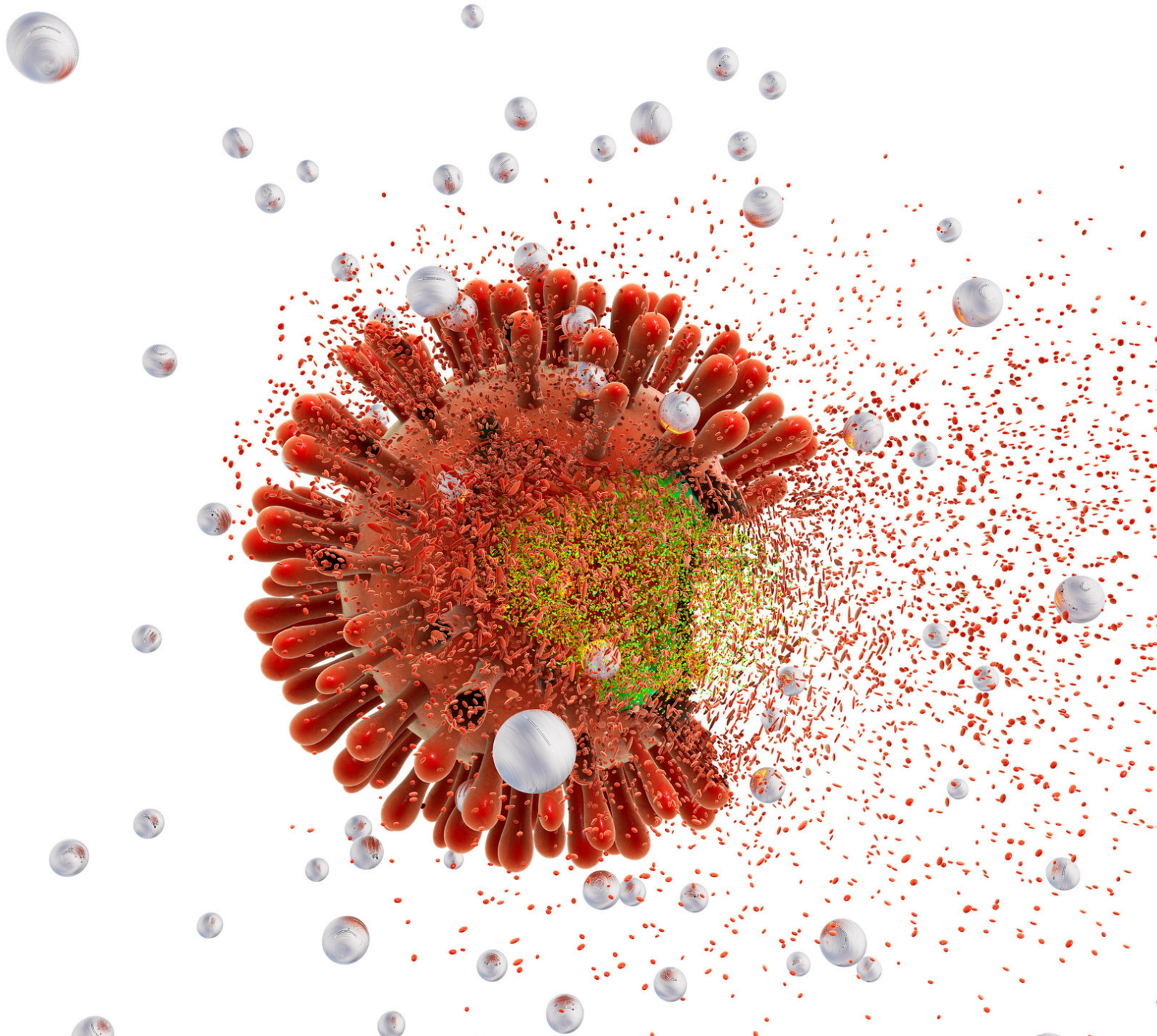
Comparison between Standard Curve of HIV-1 DNA Test and pNL4-3 plasmid in the HIV-1 DNA quantification

- The HIV-1 DNA Test is a qPCR assay to detect and quantify all forms of intracellular HIV-1 DNA in whole blood samples and PMBC.
- The kit provides a ready to use PCR mix, that allows robust and consistent performances, and a ready to use standard curve which has been demonstrated to be perfectly comparable with a pNL4-3 plasmid standard curve.



