



Department
of Health

Wadsworth
Center

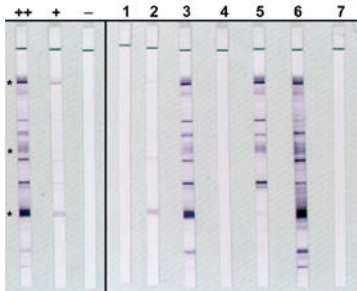
Sensitive detection and accurate quantification of HIV-2 DNA using droplet digital PCR

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Although HIV supplemental tests are becoming easier to perform, results are more complex



HIV-1 Western blot

Positive

Indeterminate

Negative



Multispot

HIV-1 Positive

HIV-2 Positive

HIV Pos (Undiff)

HIV-1 Indeterminate

Negative



Genieus

HIV-1 Positive

HIV-2 Positive

HIV-2 Positive w/ HIV-1 CR

HIV Pos (Untypable)

HIV-1 Indeterminate

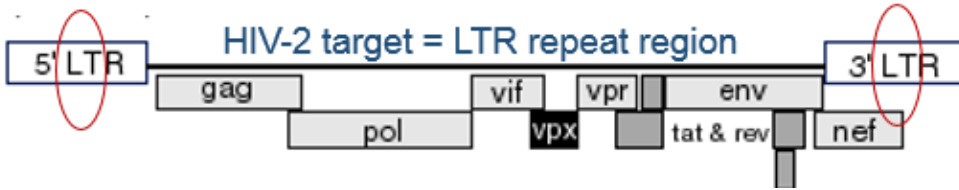
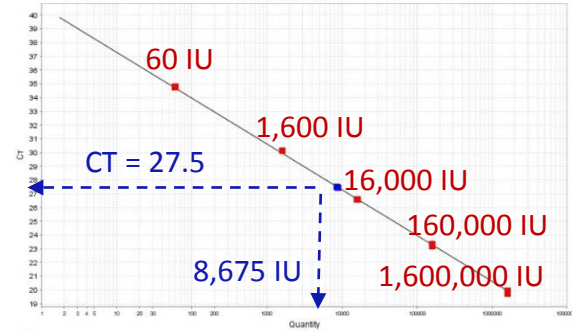
HIV-2 Indeterminate

