

A SENSITIVE CROSS-CLADE REAL-TIME QUANTITATIVE PCR ASSAY TO MEASURE TOTAL HIV-1 DNA IN INFECTED PATIENTS

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BACKGROUND

Current combination antiretroviral therapy (cART) is capable of reducing HIV-1 plasma viral load to undetectable level, however, HIV-1 proviral DNAs in infected CD4+ cells remain as latent reservoirs. Recent studies have shown that total HIV-1 DNA in infected patients is a useful marker for reservoir size to identify potential post-treatment controllers (PTCs). Low levels of HIV-1 DNA in patients under suppressive ART and high genetic variation challenge the current HIV-1 DNA quantification methods. In this study, we compared the performance of an ultrasensitive qPCR method (Ultrabio assay) to a commonly used laboratory qPCR method (GAG PCR) on both cell-line and patient PBMC samples and evaluated its ability to quantify HIV-1 of different subtypes.

MATERIALS & METHODS

The Ultrabio HIV-1 DNA Quantitative Assay:

- A multiplex real-time PCR simultaneously measuring the HIV-1 long-terminal repeat (LTR) region and human beta-actin gene.
 - Recombinant DNA, serving as quantitative standards, included to report HIV-1 DNA copies/million cells.
 - DNA extraction: Qiagen QiaAmp DNA Mini kit; qPCR: ABI QuantStudio 5.
- ### GAG Real-Time PCR Assay [1]:
- Targeting gag region; cell number estimated by Nanodrop; 8E5/LAV DNA were used to generate standard curves.

Sample list:

- Comparison of sensitivity: 8E5/LAV cells (1 proviral DNA copy/cell).
- EQAPOL Viral Diversity panel, which contained 47 strains of Group M subtypes A, B, C, D, F, G, URF_A1B, URF_BC, BF, CRF01_AE, CRF02_AG, CRF04_CPX, CRF14_BG, CRF24_BG, CRF47_BF, AD and 3 strains of Group O. Viral RNA from the panel was extracted from viral stocks with Qiagen MiniElute Virus Spin Kit using QiaCube and converted to cDNA by SuperScript IV before testing by the Ultrabio Assay. The nominal concentration was 10,000 copies/mL. An RNA (subtype B) control from Virology Quality Assurance (VQA) with the same nominal concentration was extracted and analyzed simultaneously as a positive control.
- Frozen PBMCs of HIV-1 infected individuals were obtained from the Seattle Primary Infection Program (SEAPIP, PIC) and were tested in duplicate.

Statistical analysis:

- PRISM Graphpad v8 was used to calculate correlation.
- R statistical software 3.1 was used to conduct the probit analysis.