Introduction

To date, the most widely used marker of HIV persistence in infected cells is total HIV-DNA. HIV-1 DNA forms, including unintegrated and integrated provirus, co-exist in infected cells during viral replication and their levels may vary among patients, according to the stages of HIV disease and the effectiveness of the anti-HIV therapy. Real-time PCR (qPCR) is the most common method used for HIV-1 DNA quantification. In this contest, the HIV-1 DNA Test is a qPCR assay, based on dual-labelled probes, that allows the measurement of total HIV DNA in whole blood samples from HIV positive patients. The system amplifies a highly conserved region of the HIV-1 LTR and allows to analyse 1 µg of cellular DNA without compromising the assay performance thanks to the amplification mix that neutralizes PCR inhibitors. The standard curve for the absolute quantification is obtained by diluting the Standard DNA represented by a linearized recombinant plasmid containing the HIV-1 LTR target region. Currently, no certified reference materials for the HIV-1 DNA that can be used to check the quality and to validate the performances of a molecular method are available. The only available materials are plasmids containing the HIV-1 target region used as infection molecular clone (i.e.: pNL4-3 plasmid) or non-productive cell lines harbouring a defined number of HIV-1 proviruses (such as 8E5/LAV), commonly used as calibration standard. However, it has been widely demonstrated the progressive loss of HIV-1 DNA copy from 8E5 cells during culture, unpredictably changing HIV-1 DNA levels (a,b). Contrariwise, plasmid DNA is the most common choice as standard DNA due to its high stability and reproducibility (c). The aim of the present study was to verify the accuracy of DIATHEVA HIV-1 DNA Test to quantify HIV-1 DNA by comparing the Standard DNA of the kit with the plasmid pNL4-3 calibration standard (NIH AIDS Reagent Program).

Materials and Methods

Circular and linear form plasmid DNA standards were carefully prepared from the same batch of HIV-1 pNL4-3 plasmid (Cat. Number: 114; NIH AIDS Reagent Program, **Fig.1**). Fresh prepared plasmid was used as circular form plasmid sample (C). Linear (L) form plasmid was prepared from circular plasmid using restriction endonuclease EcoRI. The complete linearization was confirmed in the agarose gel and the L plasmid was purified. The concentrations of C and L plasmids were measured by several operators, in triplicate, using three different NanoVue spectrophotometers located in two distinct laboratories and subjected to standard calibration tests.

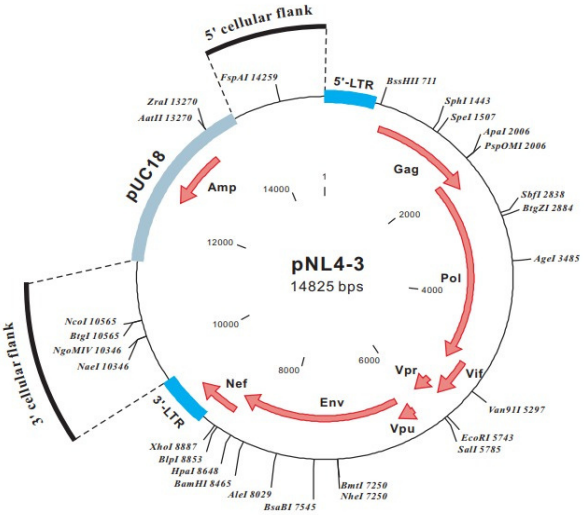
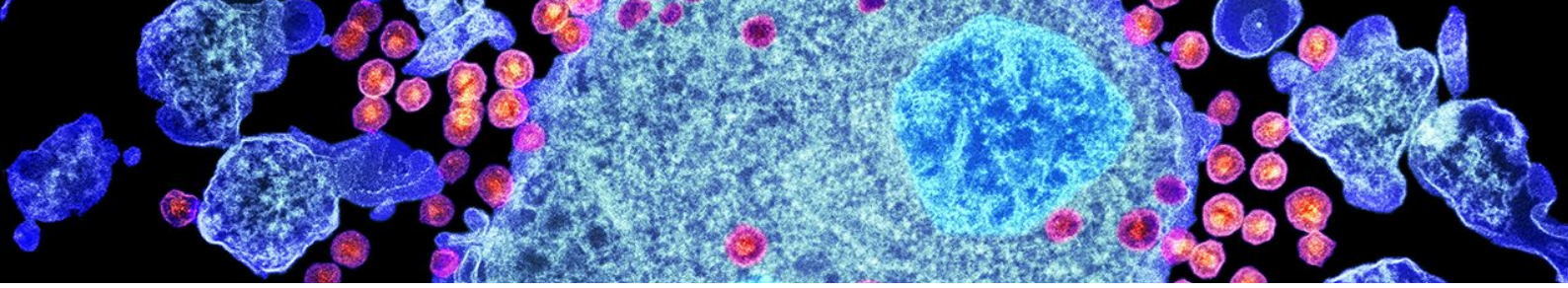


Figure 1. pNL4-3 plasmid map. Full-length, replication and infection competent chimeric DNA.

