

Assessment and Quantification of Unspliced Cell Associated HIV-1 RNA using Reverse Transcriptase Droplet Digital PCR

ZX Hu, C Becerril, NH. Lin, JZ Li, A Heisey, AMN Tsibris
and DR. Kuritzkes

Division of Infectious Disease, Brigham and Women's Hospital, Harvard
Medical School, Boston, MA, USA



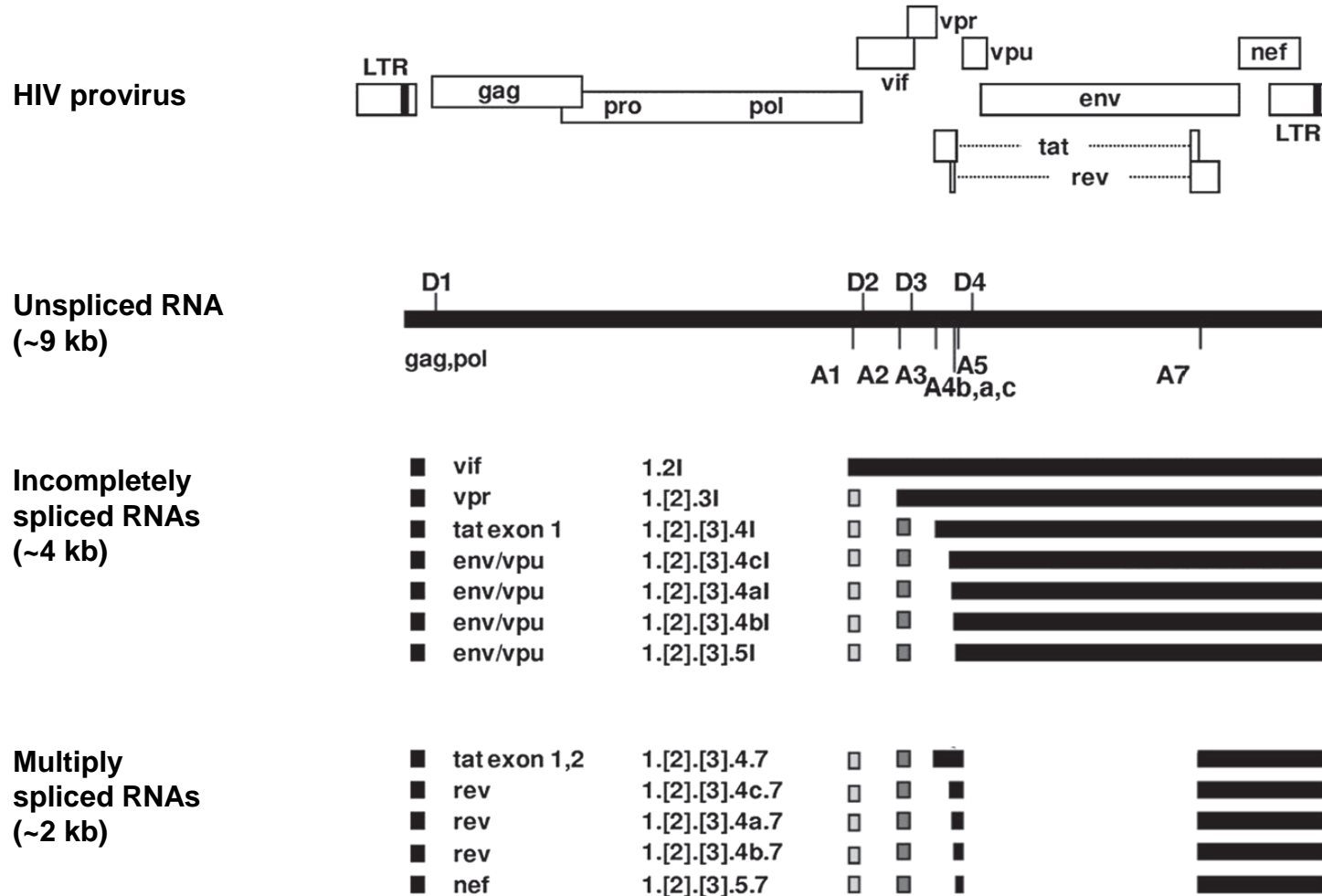
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Introduction (1)