RESULTS

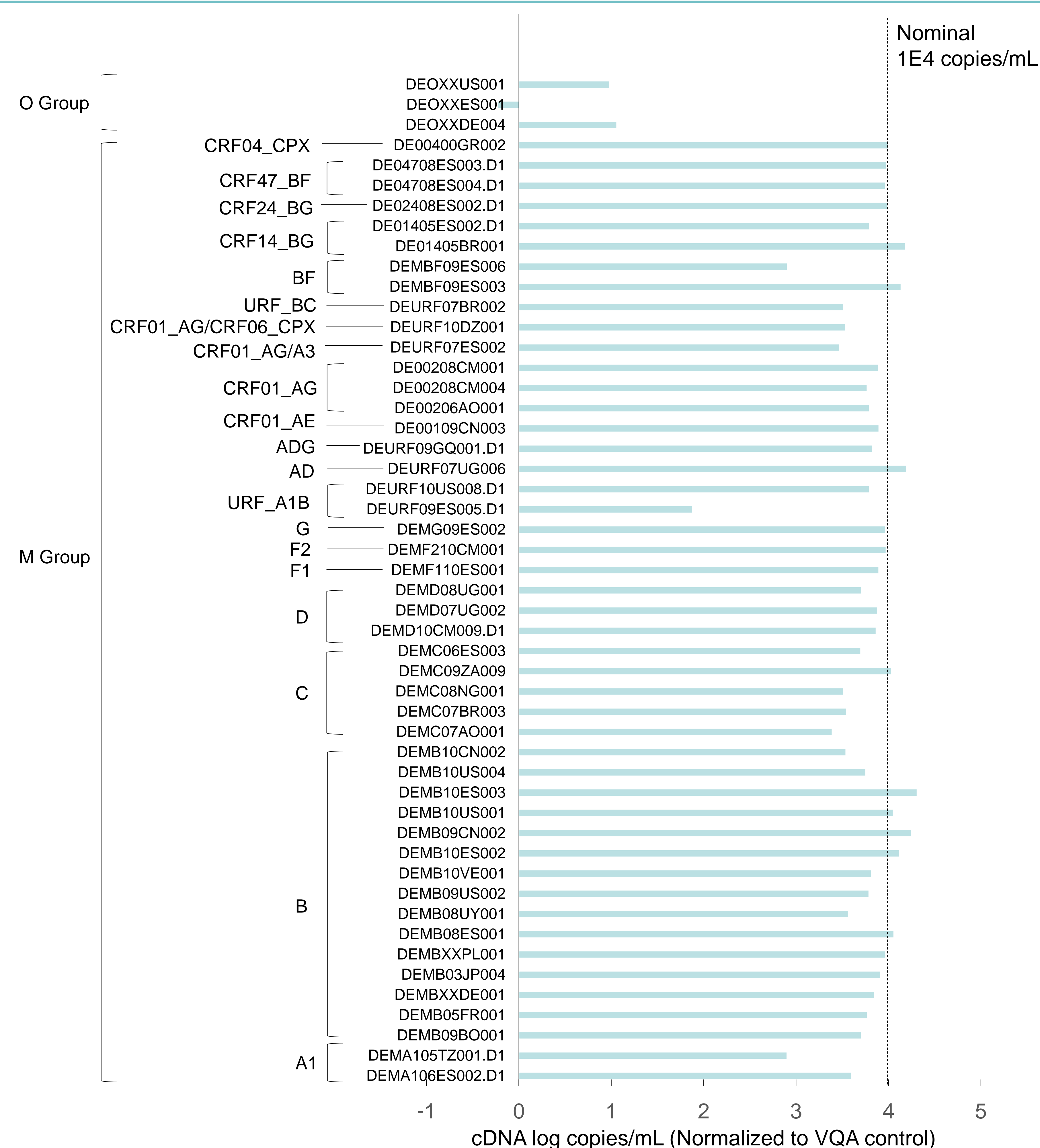
Limit of Detection (LOD) Probit analysis:

Ultrabio Assay: 4 copies /reaction; GAG PCR: 25 copies/reaction

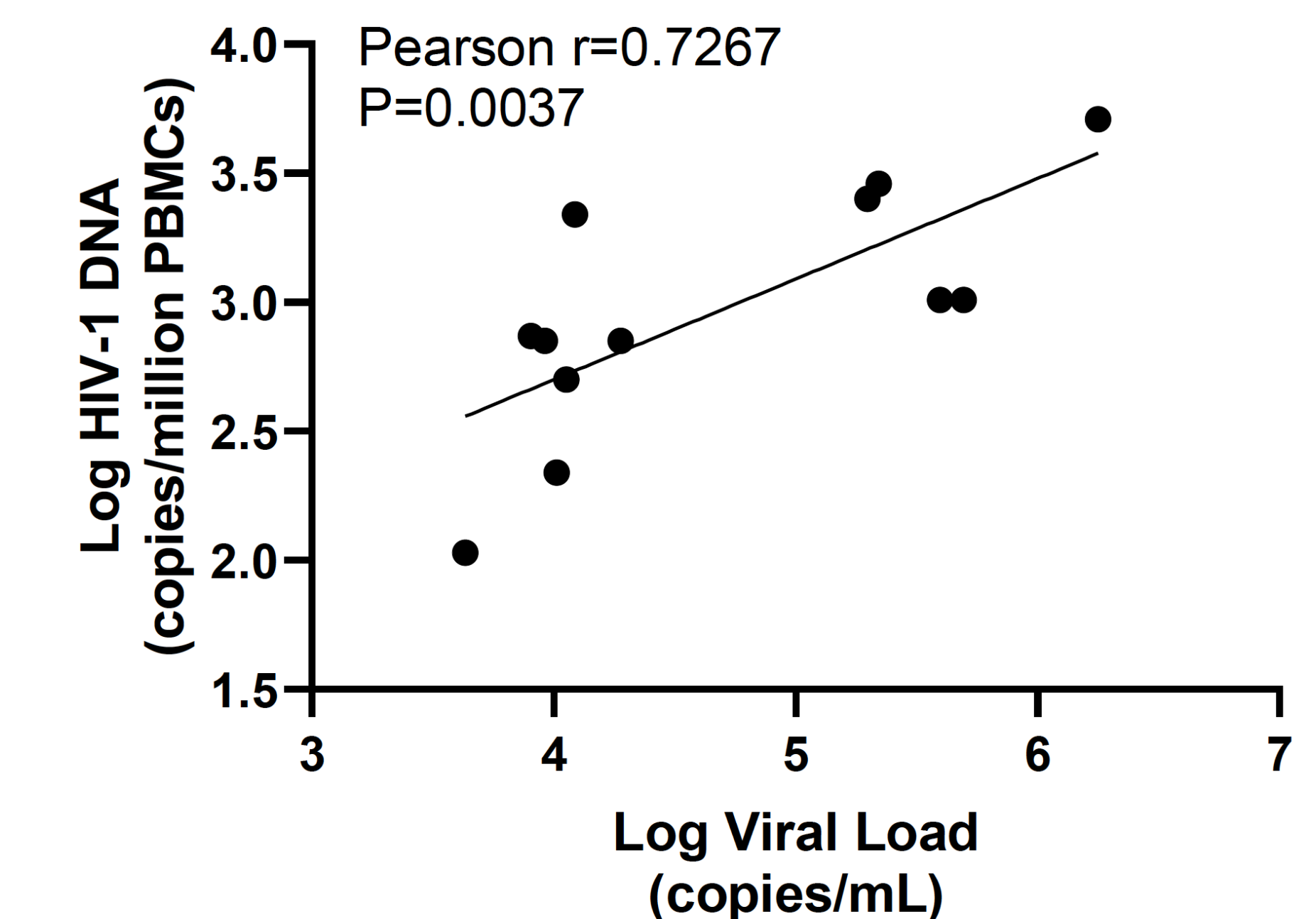
Higher precision of Ultrabio Assay at 20 copies/reaction

Nominal HIV-1 DNA copies / rxn	Ultrabio Assay			GAG PCR		
	N	Mean (SD)	% positivity	N	Mean (SD)	% positivity
1	36	1 (1)	52.78	36	1 (2)	19.44
2	36	2 (1)	80.56	36	3 (5)	36.11
3	32	2 (1)	93.75	18	3 (4)	61.11
4	32	3 (2)	96.88	18	3 (3)	66.67
5	36	3 (2)	97.22	24	2 (4)	41.67
7	36	5 (3)	100	18	4 (3)	72.22
8	12	5 (3)	100	36	5 (4)	75
10	36	8 (4)	100	30	7 (4)	90
	N	Mean (SD)	CV%	N	Mean (SD)	CV%
20	36	20 (6)	29.82	30	24 (10)	42.04
100	8	129 (15)	11.42	8	121 (15)	12.29
1000	8	1288 (101)	7.86	8	1280 (77)	6.02
10000	8	12088 (1740)	14.4	8	13783 (1891)	13.72

