

A sensitive HIV-1 DNA quantitative PCR assay for measuring the residual HIV-1 reservoir

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BACKGROUND

Sensitive and feasible methods for measuring HIV-1 reservoirs are critical to evaluate interventions for HIV-1 eradication. Though total HIV-1 DNA measurements overestimate latent reservoir size, recent studies suggest that HIV DNA reflects the size of replication-competent virus and is predictive of disease progression, response to ART, and viral rebound. Thus, HIV DNA is a valuable biomarker for HIV reservoirs (1,2).

AIM

Evaluate the performance – sensitivity, linearity, precision, accuracy and specificity – of the **UltraQ HIV-1 DNA quantitative assay**.

MATERIALS & METHODS

The UltraQ HIV-1 DNA quantitative assay:

- A multiplex PCR measuring the HIV-1 long-terminal repeat region and human beta-actin gene.
- Recombinant DNA, serving as quantitative standards, included to report HIV-1 DNA copies/10⁶ cells.
- In this study, Qiagen QiaAmp DNA blood kit and QuantStudio 5 were used to isolate DNA and perform PCR, respectively.

Sample list:

- Varied amounts of DNA extracted from 8E5/LAV cells, a single proviral DNA copy/cell, were spiked to ~1.67 µg human PBMC DNA for each PCR reaction to evaluate assay's limit of detection, linearity and precision.
- Assay specificity included viral stocks of the M group subtypes A to G and O group, HIV-2 (ROD, EHO), HCV, HBV and *Plasmodium falciparum* strain 3D7. Viral RNA or total RNA was extracted from viral stocks or *Plasmodium falciparum* culture, respectively, and converted to cDNA before testing by the Assay.
- Accuracy was evaluated by quantifying DNA in two panels with varied U1 cells/million cells by Virology Quality Assurance. **Two copies HIV-1 DNA/ U1 cell**.
- Peripheral blood of viral load suppressed patients (plasma RNA < 40 copies/mL) were tested in duplicate.

Statistical analysis:

- Microsoft Excel was used to calculate mean, SD, %CV and correlation.
- R statistical software 3.1 was used to conduct the probit analysis.

RESULTS

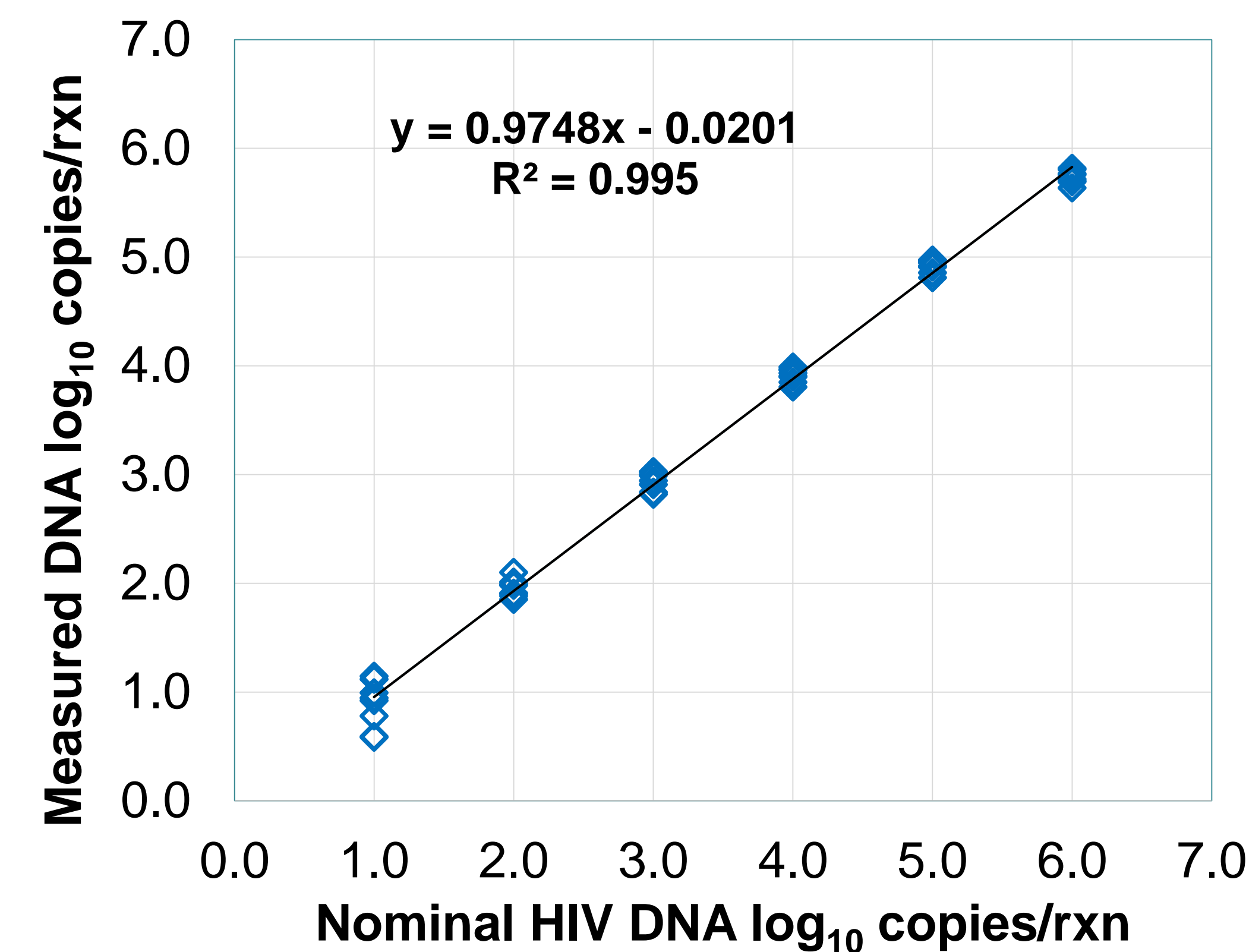
Limit of Detection (LOD)

