

Validation of Urine Collected after a Prostate Massage for Studies of Male HIV-1 Infectivity

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Abstract

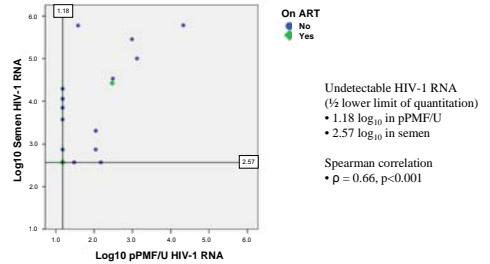
Background: Assessing male HIV-1 infectivity is difficult in many clinical settings or where semen collection by masturbation has poor acceptability. Because blood plasma viral load does not accurately predict seminal HIV-1 RNA levels, alternative genitourinary sampling methods are needed. We evaluated post-prostatic massage fluid/urine (post-PMF/U) HIV-1 RNA as a surrogate marker for seminal HIV-1 shedding.

Methods: HIV-1-seropositive Kenyan men were evaluated after 48 hours of sexual abstinence. At each visit, a clinician performed prostatic massage until expressed prostatic fluid was visualized, then post-PMF/U and blood were collected. Participants provided a semen specimen one week later, again after 48 hours of abstinence. HIV-1 RNA was quantified using a real-time PCR assay; lower limits of quantification were 30 copies/ml in post-PMF/U and plasma, 750 copies/ml in semen. Linear regression compared log₁₀-transformed semen and post-PMF/U HIV-1 RNA levels, adjusting for factors that may influence shedding. Longitudinal analysis with generalized estimating equations (GEE) was performed to evaluate change over 2 visits.

Results: 30 men provided paired semen and post-PMF/U samples, including 11 (37%) on antiretroviral therapy. A model including both plasma viral load and post-PMF/U fitted this cross-sectional study best, with a change in log₁₀ post-PMF/U RNA per change in log₁₀ semen RNA at 0.75 (95% CI 0.50, 1.0, p<0.001, R²=0.67). Nineteen men provided samples at 2 visits. GEE analysis showed that log₁₀ post-PMF/U RNA alone was the best predictor of log₁₀ semen RNA (beta = 1.0, 95% CI 0.63-1.37, p<0.001).

Conclusions: At a single visit, plasma viral load and post-PMF/U HIV-1 RNA levels accounted for 67% of the variation in seminal HIV-1 RNA levels. Over time, seminal HIV-1 RNA levels were more closely associated with post-PMF/U than with plasma viral load. For studies requiring repeated genitourinary sampling, post-PMF/U is a better surrogate for seminal HIV-1 infectivity than blood plasma.

Figure 2. Log₁₀ copies/mL semen vs. pPMF/U HIV-1 RNA



Introduction

- Semen is the primary vehicle for HIV-1 transmission from infected men to their partners
- Blood plasma viral load does not accurately predict seminal HIV-1 RNA levels
- Direct assessment of male HIV-1 infectivity is difficult due to
 - Poor acceptability of semen collection by masturbation
 - Need for rapid sample processing after collection
- Alternative genitourinary sampling methods are needed

Objective

- To evaluate urine collected after a prostate massage (post-prostatic massage fluid/urine or pPMF/U) as a potential marker for HIV-1 infectivity that could be used in place of semen
 - Reliability as a method for regular collections
 - Acceptability (see poster WEPEA107)
 - Ease of laboratory processing (see poster WEPEA107)

Background

- Extensive investigation of seminal HIV-1 sources (Coombs *et al.*, 2004)
 - pPMF/U HIV-1 RNA only independent predictor of seminal HIV-1 RNA levels
- Potential sources of virus in pPMF/U
 - Prostate gland
 - Seminal vesicles
 - Perirethral (Littré) glands
 - Bulbourethral (Cowper) glands
 - Perirethral submucosal mononuclear cells

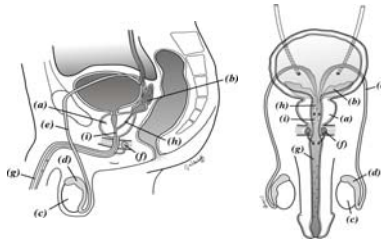


Figure 1. Male genitourinary tract.

