



# Longitudinal measurements of ultra-sensitive plasma HIV RNA and quantitative antibody levels in HIV-1 infected individuals who naturally maintain viral loads below the limit of conventional detection

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## Background

HIV-seropositive individuals who are able to maintain plasma HIV RNA levels below the limit of conventional detection in the absence of antiretroviral therapy ("elite" controllers) should provide insights into novel mechanisms of viral control, and may serve as a platform for studies aimed at HIV eradication.

No prior study has systematically characterized the level of viremia over time in a large cohort of controllers. We also measured longitudinal HIV antibody levels, assuming that a slow decay in antibody levels might reflect a fully controlled virus.

## Methods

### Study Participants

We have recruited and are now characterizing the natural history of a cohort of "elite" controllers, which we define as:

1. HIV antibody-positive (defined by at least 2 serologic determinations with both EIA and confirmatory Western blot); and
2. No ARV in preceding 24 months; and
3. At least 3 plasma HIV-1 RNA levels < 75 copies/mL (bDNA version 3.0, Bayer), all spanning at least 12 months.

### Ultra-sensitive Plasma HIV-1 RNA (TMA) assay

The isothermal Transcription Mediated Amplification (TMA) (Aptima, Gen-Probe) assay was used to obtain longitudinal measurements of plasma HIV RNA. The sensitivity of the TMA assay is approximately 3 RNA copies/mL when 4 replicates are performed.

### HIV-1 antibody assay (LS-EIA)

In addition, a "detuned" or less-sensitive EIA (LS-EIA) (Organon Technica Vironostik [OTV], BioMerieux) assay was used to obtain quantitative HIV antibody levels (measured as standardized optical density [SOD]) over time. The OTV is a second-generation ELISA that detects IgG and IgM antibodies to HIV-1 and is FDA-approved for diagnostic testing; the less sensitive modification (LS-EIA) involves testing 1:20,000 dilutions of plasma under abbreviated incubation conditions. Specimens with SOD<0.2 on the LS-EIA underwent additional antibody testing using a standard third-generation EIA.

Table 1. Baseline Characteristics (n=46)

|  |                       |
|--|-----------------------|
| Gender   | 61% Male / 39% Female |
| Age (years)  | 46 (43-49)            |
| Self-reported duration of HIV infection (years)                | 13 (8-17)             |
| CD4+ T cell count (cells/mm <sup>3</sup> )                     | 753 (537-1039)        |
| Self-reported nadir CD4+ T cell count (cells/mm <sup>3</sup> ) | 571 (400-694)         |
| Plasma HIV RNA (copies/mL)                                     | 75 (75-75)            |
| Duration of follow-up (months)                                 | 16 (7-25)             |

Data are medians with interquartile ranges (IQR).

Figure 1. Plasma HIV-1 RNA (n=46)

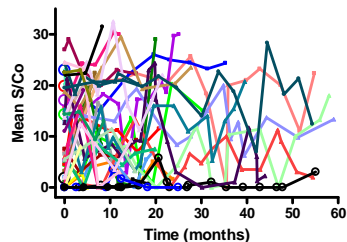


Figure 1. Raw data for plasma HIV-1 RNA using TMA assay (n=46).  
Figure 2. Repeated measures analysis showing an average of 0.08 increase in signal:cutoff ratio (S/Co) per month (p=0.08).  
Figure 3. Repeated measures analysis showing an average of 0.01 increase in standardized optical density (SOD) per month (p=0.40).

Figure 2. Plasma HIV-1 RNA (TMA)

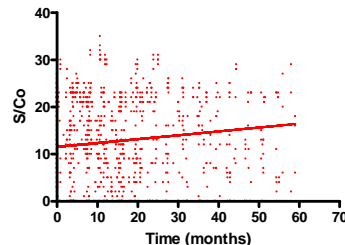
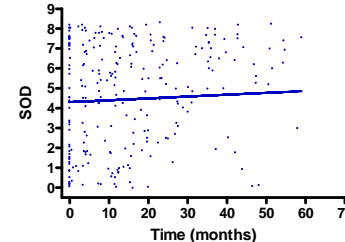


Figure 3. HIV-1 Antibody (LS-EIA)



## Results

### Ultra-sensitive Plasma HIV-1 RNA Levels

A total of 1117 TMA assays were performed on 311 specimens in 46 controllers (median 4 replicates per specimen) (Figure 1). 15/46 (33%) subjects had all TMA assays positive (signal:cutoff [S/Co] value>1). Only 1 subject had all TMA assays negative across all available (n=5) specimens, spanning a period of over 1 year. A repeated measures analysis showed an average of 0.08 increase in S/Co per month (p=0.08) (Figure 2).

Cell-associated RNA levels were also measured using the TMA assay and was detectable in the majority (25/29) of controllers. Proviral DNA was also detectable in most (21/29) individuals (median 16 copies DNA/10<sup>6</sup> PBMCs, IQR 6-48) and levels remained stable over time (p=0.45).

### HIV-1 antibody levels

A total of 249 LS-EIA's were performed on the same 46 controllers (median 4 specimens per subject). The median SOD was 4.5, which was higher than that observed in untreated individuals (n=543, median SOD=3.7, p=0.03). Two subjects had all LS-EIA's negative (SOD<0.2), one of whom was the same subject who had all TMA assays negative; all LS-EIA negative specimens were positive by standard EIA. Repeated measures analyses showed no strong evidence of change in SOD over time (an average of 0.01 increase in SOD per month, p=0.40) (Figure 3).

## Conclusions

• The vast majority (98%) of "elite" controllers have measurable plasma HIV RNA, albeit at very low levels.

• In contrast to what has been observed with long-term HAART suppressed patients, there was individual variability in the level of viremia over time (there did not appear to be a steady-state for each subject; Figure 1). Although this could be due to assay variability, it may also represent true biologic variability and reflect "predator-prey dynamics" between the host and the virus.

• Persistent viremia suggests the presence of replication-competent virus and a failure to eradicate the virus, even in these rare individuals who appear to be able to control the virus without antiretroviral therapy.