Persistence of HIV DNA in Seminal Plasma Fraction after ART among Men who have Sex with Men and Transgender Women in the Thailand Test &Treat Cohort

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Background
Semen has been suggested as a potential reservoir for latent HIV infection; however, few studies have investigated the presence of HIV DNA in semen or persistence after anti-retroviral therapy (ART). We hypothesized that HIV DNA would be detectable in semen specimens after ART initiation and for a longer period afterward compared to HIV RNA.

Materials & Methods
- Semen specimens were previously collected for the Thai MSM/TG Test and Treat Study per Chulalongkorn University IRB guidelines. Inclusion criteria:
  - Thai nationals ≥ 18 years of age
  - Men or transgender women who have sex with men (MSM/TG) who engaged in anal intercourse without condom or had >3 sex partners in last 6 months
  - No previous HIV-positive test
  - Signed consent
- Amendment for NPGR substudy was approved by Chulalongkorn University IRB; and reviewed and exempted by University of Hawaii IRB.
- DNA from semen specimens collected at ART initiation as well as month 6 and month 12 afterward were extracted by differential lysis into separate seminal plasma and sperm fractions (Figure 1):

![Figure 1. Differential Lysis DNA Extraction](image)

- With specific primers/probes, HIV subtype A/E gag and β-globin DNA were amplified by real-time PCR and quantitated in comparison to standard curves to calculate HIV DNA copies per million cells (qPCR). DNA from OM10.1 cells was used as a positive control. The negative control was water. Assays were performed in triplicate (Figure 2):

![Figure 2. qPCR for HIV DNA](image)

- HIV DNA detection in paired samples was analyzed by McNemar’s test.

Results
From 91 participants in Bangkok, 160 semen specimens had DNA extracted by differential lysis into separate seminal plasma and sperm fractions and assayed for HIV DNA copy numbers (Figure 3):

![Figure 3. HIV DNA Copy Number at Timepoints Relative to ART Initiation](image)

Detection of HIV DNA was significantly different between paired sperm and seminal plasma fractions at ART initiation by McNemar’s test (p=0.02).

Most participants with detectable seminal plasma HIV DNA and semen specimens at more than one timepoint exhibited declining HIV DNA copy numbers. However, a few displayed detectable HIV DNA at month 12 after ART initiation despite non-detectable HIV DNA at ART initiation and/or month 6 (Figure 4):

![Figure 4. Seminal Plasma HIV DNA Copy Number over Time](image)

HIV RNA was detected in only 1 of 5 seminal plasma specimens with measurable HIV DNA at month 6 and in 0 of 3 seminal plasma specimens with measurable HIV DNA at month 12.

Conclusions
HIV DNA was measurable in seminal plasma fractions at least to month 12 after ART initiation while HIV RNA became undetectable in the same specimens. Further study is necessary to characterize semen as a HIV reservoir and to determine reactivation potential and transmissibility.

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