HIV Indeterminate

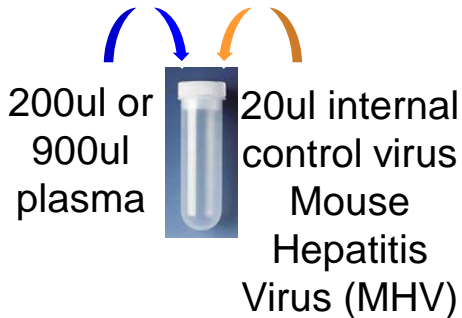
Negative

Requires NAT

Our lab developed qualitative and quantitative HIV-2 RNA Real Time PCR assays



LYSIS



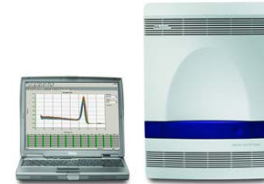
EXTRACTION



REVERSE TRANSCRIPTION



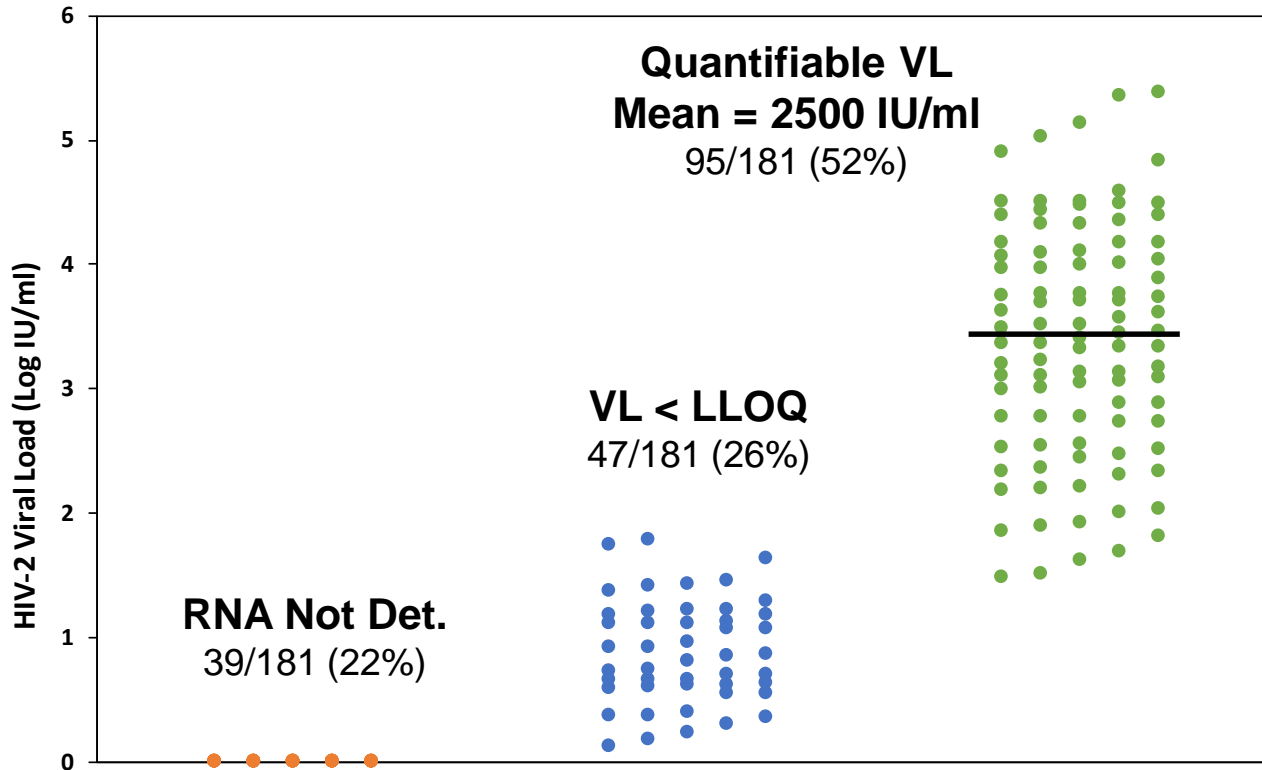
DUPLEX REAL TIME PCR



HIV-2

MHV

We have tested 181 samples from 59 HIV-2 Ab positive patients on the HIV-2 viral load assay



Detected HIV-2 RNA in ≥ 1 sample from 50/59 (85%)

Problem:
HIV-2 RNA not detected in 9/59 (15%)