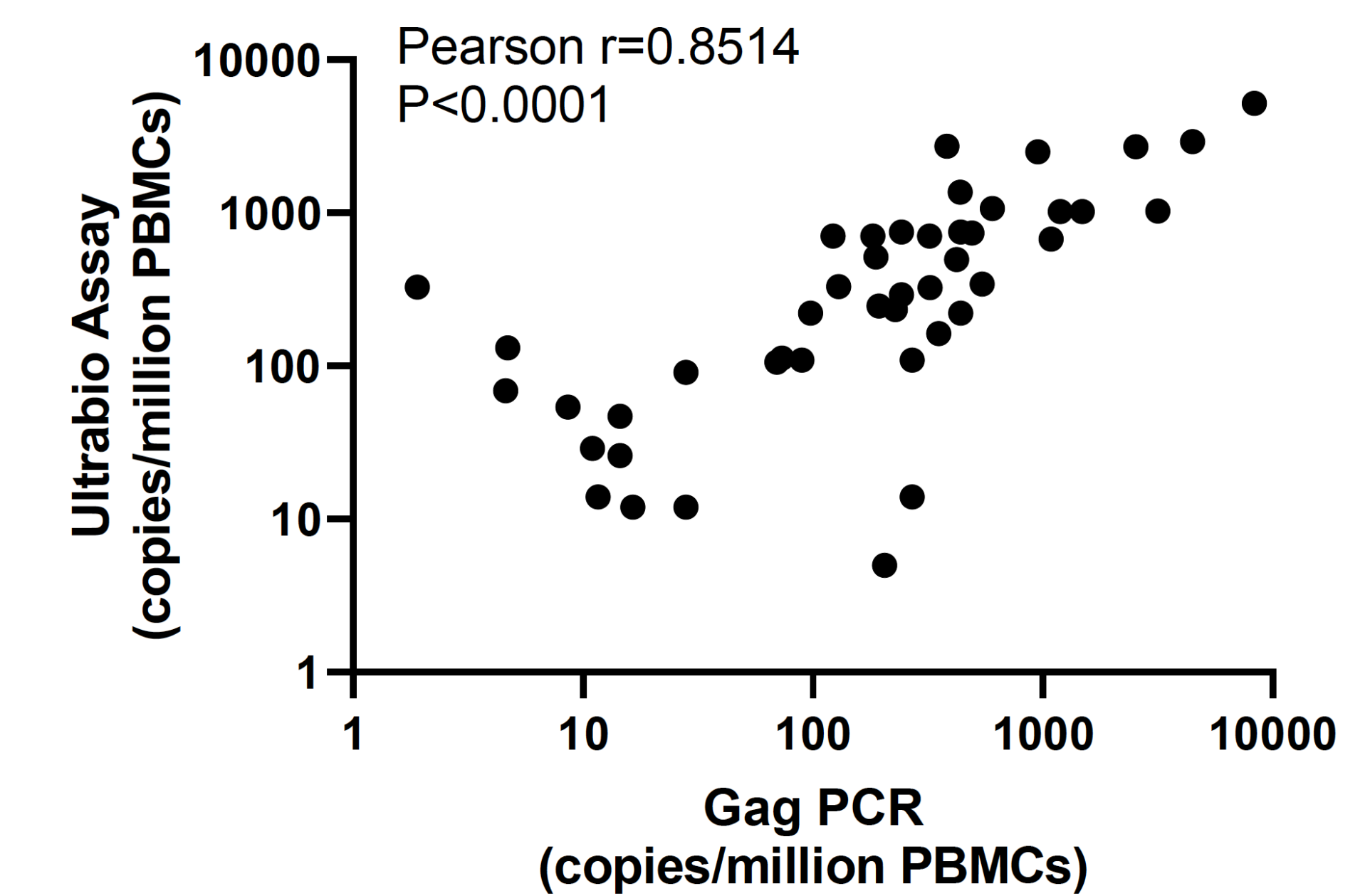
Quantification and Detection of EQAPOL Diversity Panel