Cell-associated HIV RNA could be a dynamic biomarker of the latent HIV infection and reservoirs



Introduction (2)

Techniques available to study cell associated HIV-1 RNA

- In situ hybridization-based assay
- Transcription-Mediated Amplification
- Real time RT-PCR
- Droplet Digital PCR (ddPCR)

- *Workflow of ddPCR:*



- *Advantages of ddPCR:*

1. Absolute quantification for DNA/RNA –(no standard curve is need)
2. Highly precise - (+/- 10% precision)
3. Likely more robust to primer/probe sequence mismatches

Introduction (3)

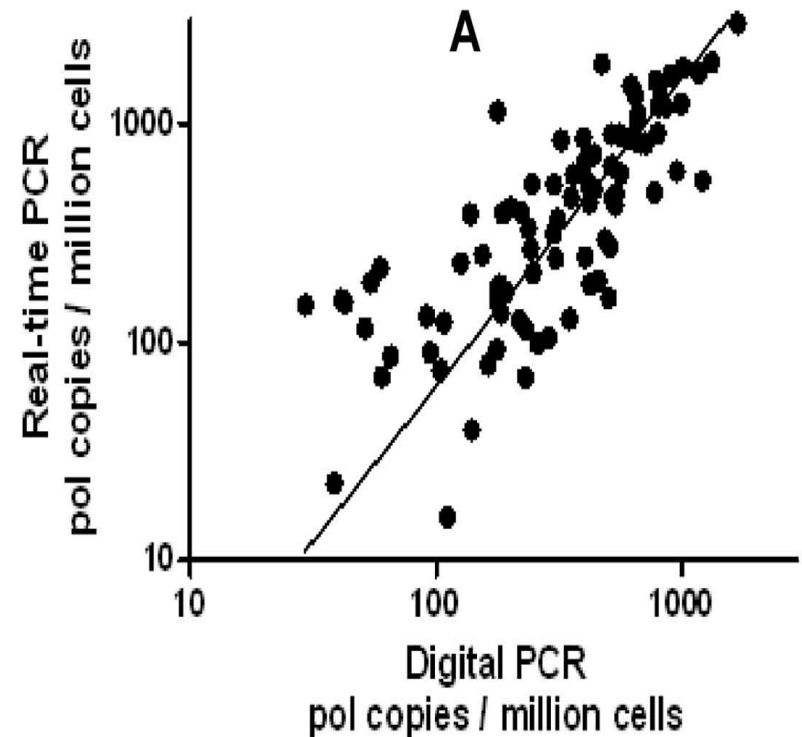
- ddPCR is comparable to real time-PCR methods for quantifying HIV-1 DNA.

-Strain *et al.*, *PLoS ONE* 2013

- ddPCR for the quantification of low-levels of plasma HIV-1 RNA using two step protocols.

- Anderson *et al.*, *CROI* 2012;

- Shen *et al.*, *J Am Chem Soc* 2011;



- One-step RT-ddPCR for the measurement of cell-associated HIV-1 RNA has not been reported.