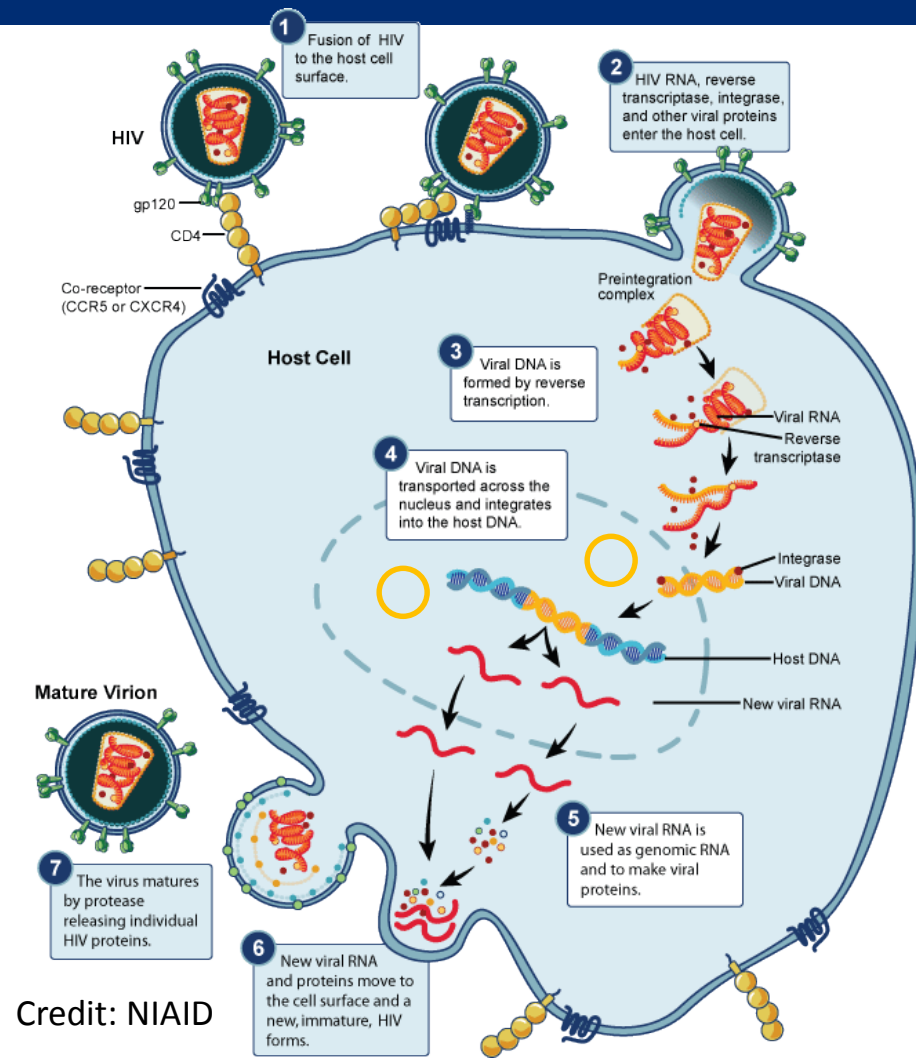
GOAL: Assay to detect HIV-2 DNA

DNA provides better diagnostic target

- More abundant marker of infection
- Detectable in treated patients

Detect all forms of HIV-2 DNA

- Linear cDNA
- 1-LTR and 2-LTR circles
- Integrated proviral DNA



Credit: NIAID

Droplet digital PCR provides better detection of 'rare' HIV-2 DNA and absolute quantification

Real time PCR (relative quantification)



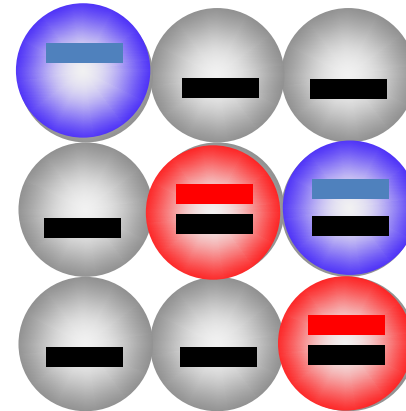
One measurement



Droplet Digital PCR (absolute quantification)



Many thousands of discrete measurements



■ Human DNA
■ HIV-2
■ Internal control

Partition sample, amplify DNA and count '+' partitions



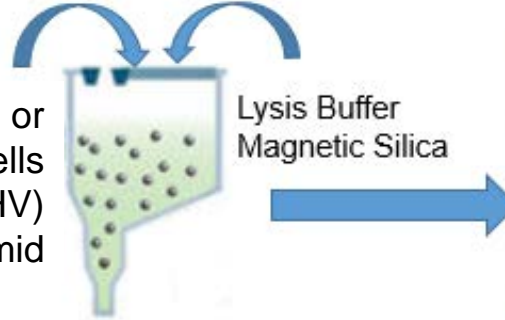
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Methods

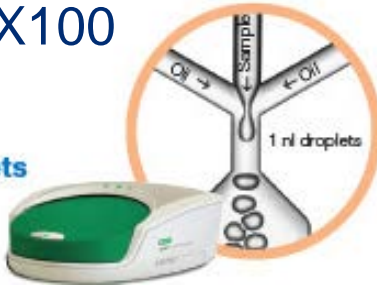
DNA Extraction: EasyMAG Specific B Protocol

