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# HIV-DNA quantification as prediction of GRT SUCCESS in HIV-DNA NGS

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## Background

- Total HIV-DNA, including stable integrated proviruses and unintegrated (extrachromosomal and linear) forms, represents the viral reservoir of HIV.
- It is widely considered as one of the most important markers of persistence of HIV inside infected cells, reflecting the history of infection, and its quantification, besides being linked to both progression to AIDS and response to treatment, could also predict the feasibility of genotypic testing.
- Resistance associated mutations (RAMs) detection in proviral DNA has been increasingly used in people living with HIV (PWH) with undetectable virus and without a historical genotype when a treatment switch is desirable.
- In this study a correlation between HIV-DNA levels and success of Next Generation Sequencing Genotype Resistance Testing (NGS-GRT) on HIV-DNA has been evaluated.

## Materials & Methods

- Viral DNA was extracted from frozen blood samples with ELITe InGenius® cartridge SP1000.
- Library for NGS was prepared using the commercial kit AD4SEQ HIV-1 Solution v2 (Arrow Diagnostics).
- Coverage analysis of FastQ files, obtained on iSeq100 sequencer (Illumina), was carried out by means of SmartVir (SmartSeq S.r.l.) software and HIV Drug Resistance Database (Stanford University).
- HIV-DNA was quantified with HIV-1 DNA Test Pro (Diatheva) adapted on ELITe InGenius® device.
- Linear regression was used as statistical method, minimizing the Akaike's information criterion.

## Results

- For this study, 26 samples were processed. The demographic and clinical characteristics are summarized in Table 1.
- Inclusion criteria were undetected or inferior to detection limit HIV-RNA.
- The median detected HIV-DNA was 192 [IQR: 105 – 374] DNA cp/10^6 cells.
- The overall mean sequencing coverage for Protease (PR), Retro transcriptase (RT) and Integrase (INT) was 87.76 %, 80.48%, and 75.16% respectively, with a 76% of GRT success.
- In samples with at least 100 copies of HIV-DNA/10^6 cells, coverage data increased to 99.6%, 91.7% and 81.3%, with GRT success rate of 94.7%.

***References***

- Samples stratifications per HIV-DNA quantification are shown in Figures 1 and 2.

| Demographic and clinical characteristics | Median (IQR) | Percentage |
|---|---|---|
| Age (years) | 57 (52-63) | |
| Male | | 62% |
| HIV-1 subtype (% of subtype B) | | 89% |
| Time since HIV-1 diagnosis (years) | 29 (21-32) | |
| CD4 cell count (cells/mL) nadir | 238 (148-329) | |
| HIV-RNA zenit | 203250 (34250 - 320475) | |
| Suppression Time (months) | 189 (128-224) | |
| | 26 (17-28) | |
| Therapeutics lines number | 8 (5-12) | |
| Exposure to NRTI (months) | 105 (77-188) | |
| Exposure to NNRTI (months) | 73 (0-183) | |
| Exposure to PI (months) | 272 (190-302) | |
| Exposure to INI (months) | 6 (3-54) | |
| Virological Failure | | 35% |
| HCV co-infection / HBV co-infection | | 26.9% / 3.85% |

*Table 1. Demographic and clinical characteristics.*

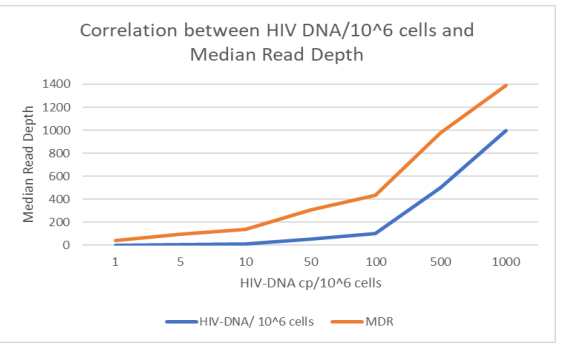


*Figure 1. Correlation between copies of HIV DNA per million of cells and MRD.*

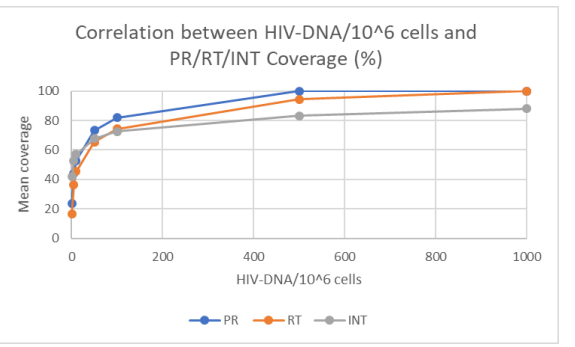


*Figure 2. Correlation between HIV-DNA/10^6 cells and PR/RT/INT Coverage (%).*

## Results of 2

- The association between mean PR/RT/INTvcoverage and independent variables is shown in Table 2.
- Data suggest expected positive correlation between overall coverage, HIV-DNA content and CD4+ cells count nadir. In particular, the INT coverage strongly correlates with previous virologic failures, whereas a negative correlation was found between INT coverage and suppression duration.

| Variable | Reverse Transcription | | Integrase | | Protease | |
|---|---|---|---|---|---|---|
| | Coef (ES) | P value | Coef (ES) | P value | Coef (ES) | P value |
| Intercept | -181.03 (46.44) | <0.001 | -127.42 (49.47) | 0.02 | -72.79 (26.59) | 0.015 |
| Log10 DNA copies per 106 cells | 25.16 (6.30) | <0.001 | 23.14 (5.95) | 0.001 | – | – |
| Age,increase | 0.61 (0.29) | 0.049 | – | – | 1.89 (0.39) | <0.001 |
| CD4+ nadir, n | 0.04 (0.02) | 0.037 | 0.03 (0.02) | 0.13 | 0.11 (0.03) | 0.003 |
| Suppression time (months) | – | – | -0.17 (0.07) | 0.035 | – | – |
| Previous virologic failures | – | – | 15.78 (7.73) | 0.057 | – | – |
| Co-Infections (HBV, HCV) | – | – | -11.85 (7.63) | 0.14 | -15.66 (10.44) | 0.15 |
| Therapeutic lines | – | – | – | – | 2.14 (1.65) | 0.21 |
| INSTI exposition (months) | 0.05 (0.03) | 0.088 | 0.13 (0.05) | 0.017 | -0.13 (0.08) | 0.15 |
| NRTI exposition (months) | – | – | – | – | 0.23 (0.09) | 0.018 |
| NNRTI exposition (months) | – | – | – | – | 0.19 (0.08) | 0.028 |
| R2 | 0.72 | | 0.59 | | 0.74 | |

*Table 2. Association between mean coverage of HIV-1 and indipendent variables of interest.*

## Conclusion

- As the NGS technique is relatively new and complex, predicting GRT success by knowing the HIV-DNA content could be particularly useful to save costs and time. In order to do so, a lot of variables should be taken into considerations.
- The simplest correlation between HIV-DNA and GRT success suggests that samples with less than 100 copies of HIV-DNA/10^6 cells should be not tested with this method, or at least, previously enriched to increase NGS positive outcome.

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2. Teo CHY, Norhisham NHB, Lee OF, Png S, Chai CN, Yan G, Tang JW, Lee CK. Towards Next-Generation Sequencing for HIV-1 Drug Resistance Testing in a Clinical Setting. Viruses. 2022 Oct 7;14(10):2208. doi: 10.3390/v14102208. PMID: 36298763; PMCID: PMC9608942.