- Prostate gland
- Seminal vesicle
- Testis
- Epididymis
- Vas deferens
- Bulbourethral gland
- Perirethral gland

Methods

Clinical

- HIV-1-seropositive Kenyan men examined after 48 hours of sexual abstinence
- Prostatic massage performed until prostatic fluid expressed, then post-PMF/U and blood collected
- Semen specimens collected 1 week later after 48 hours of sexual abstinence

Laboratory

- HIV-1 RNA quantified using real-time PCR
- Lower limits of quantification
 - 30 copies/mL in post-PMF/U and plasma
 - 750 copies/mL in semen
- Values below limit set to 1/2 the lower limit of detection for that specimen type

Statistical

- Linear regression compared log₁₀-transformed semen and post-PMF/U HIV-1 RNA levels, adjusting for factors that may influence shedding
- Longitudinal analysis with generalized estimating equations (GEE) performed to evaluate change over 2 visits

Results

Thirty men contributed paired semen and post-PMF/U specimens at a single visit, of whom 16 also attended a second visit.

Table 1. Participant Characteristics (N = 30)

Characteristic	Median, range or N (%)
Age, years	29, 18 – 53
CD4 cell count, cells/μL	374, 128 – 1,195
Plasma HIV-1 RNA, copies/mL	625, 15 – 29,847
Taking ART	11 (37%)

Table 2. Factors Associated with Log₁₀ Seminal HIV-1 RNA (n = 30 men): Cross-Sectional

Factor	Univariate Analysis		Multivariate Analysis*	
	Beta (95% CI)	P value	Beta (95% CI)	P value
Log ₁₀ blood plasma RNA	0.6 (0.3–0.9)	<0.001	0.4 (0.2–0.6)	0.002
Log ₁₀ post-PMF/U RNA	1.0 (0.6–1.4)	<0.001	0.8 (0.4–1.1)	<0.001
ART (yes/no)	-0.9 (-1.7 – -0.1)	0.02		
CD4 categories				
<200 cells/μL	0.1 (-1.0–1.2)	0.9		
200–350 cells/μL	0.1 (-0.9–1.1)	0.8		
>350 cells/μL	Reference			

* Predictors retained if p<0.10 in final model
R² = 0.67 for final multivariate model

Figure 3. Change over Two Visits, Selected Patients

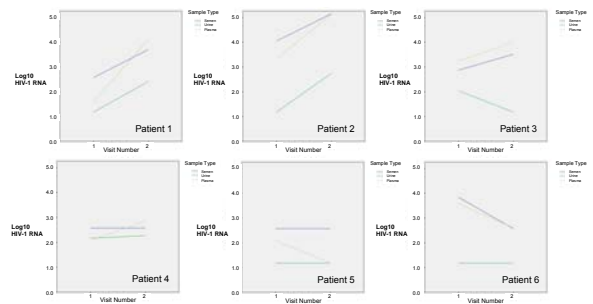


Table 3. Factors Associated with Log₁₀ Seminal HIV-1 RNA (n = 19 men): Longitudinal with GEE

Factor	Univariate Analysis		Multivariate Analysis*	
	Beta (95% CI)	P value	Beta (95% CI)	P value
Log ₁₀ blood plasma RNA	0.4 (0.3–0.6)	<0.001	0.3 (0.1–0.4)	0.007
Log ₁₀ post-PMF/U RNA	0.8 (0.6–1.0)	<0.001	0.6 (0.2–1.0)	0.002
ART (yes/no)	-0.9 (-0.2 – -1.6)	0.01		
CD4 categories				
<200 cells/μL	0.2 (-0.4–0.7)	0.5		
200–350 cells/μL	0.2 (-0.3–0.7)	0.5		
>350 cells/μL	Reference			

* Predictors retained if p<0.10 in final model

Summary

- At one visit, plasma viral load and post-PMF/U HIV-1 RNA levels accounted for 67% of the variation in seminal HIV-1 RNA levels.
- Seminal HIV-1 RNA levels were more closely associated with post-PMF/U than with plasma viral load over time.
- For studies requiring repeated genitourinary sampling, post-PMF/U is a better surrogate for seminal HIV-1 infectivity than blood plasma.

Conclusions

- In conclusion, pPMF/U is a potential marker for HIV-1 infectivity in place of semen
 - Reliable as a method for regular collections
 - Acceptable to men (see poster WEPEA107)
 - Easier laboratory processing

Literature cited

Coombs RW, Lockhart D, Ross SO, *et al.* Lower genitourinary tract sources of seminal HIV-1. *J Acquir Immune Defic Syndr* 2006;41(4):430-8.

PPMF/U acceptability and feasibility study: please see Poster WEPEA107

Acknowledgments

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