Based on the concentration value and the DNA sequence, the copy number of DNA molecules per unit volume was calculated.
Decimal dilution ranges starting from 5 to 5 x 10^4 copies µl-1 were prepared using the C or L HIV-1 pNL4-3 plasmids and the Standard DNA provided in the HIV -1 DNA Test. DNA from 4 whole blood samples of HIV-1 positive patients were extracted and purified using the QIAamp DNA Blood Mini kit (QIAGEN) for analyses in qPCR.
The amplification assay was performed using the "HIV-1 DNA Test" (MBK0086, Diatheva) following manufacturer's instructions.



Results and Discussion

Purified plasmid DNAs are most commonly found as circular molecules, and supercoiled (circular) plasmid is also the most popular form.
Therefore, we compare the standard curves prepared using the Standard DNA provided in the HIV-1 DNA Test or the circular pNL4-3 plasmid in the same amplification run (**Fig.2**). At any concentration applied (5 to 50,000 HIV-1 copies (cps) per reaction), the circular pNL4-3 plasmid DNA increased Ct values compared to the standard curve of HIV - 1 DNA Test.

Scientific studies demonstrate that DNA standard curves shifted significantly among plasmid standards with different DNA conformations. In particular, supercoiled plasmid standards cause significant overestimation in qPCR quantification, so the circular plasmid DNA is unsuitable as a standard (c,d).
Therefore, the pNL4-3 plasmid was linearized to obtain a more comparable and actual result. The comparison of the standard curves using the Standard DNA provided in the "HIV-1 DNA Test" and the linearized pNL4-3 plasmid was performed in the same amplification run.
The amplification plot shows a perfect overlap of the two distinct standard curves (**Fig. 3**).

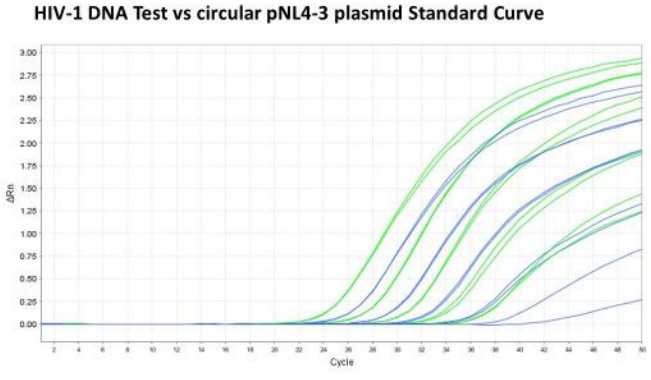


Figure 2. Amplification plot of HIV-1 DNA Test and circular pNL4-3 plasmid Standard Curves ranging from 5 to 50,000 cps/reaction.

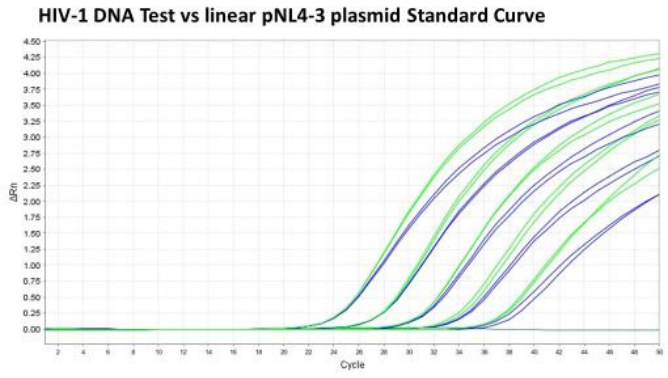
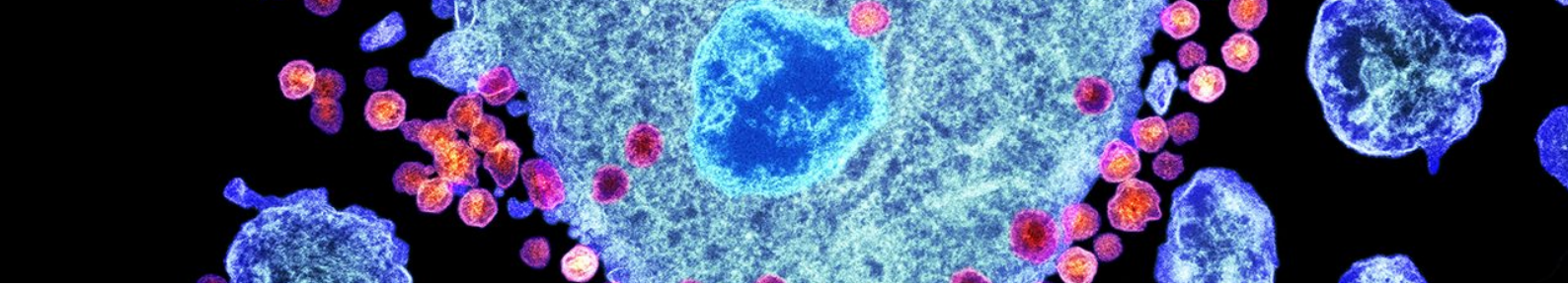