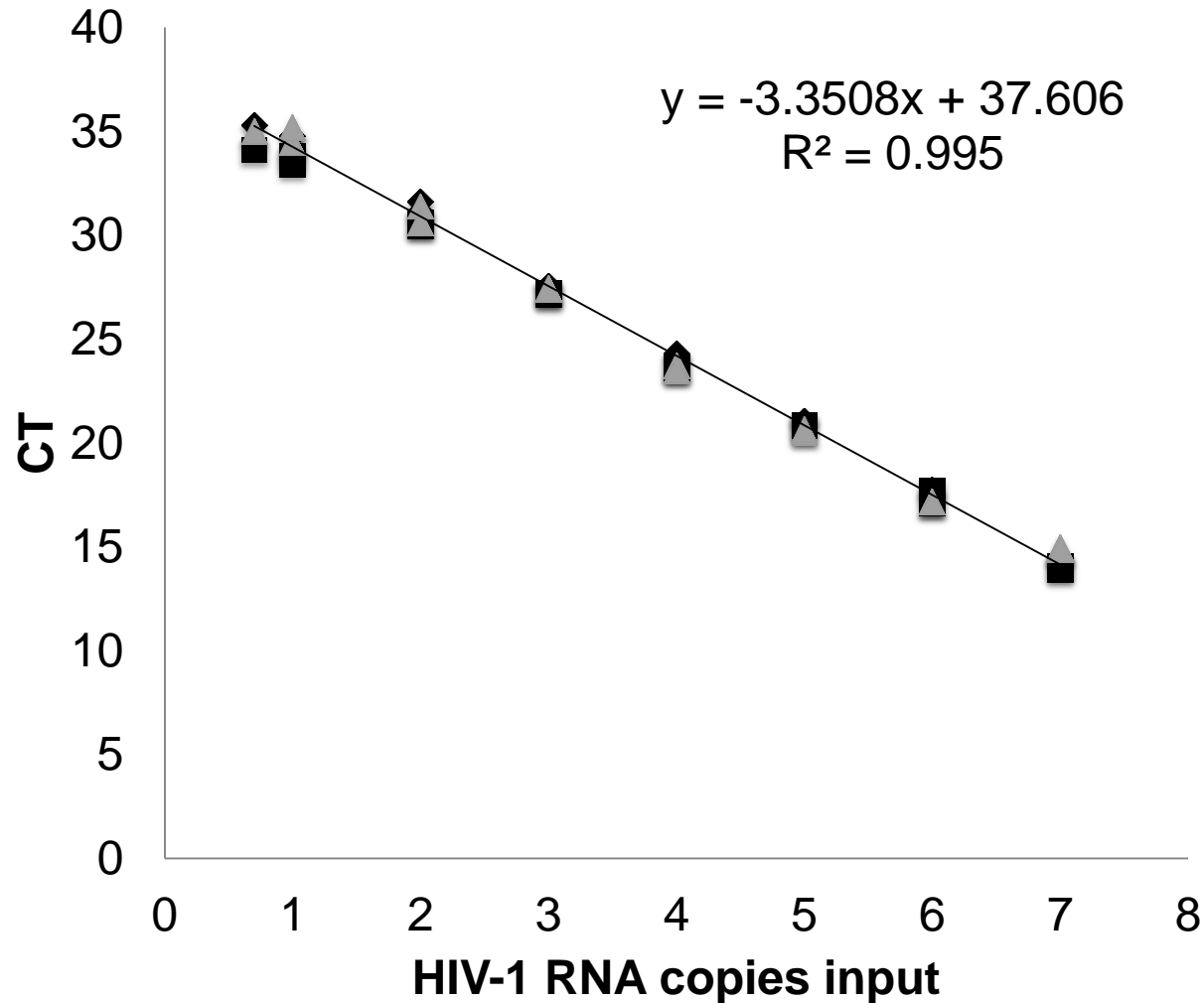
Aims and Study Design

Aims: To develop and evaluate the applicability of RT-ddPCR and compare to real time RT-PCR for measurement of cell-associated HIV-1 RNA.

Study Design:

1. Synthesizing standard-HIV-1 RNA
2. Validation of the standard-HIV RNA
3. Assessment of RT-ddPCR
 - ❖ in vitro RNA transcripts
 - Sensitivity,
 - linear range,
 - accuracy and repeatability
 - ❖ in artificially seeded (ACH2 + MT2 cell lines) samples
 - ❖ in samples from patients on suppressive ART