200ul Whole Blood or
90ul Blood Cells
Internal Control (MHV)
DNA Plasmid



Droplet Digital PCR: Bio-Rad QX100

1 Make droplets



2 PCR DNA
in droplets



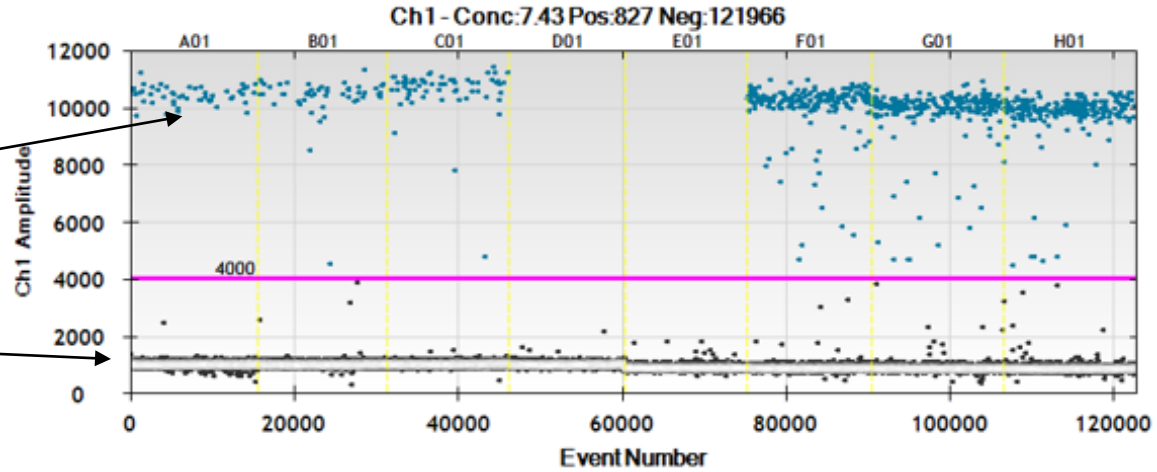
3 Read and
analyze results



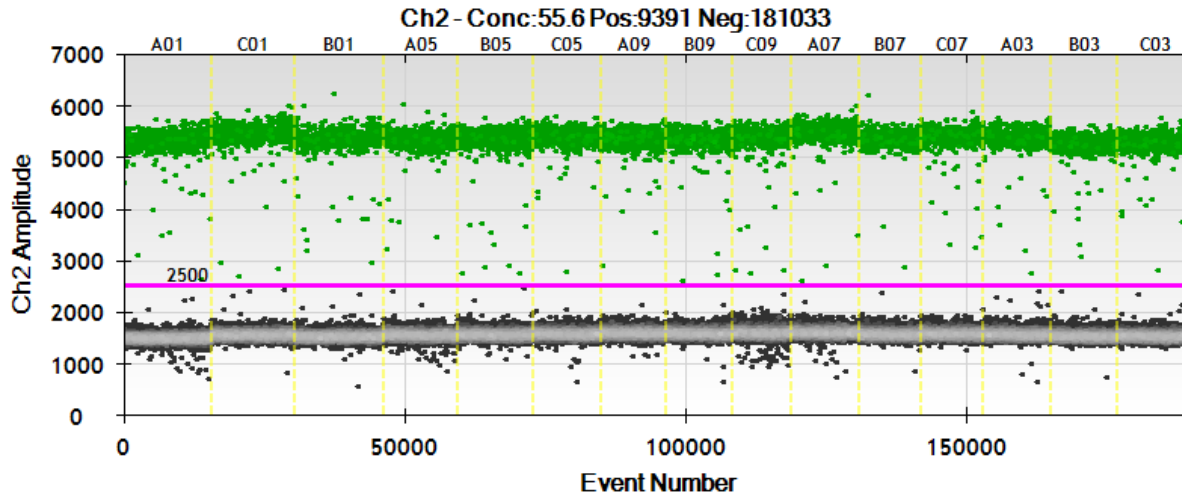
Methods

Positive droplets

Negative droplets



HIV-2

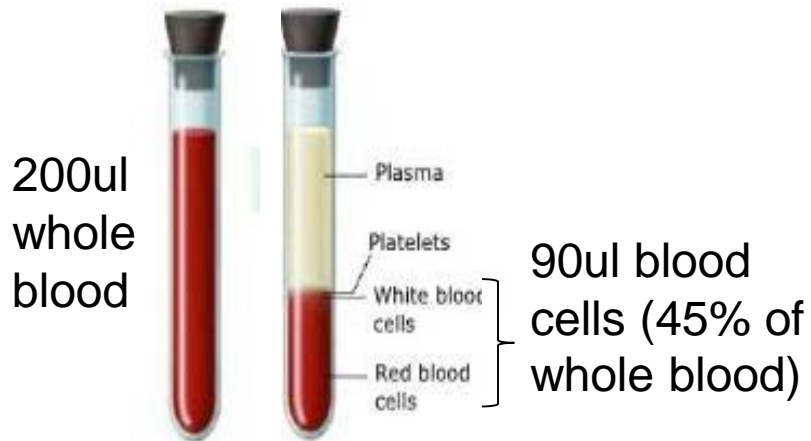


Internal Control
(MHV plasmid)