Probit analysis: LOD = 4 (95% CI, 2-6) copies HIV-1 DNA/reaction

Nominal HIV copies/reaction	Number of tested	Number of detected	Percent detected
10	20	20	100%
5	20	19	95%
3	20	19	95%
1	20	8	40%
0	20	0	0%

Linearity and precision of HIV-1 DNA per reaction

HIV DNA copies/ reaction			
Nominal	Mean of observed	SD	%CV
10,000	8522	1155	13.56
1,000	936	171	18.31
100	82	19	22.74
30	22	6	28.76
10	8	3	37.39

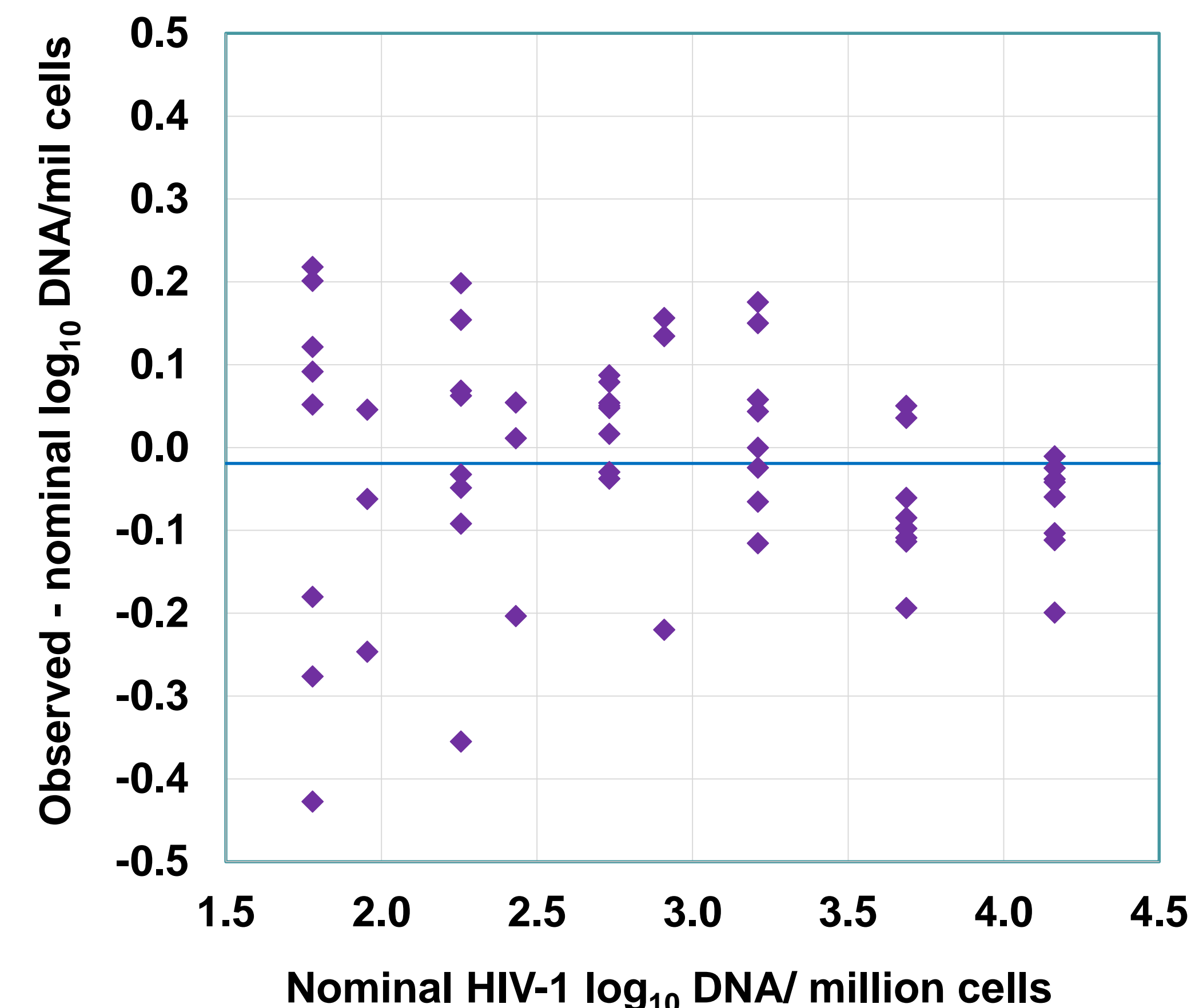
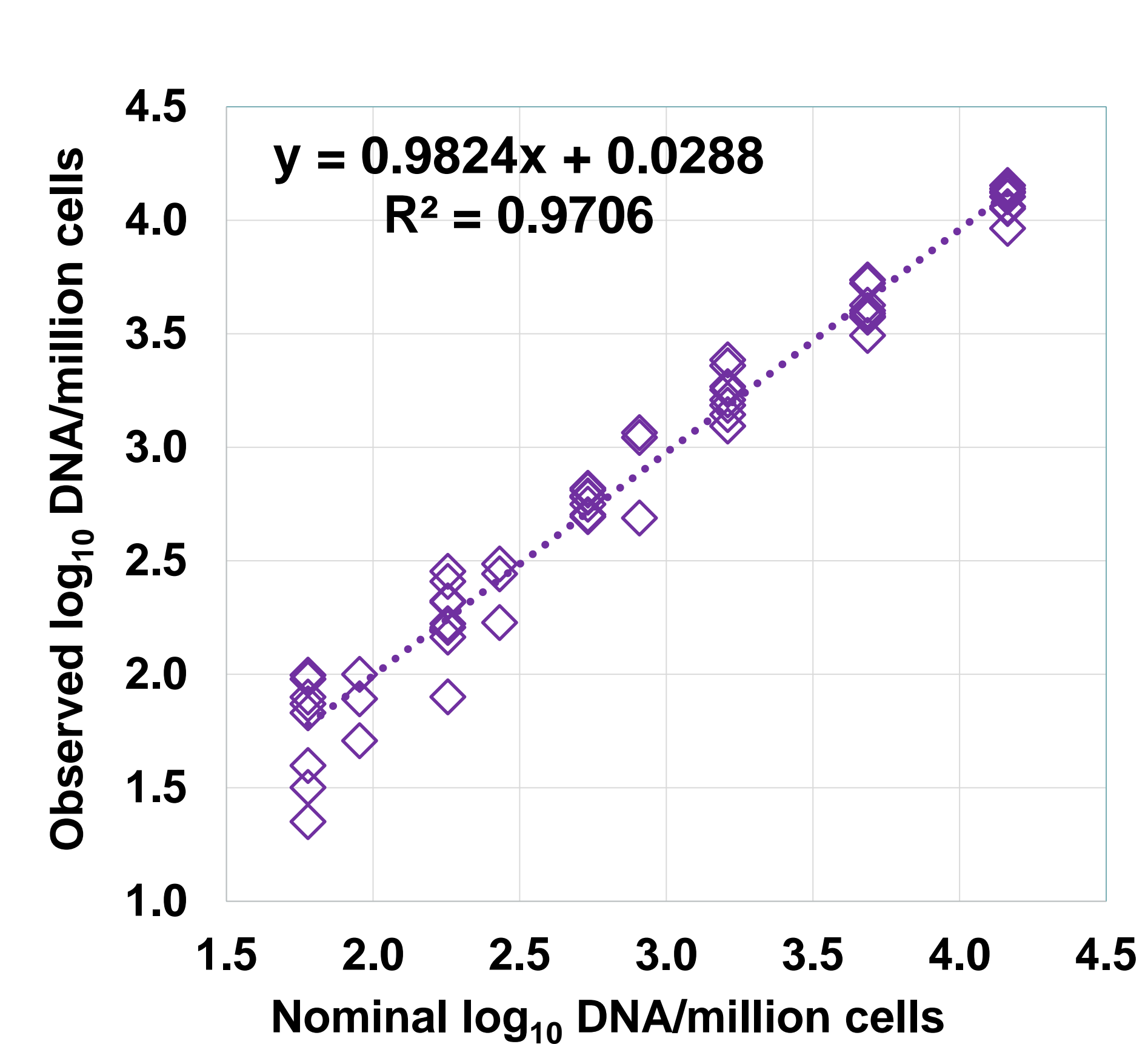