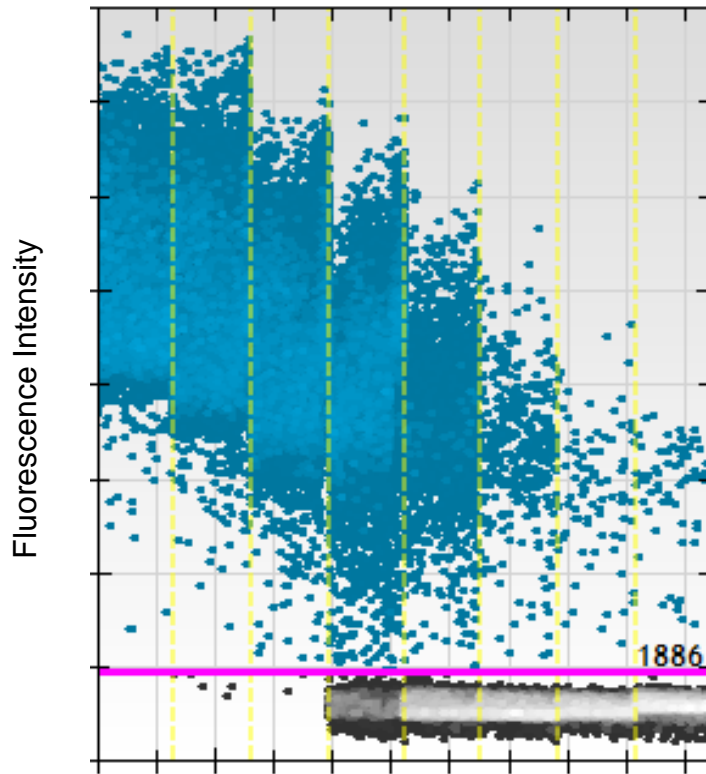
Validation of HIV-1 RNA transcripts by real time RT-PCR



