Methods of low-level detection of HIV-1: Validation of a real time PCR for total DNA quantification and a sensitive subtype independent plasma RNA PCR

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Background
Treatment of HIV-1 infection with effective combination antiretroviral therapy (cART) reduces plasma viral load to undetectable levels measured with clinical assays. However, stable viral reservoirs established early in infection are obstacles for eradication. Correct measurements of low-grade viremia and of the reservoir size are crucial in HIV-1 eradication trials and there is a need for sensitive, automated and subtype-independent assays.

Methods
We intended to enable subtype-independent HIV-1 RNA quantification at very low viral levels in plasma. Furthermore, we aimed to validate an assay for ultrasensitive measurement of total HIV-1 DNA in peripheral blood mononuclear cells (PBMC), and with those assays explore the potential correlation between total HIV-1 DNA and plasma viral load in long-term treated patients on cART.

Purpose
We intended to enable subtype-independent HIV-1 RNA quantification at very low viral levels in plasma. Furthermore, we aimed to validate an assay for ultrasensitive measurement of total HIV-1 DNA in peripheral blood mononuclear cells (PBMC), and with those assays explore the potential correlation between total HIV-1 DNA and plasma viral load in long-term treated patients on cART.

Results
HIV-1 RNA assay: A plot of calculated concentrations versus measured copy numbers per reaction demonstrated good agreement between expected and the measured values for 4 independent serial dilutions of the control RNA transcripts (R²=0.98), Figure 1. The ultrasensitive assay showed greater sensitivity compared to the standard assay, consistently detecting HIV-1 RNA at all dilutions and all samples but two, reaching a sensitivity of 3-3 copies/ml (lowest copy number 2.4 copies/ml.) (table 2a and b).

Conclusions
By modifications of a commercial assay, we developed a sensitive assay for quantification of plasma HIV-1 RNA down to 3 copies/ml. We also present a validation of a sensitive assay for quantification of total HIV-1 DNA in PBMC. The lack of correlation between plasma HIV-1 RNA and total DNA could be explained by methodological problems enhanced by measurement of non-viable virions and long-term cART. It could also be partly explained by other sources than solely resting CD4+ cells contributing to residual plasma viremia in patients on effective treatment. The assays were subtype-independent, which makes them useful for research and possible clinical use in settings with a variety of HIV-1 sub-types present.