Software
calculates
copies/ul

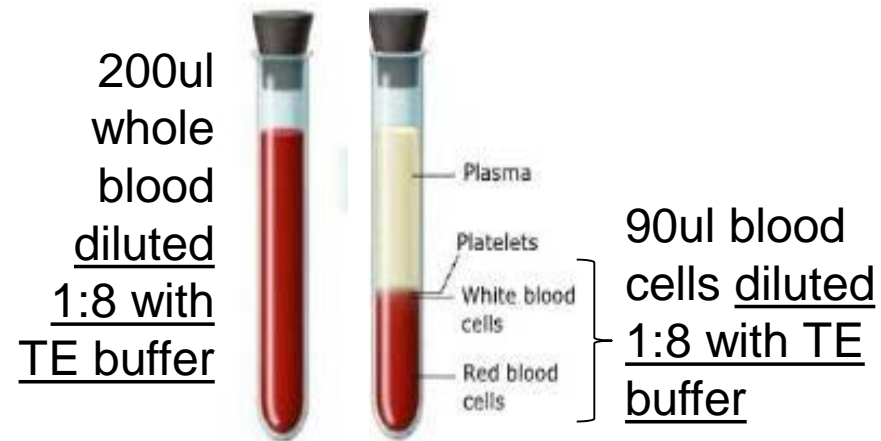
Tested whole blood and blood cells spiked with HIV-2 LTR plasmid DNA – Group A and Group B

Qualitative HIV-2 DNA ddPCR



Lower limit of detection...BUT inaccurate quantification & occasional inhibition

Quantitative HIV-2 DNA ddPCR



Accurate & reproducible quantification, no inhibition...BUT not as sensitive

Limit of Detection: **Qualitative** HIV-2 DNA ddPCR

Whole Blood

		Undiluted Whole Blood		
Expected Copies/200ul	# Positive	# Tested	% Positive	
Group A Strain	25	2	2	100
	50	3	3	100
	100	3	3	100
Group B Strain	25	2	2	100
	50	3	3	100
	100	3	3	100

Blood Cells

		Undiluted Blood Cells		
Expected Copies/90ul	# Positive	# Tested	% Positive	
Group A Strain	25	2	3	67
	50	4	4	100
	100	4	4	100
Group B Strain	25	3	3	100
	50	4	4	100
	100	4	4	100

LOD: 50 cps/200ul WB or 90ul BC

Limit of Detection: **Quantitative** HIV-2 DNA ddPCR

1:8 Diluted Whole Blood

Expected Copies/200ul	# Positive	# Tested	% Positive	
Group A Strain	125	4	7	57
	250	7	7	100
	500	6	6	100
Group B Strain	125	6	7	86
	250	7	7	100
	500	6	6	100

1:8 Diluted Blood Cells

Expected Copies/90ul	# Positive	# Tested	% Positive	
Group A Strain	125	6	6	100
	250	6	6	100
	500	6	6	100
Group B Strain	250	6	7	86
	500	6	6	100
	1000	6	6	100

LOD: 500 cps/200ul WB or 90ul BC

Accuracy: **Qualitative** HIV-2 DNA ddPCR

Whole blood and blood cells spiked with Group A and Group B plasmids

Whole Blood

		Actual		
		Positive	Negative	Total
Expected	Positive	33	0	33
	Negative	1*	17	18
Total		34	17	51

Blood Cells

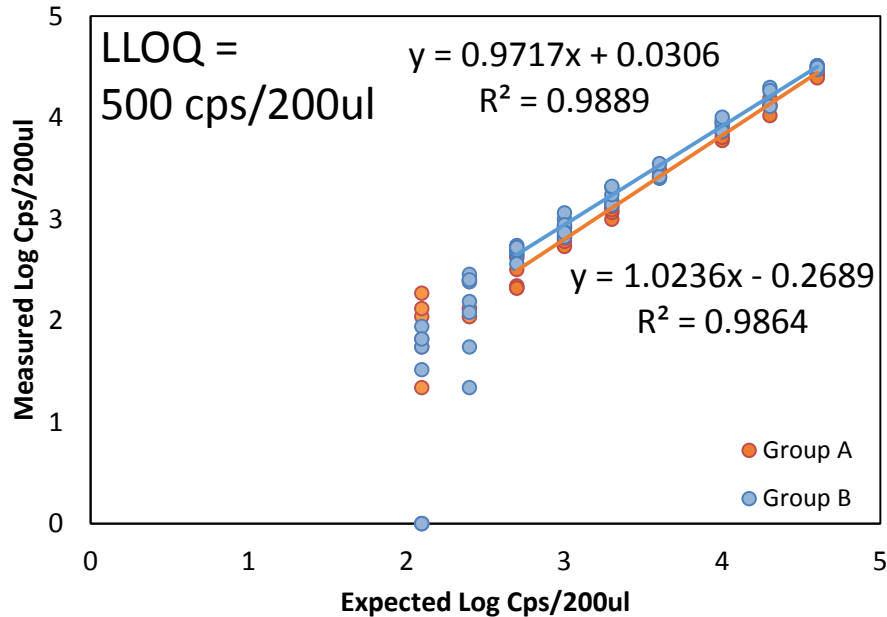
		Actual		
		Positive	Negative	Total
Expected	Positive	50	0	50
	Negative	0	10	10
Total		50	10	60