HIV-1 RNA transcripts measured by RT-ddPCR

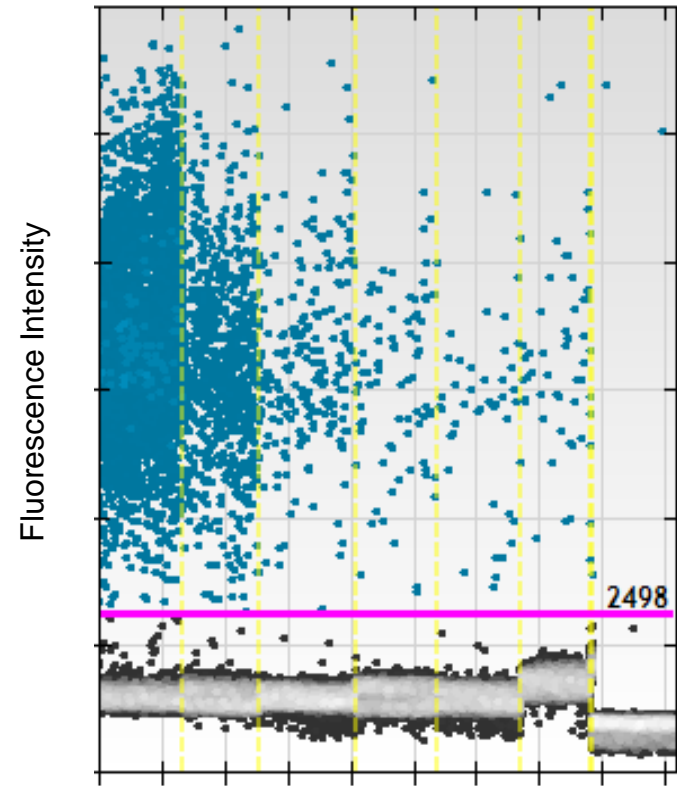
HIV RNA standards input

10⁸ 10⁷ 10⁶ 10⁵ 10⁴ 10³ 10² 10