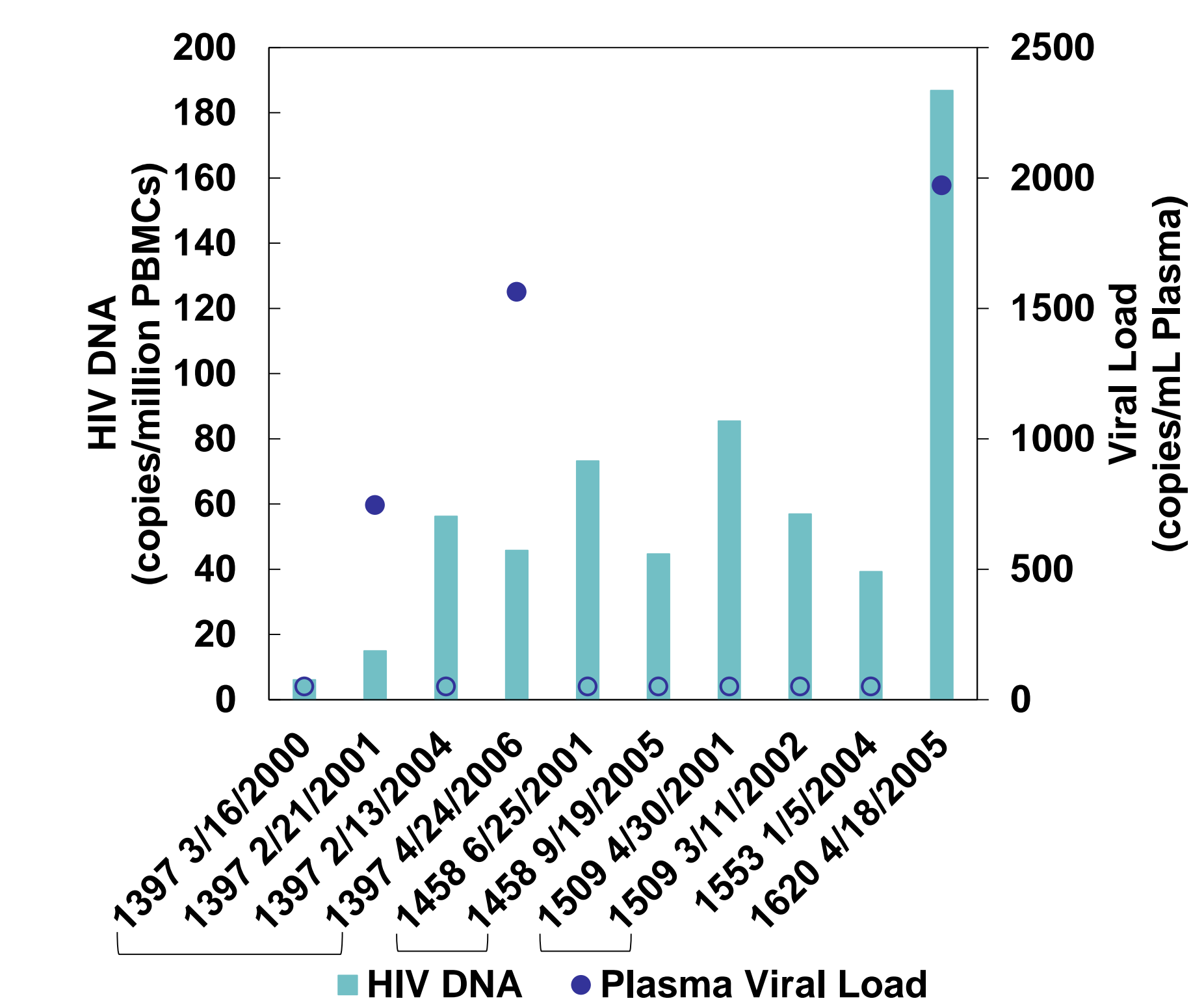
Quantification of HIV-1 DNA in patient PBMCs



Positive correlation of HIV-1 DNA copy numbers in PBMCs and plasma viral load in treatment-naive viremic patients



Correlation of Ultrabio assay and GAG PCR; Ultrabio assay detected 54/54 (100%) samples while GAG PCR detected 50/54 (93%) samples.



The range of HIV DNA level in five non-progressors (maintained VL<2000 and CD4>500 without ART for at least 1.5 years) quantified by Ultrabio assay was 6-187 copies/million cells.

CONCLUSIONS

- Compared to the GAG PCR, the Ultrabio assay showed better sensitivity and precision for low levels of HIV-1 DNA present in 8E5/LAV cells as well as gave higher positivity rate for infected PBMCs samples.
- The Ultrabio assay was able to quantify low levels of HIV-1 DNA in PBMCs
- The Ultrabio assay was able to cover a wide range of subtypes in Group M.

[1] Coombs, R.W., Lockhart, D., Ross, S.O., Deutsch, L., Dragavon, J., Diem, K., Hooton, T.M., Collier, A.C., Corey, L. and Krieger, J.N., 2006. Lower genitourinary tract sources of seminal HIV. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 41(4), pp.430-438.