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Success rate of HIV DNA Sanger sequencing in PBMC and whole blood samples.

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Introduction

The new Long-acting injectable dual therapy (cabotegravir + rilpivirine) has been shown to improve quality of life and/or adherence in virologically controlled patients already undergoing antiretroviral therapy (ART). However, several risk factors of virological failure upon switching to this treatment have been identified, including HIV subtype and resistance mutations to cabotegravir and/or rilpivirine detected in either HIV RNA or DNA. When HIV subtype and genotypic history is missing or incomplete, HIV DNA sequencing should be performed.
HIV DNA sequencing is now routinely used for HIV-infected individuals on ART with or without genotypic history. Successful amplification of HIV pol gene (Protease, reverse transcriptase and integrase) has not yet correlated with HIV DNA levels. Since plasma HIV RNA predicts the success of HIV sequencing, it can be assumed that HIV DNA load in blood cells is likewise associated with HIV DNA sequencing success.

Objective

Aim of this work was i) to analyze the performances of HIV proviral Sanger sequencing in two different matrices: peripheral blood mononuclear cell (PBMC) and whole blood (WB); ii) to assess the relationship between HIV DNA viral load and HIV DNA sequencing results.

Methods

We analyzed 451 HIV DNA sequences derived from 399 persons living with HIV-1. We tested 358 HIV-DNA sequences starting from WB samples and 93 sequences from PBMC samples.

Sanger HIV-DNA sequencing was performed using a home-made method. Protease (PR), reverse transcriptase (RT) and integase (INT) genes were amplified using a nested PCR and sequenced using two sets of specific primers.
In 124 samples (87 WB and 37 PBMC) from 124 persons living with HIV) we quantified total HIV-DNA with the HIV-1 DNA Test PRO (Diatheva) processed in automation with Elite InGenius platform (Elitech).

Table 1. Mean levels of total HIV-DNA correctly sequenced and failed in 87 WB and 37 PBMC samples.

	WB		PBMC	
	Sequenced	Failed	Sequenced	Failed
Mean	524.67	595.14	435.55	378
Sd	283.35	442.81	467.66	315.31
N	43	44	29	8

Results

The success rates of HIV DNA Sanger sequencing in the 358 WB samples were 66%, 66%, and 67% for PR, RT and INT amplicons, respectively.
In the 93 PBMC samples, the sequencing success rates were 95%, 94% and 82% for PR, RT and INT genes, respectively (**Fig.1**).

Total HIV-1 DNA was detected in 124/124 samples and HIV-DNA levels were compared with success rate of HIV-DNA sequencing: mean levels of HIV-DNA were 510 and 573 copies/10^7 cells for HIV-DNA successfully sequenced and failed, respectively. Between the two groups, no statistically significant difference was observed (T test, p=0.38).

In particular 43/84 (51%) WB and 29/37 (78%) PBMC were successfully sequenced; mean levels of HIV-DNA correctly sequenced were 550 in WB and 451 in PBMC and 608 and 378 in failed sequenced for WB and PBMC, respectively (**Table. 1**). Between the two groups (sequenced and failed) in the WB and PBMC no statistically significant differences were observed (T test, *p=0.462* and *p=0.682*). (**Fig. 2**)

Figure 1. Sanger sequencing succes rate (%) in 358 WB and 93 PBMC samples.

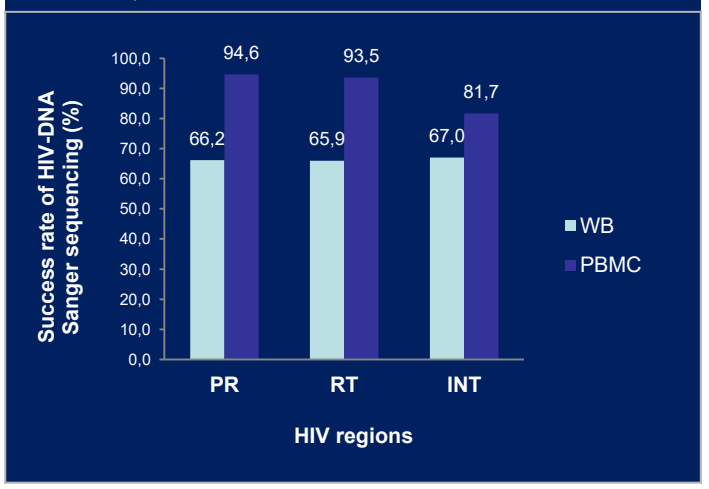
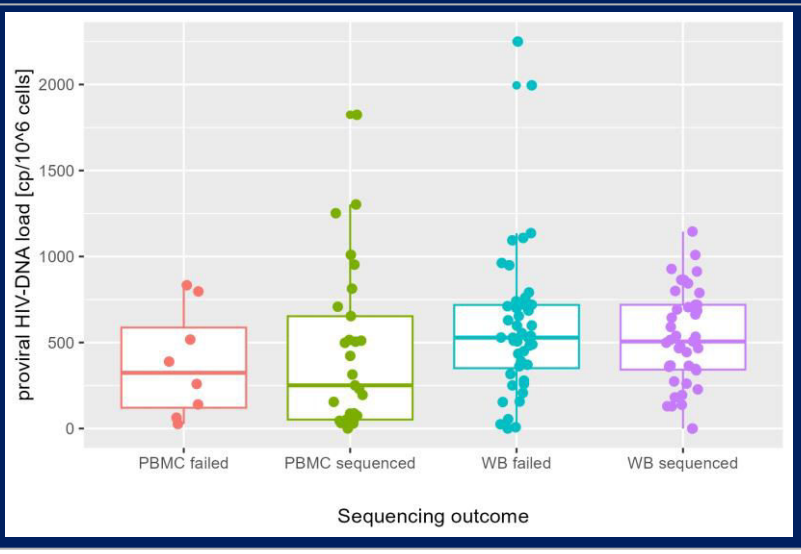


Figure 2. Comparison between HIV-DNA load in WB and PBMC succefull and failed sequenced



Conclusion

Our results showed that success rate of HIV DNA sequencing is higher with PBMC as starting material. No correlation between HIV-DNA quantity and HIV DNA Sanger sequencing success was observed.

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