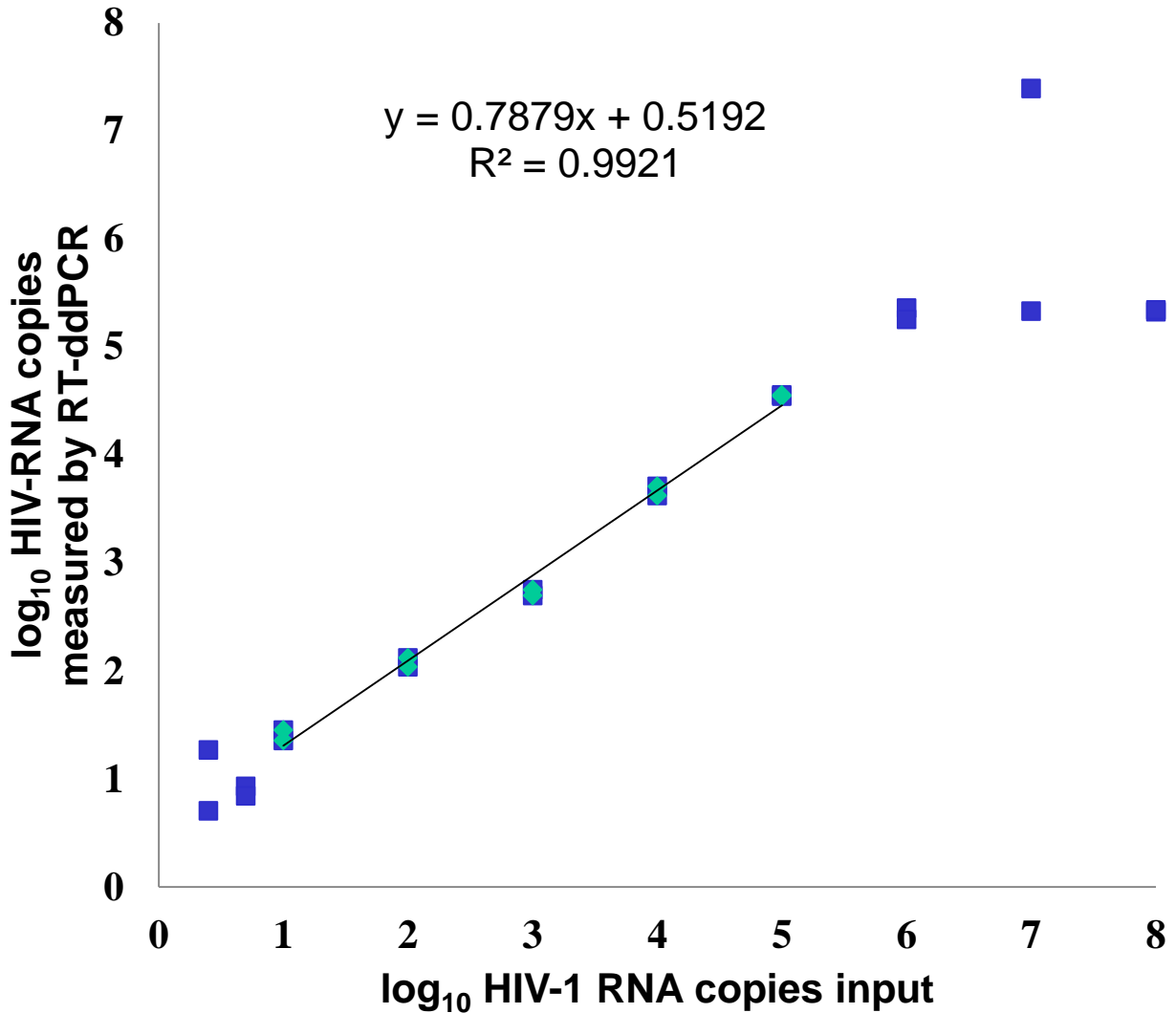


HIV RNA standards input

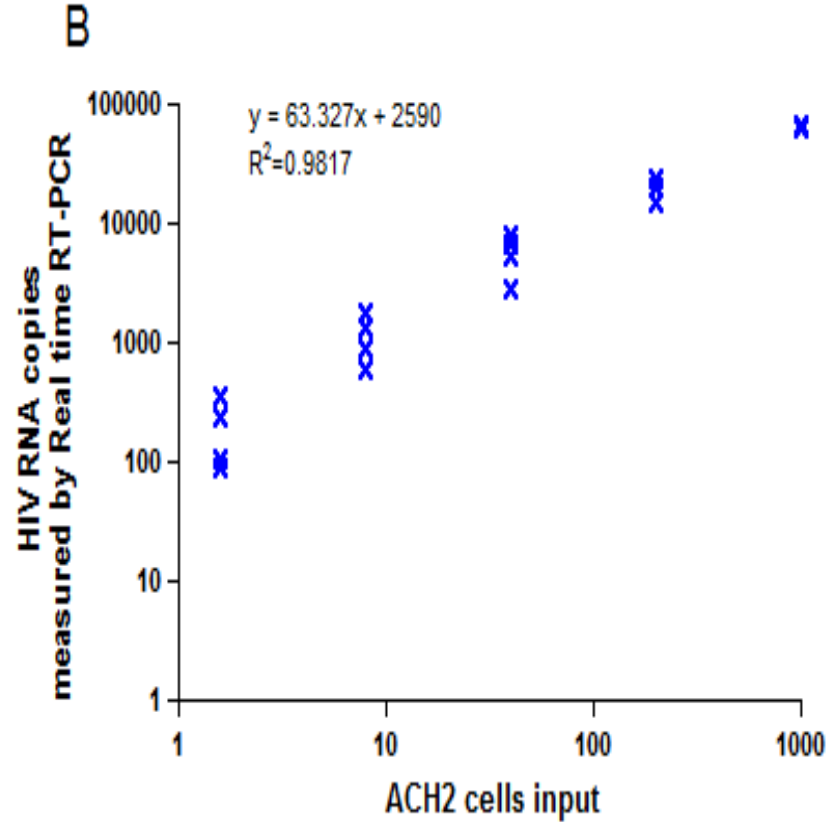
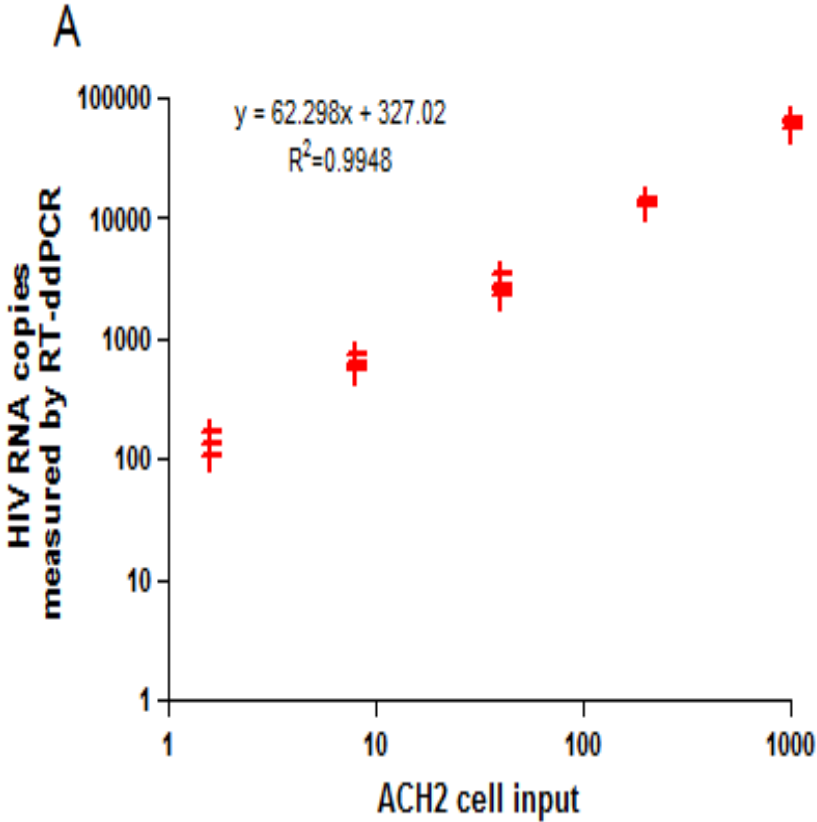
10⁴ 10³ 10² 10 5 2.5 NC