Quantification and Detection of HIV-1 DNA in VQA U1 cells per million PBMCs:

All replicas with nominal ≥ 30 U1 were detected; 8 replicas (in black) and 3 replicas (in blue) were measured.

Nominal number U1 cells	Nominal log ₁₀ copies/ million cells	Observed log ₁₀ copies/ million cells	SD log ₁₀ copies/ million cells
7290	4.16	4.09	0.06
2430	3.69	3.61	0.08
810	3.21	3.24	0.09
405	2.91	2.93	0.17
270	2.73	2.77	0.04
135	2.43	2.39	0.11
90	2.26	2.25	0.16
45	1.95	1.87	0.12
30	1.78	1.75	0.22

Nominal number U1 cells	Nominal HIV DNA copies in total	Nominal HIV DNA copies per reaction	Positivity per reaction
15	30	3	75% (9/12)
0	0	0	0% (0/12)



Peripheral blood viral-load suppressed patients

Sample ID	HIV DNA copies/million cells (mean ± SD)
0002	281 ± 36
0003	223 ± 34
0004	227 ± 13
0005	297 ± 11
0006	33 ± 1
0008	92 ± 6
0010	235 ± 59
014	438 ± 98
015	91 ± 32
016	227 ± 84
020	222 ± 13

HIV-1 groups and subtypes testing

Nominal 1E7 copies/ mL for viral stocks

Group	Sub-type	Strain	Measured copies/mL
M	A	CM238	5.11E6
M	A	UG273	2.50E6
M	B	BZ167	6.19E6
M	B	US2	1.29E7
M	C	ZAM18	1.01E7
M	C	UG268	3.41E6
M	D	SE365	7.44E6
M	D	SE365	1.17E7
M	E	POC30506	2.98E6
M	F	BZ163	1.38E7
M	F	BZ162	6.17E6
M	G	HH8793	9.63E6
M	AE	CM235	8.74E5
M	A/G	ZACH	6.90E6
N	N	YBF30	6.31E6

The assay gave undetected results for HIV-2 (ROD, EHO), HCV, HBV and *Plasmodium falciparum*.

CONCLUSIONS

Key features of the UltraQ HIV-1 DNA quantitative assay

- The assay's LOD is 4 copies (95% CI, 2-6) HIV-1 DNA/ reaction in the presence of human genome equivalent to 200,000 cells.
- It potentially quantifies HIV-1 DNA in PBMC from patients infected with HIV-1 strains of M and N groups.
- Quantification of HIV-1 DNA in U1 cells ranging from 30 to 7290 per million cells –corresponding to 1.78 to 4.16 log₁₀ HIV-1 DNA copies/ million cells, respectively – is highly correlated with nominal values provided by VQA. SD is less than 0.22 log₁₀ copies/ million cells.
- The UltraQ assay has potential ability to estimate HIV-1 DNA reservoir size and assess the efficiency of HIV-1 therapeutic strategies.

Reference: 1. Avettand-Fènoël V et al. Total HIV-1 DNA, a Marker of Viral Reservoir Dynamics with Clinical Implications. Clin Microbiol Rev. 2016 Oct;29(4):859-80.
2. Rouzioux C, Avettand-Fènoël V. Total HIV DNA: a global marker of HIV persistence. Retrovirology. 2018 Apr 3;15(1):30.

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