well plate and amplify DNA by PCR



PCR DNA within droplets

4. Read fluorescence of each droplet and analyze results





$$\rightarrow \frac{\#\ \text{positive}}{\text{total}} \rightarrow \frac{\text{cps}}{\mu\text{l}}$$

Results: Blood Cells Spiked with HIV-2 Plasmids

Sensitivity for Group A and Group B HIV-2

Strain	Expected Cps/90ul	# Positive	# Tested	% Positive
Grp A (A1958)	500	6	6	100%
	250	6	6	100%
	125	6	6	100%
Grp B (310319)	500	6	6	100%
	250	6	7	86%
	125	5	6	83%

Table 1- Blood cells spiked with HIV-2 plasmids from Group A and B viruses. Limit of detection is 125 cps/90ul for Group A and 500 cps/90ul for Group B

Linearity

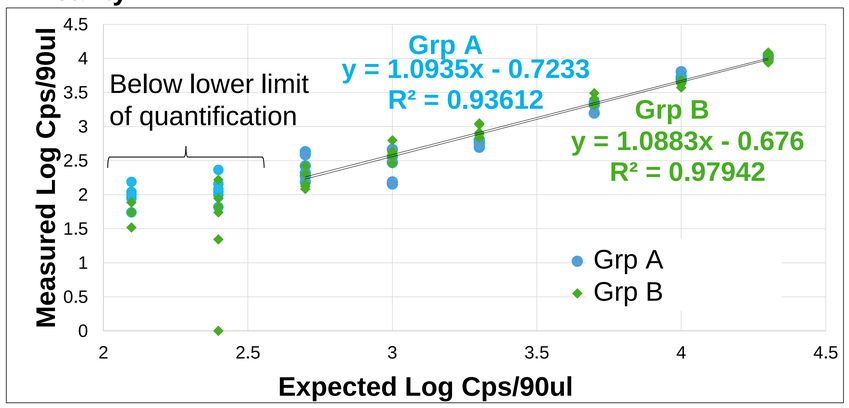


Figure 1- Blood cells spiked with HIV-2 plasmids from Group A (A1958) and Group B (310319) viruses. The assay produced linear quantitative results, with coefficients of determination (R^2) and slopes were very close to 1.

Intra-Assay (Within Run) Reproducibility

Strain	Expected Cps/ 90ul	Rep 1	Rep 2	Rep 3	Ave	%CV
Grp A	10,000	5390	5060	4950	5133	5
	2000	583	627	638	616	5
	500	209	264	187	220	18
Grp B	10,000	4510	4840	4180	4510	7
	2000	814	770	682	755	9
	500	500	198	143	264	30

Inter-Assay (Between Run) Reproducibility

Strain	Expected Cps/ 90ul	Run 1	Run 2	Run 3	Ave	%CV
Grp A	10,000	6380	5060	4620	5353	17
	2000	495	627	528	550	13
	500	209	429	385	341	34
Grp B	10,000	4510	4290	3740	4180	10
	2000	1111	1067	770	983	19
	500	121	132	198	150	28

Tables 2 and 3- Blood cells spiked with Group A and Group B plasmids. The assay produced similar quantitative results when tested within the same run or between runs, with coefficients of variation (%CV) below 35%.

Results: Patient Blood Cells

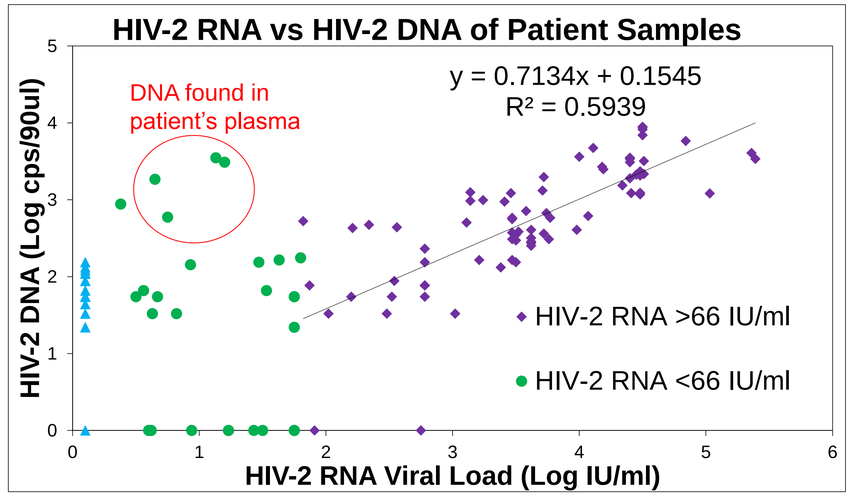


Figure 2- HIV-2 DNA and previously quantified HIV-2 RNA levels have a positive relationship in HIV-2 infected patients. For samples with RNA values >66 International Units (IU)/ml, the R^2 value is 0.594 and the slope is 0.713. For 10/12 samples with undetected RNA, DNA was detected.

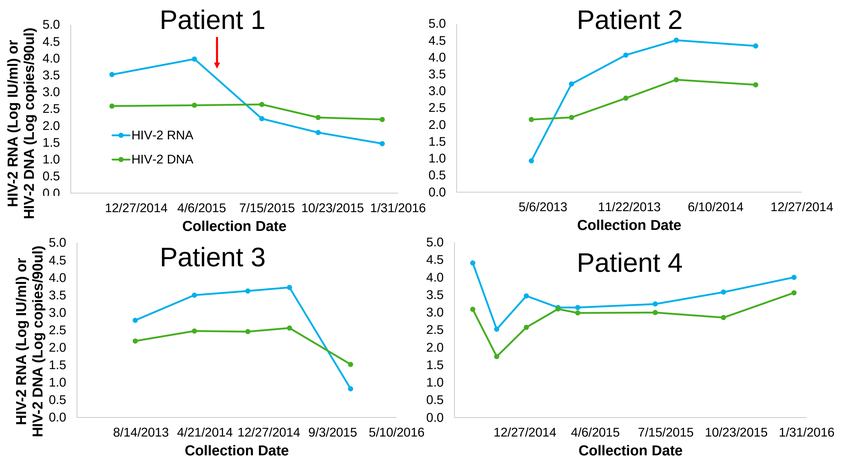


Figure 3- In four HIV-2 infected patients, HIV-2 DNA and RNA levels are correlated. In Patient 1, DNA levels decreased ~3 months after RNA. Red arrow indicates known treatment start date.

Conclusions

The quantitative HIV-2 DNA ddPCR assay is sensitive, linear, and reproducible. Accurate quantification of HIV-2 DNA will provide a second measure of treatment response and will improve our understanding of HIV-2 infection dynamics.

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