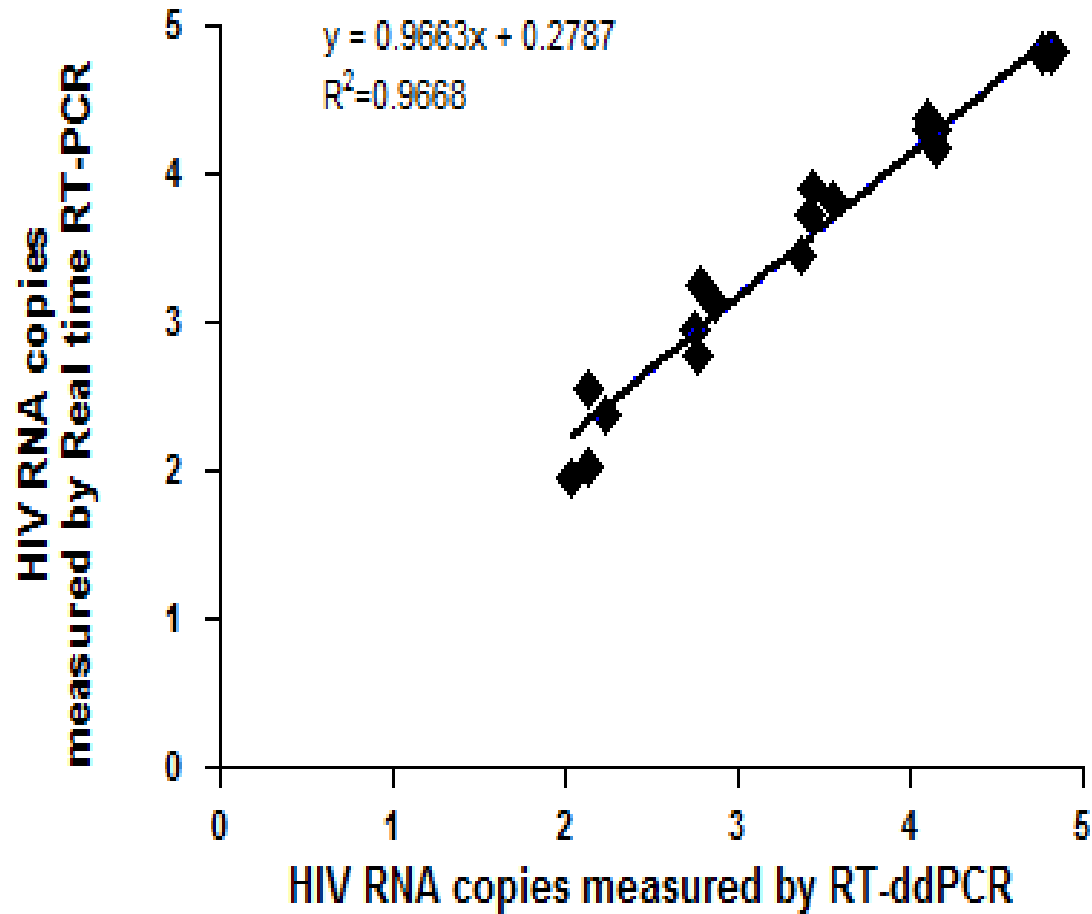
Sensitivity, dynamic range and accuracy of RT-ddPCR



Measurement of cell associated HIV-1 RNA in samples from MT2 spiked with latent HIV infected ACH2 cells by the two methods



Correlation of RT-ddPCR and real time RT-PCR



Detection and quantification of cell associate HIV-1 RNA in clinical patients' samples.

Patient-ID	HIV-1 ca-RNA detected by RT-ddPCR	HIV-1 ca-RNA detected by Real Time RT-PCR
B004	undetectable	undetectable
B011	18.0	undetectable
M302	undetectable	undetectable
M305	135.9	116.9
M308	86.6	68.9
M314	undetectable	undetectable
M316	151.7	108.2
M318	20.5	52.1
M321	undetectable	undetectable
M327	80.9	112.3
M332	undetectable	undetectable
M330	74.1	57.5
M329	5.7	undetectable
M328	83.0	99.9
M326	66.9	112.9
M313	undetectable	undetectable
M309	3.8	5.5
M301	23.9	8.7
B013	34.8	65.9
W102	283.9	479.3
20 samples	+ 14/20 4- 284 copies/million PBMC	+ 12/20 6- 479 copies/million PBMC

Conclusion

- The RT-ddPCR assay detected and accurately quantified cell associated HIV-1 RNA with a dynamic range down to 10 copies per reaction.
- RT-ddPCR demonstrated similar sensitivity and accuracy compared to real-time RT-PCR in the measurement of cell-associated HIV-1 RNA in both cell-lines spiked with HIV-infected cells and in patient samples.
- RT-ddPCR is a promising tool for the quantification of cell-associated-RNA in the latent HIV infection and reservoirs.
- Further optimization needed to improve the precise for quantification of samples with <10 RNA copies.