Figure 3. Amplification plot of HIV-1 DNA Test and linearized pNL4-3 plasmid Standard Curves ranging from 5 to 50,000 cps/reaction



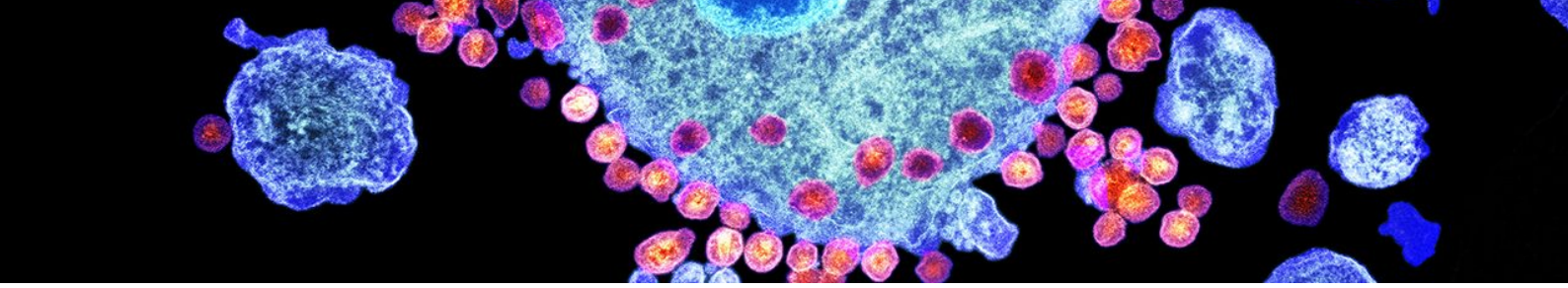
Both standard curves show good Efficiency and R2 values allowing to obtain an accurate quantification (**Tab. 1**).

		HIV-1 DNA Test Standard Curve	pNL 4-3 plasmid Standard Curve
Amplification Assay Performances	**Efficiency**	102.2%	98.3%
	R^2	1	1
	Slope	-3.27	-3.36
	Y-Intercept	39,09	40,40
	50,000 cps/reaction (Ct)	24.5 ± 0.012	24.6 ± 0.020
	5,000 cps/reaction (Ct)	27.8 ± 0.046	27.9 ± 0.009
	500 cps/reaction (Ct)	31.1 ± 0.017	31.4 ± 0.129
	50 cps/reaction (Ct)	34.3 ± 0	34.7 ± 0.132
	5 cps/reaction (Ct)	37.6 ± 0.087	38.0 ± 0
HIV-1 Positive Samples (HIV-1 DNA cps/reaction)	**A**	16.1 ± 4.2	20.9 ± 5.3
	B	36.2 ± 6.8	45.9 ± 8.5
	C	12.2 ± 0.6	16.0 ± 0.8
	D	4.9 ± 0.5	6.5 ± 0.64

Table 1. Amplification assay performances of the "HIV-1 DNA Test " and pNL4-3 Standard Curves, and quantification results of HIV-1 positive samples relating to the both standard curves. Samples were amplified in duplicate and 1 µg of DNA sample was loaded per reaction.

Conclusion

The quantification of HIV-1 DNA copies in real HIV-1 positive samples, obtained using the standard curve produced with L pNL4-3 plasmid, is similar to the quantification obtained with the Diatheva's Standard Curve.



References

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d Lin C.H., Chen Y. C., Pan T.M. (2011) Quantification bias caused by plasmid DNA conformation in quantitative real-time PCR assay. PLoS One, 6, e29101.

Ordering information

PRODUCT CODE	PRODUCT DESCRIPTION	SIZE
MBK0086	**HIV-1 DNA Test**	**96 tests**
MBK0086-32T	**HIV-1 DNA Test**	**32 tests**
MBK0087	**HIV-1 DNA Test PRO**	**96 tests**
MBK0087-32T	**HIV-1 DNA Test PRO**	**32 tests**

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Visit the page **https://www.diatheva.com/it/prodotto/hiv-1-dna-test-pro-96/**

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