* 1 low amplitude positive drop in neg WB

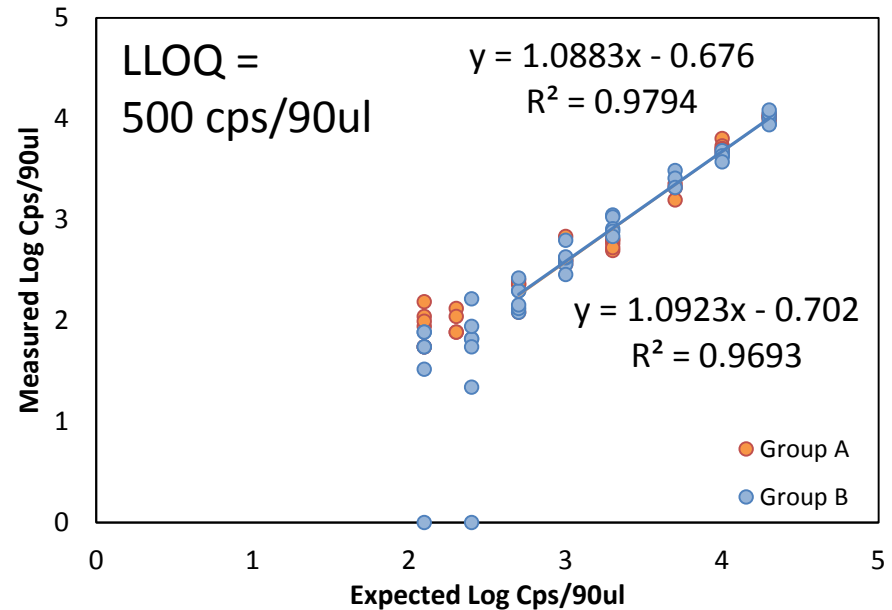
Accuracy: Quantitative HIV-2 DNA ddPCR

Whole blood and blood cells spiked with Group A and Group B plasmids

1:8 Diluted Whole Blood



1:8 Diluted Blood Cells



Reproducibility: Quantitative HIV-2 DNA ddPCR

1:8 Diluted Whole Blood

	Cps/ 200ul	Run 1	Run 2	Run 3	Ave	%CV
Grp A	500	220	319	209	249	24
	2000	1,001	1,243	1,331	1,192	14
	10,000	7,040	6,380	7,260	6,893	7
Grp B	500	451	484	363	433	15
	2000	1,518	1,386	2,057	1,654	22
	10,000	9,020	7,370	10,120	8,837	16

1:8 Diluted Blood Cells

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

HIV-2 Ab + Patient Blood Cells: Qualitative Results

NYC: Plasma for HIV-2 viral load and blood cells – paired RNA/DNA – tested undil and 1:8 dil

Undiluted Blood Cells

		HIV-2 DNA ddPCR		
		Detected	Not Detected	Total
HIV-2 RNA Viral Load	Detected	71	3	74
	Not Detected	13	0	13
Total		84	3	87

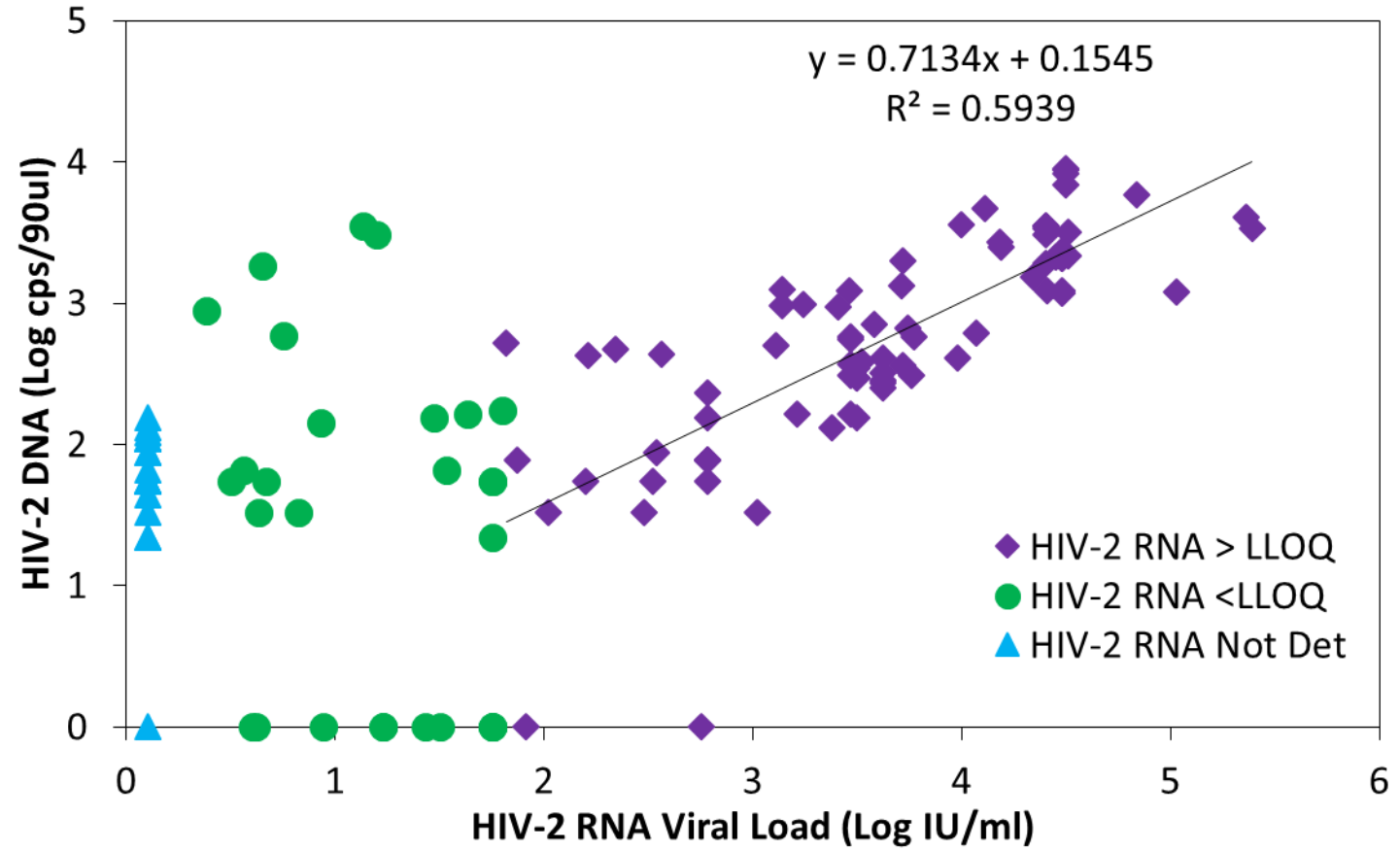
Detect DNA in 71/74 (96%) RNA pos samples
 Detect DNA in 13/13 (100%) RNA neg samples

1:8 Diluted Blood Cells

		HIV-2 DNA ddPCR		
		Detected	Not Detected	Total
HIV-2 RNA Viral Load	Detected	66	8	74
	Not Detected	12	1	13
Total		78	9	87

Detect DNA in 66/74 (89%) RNA pos samples
 Detect DNA in 12/13 (92%) RNA neg samples

HIV-2 Ab + Patient Blood Cells: Quantitative Results



The HIV-2 DNA droplet digital PCR assay is...

- **Sensitive:** LOD of 50 cps/200ul whole blood or 90ul blood cells
- **Accurate:** Qual: 50/51 correct, Quant: values linear >500 cps
- **Reproducible:** Interassay CV <35%

In blood cells from HIV-2 infected people...

- Detected HIV-2 DNA in >90% of samples without RNA
- Moderate correlation between HIV-2 RNA and DNA levels

The HIV-2 DNA ddPCR assay is a valuable diagnostic tool to detect and/or rule-out HIV-2 infection. May provide important insights into quantitative dynamics of HIV-2 RNA and DNA.

Acknowledgements

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