Comparison of Three Female Genital Tract Sampling Techniques for HIV RNA Recovery

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BACKGROUND

- There is no established "gold standard" female genital tract sampling method to measure HIV RNA and secreted inflammatory markers.[1] Cervicovaginal lavage (CVL) and endocervical swab (ECS) collection, although widely used, have varying advantages and disadvantages.[2-4]
- More recently, the menstrual cup (MC) - an inert, atraumatic collection receptacle allowing high volume undiluted specimen recovery (500 µL MC vs. 50-200 µL for swabs and Weck-Cell sponges) appears to be a promising mucosal sampling method. Data comparing MC performance against more established genital sampling methods to recover HIV RNA is critical prior to scaling up use.[1]

OBJECTIVE

To compare the performance of the MC, ECS, and swab-enriched cervicovaginal lavage (eCVL) for HIV RNA detection.

METHODS

- This study, nested within an ongoing trial assessing IUD safety & acceptability for HIV-infected women CD4>350 cells/mm^3, collected MC (Instead Softcup™), ECS, & eCVL genital tract samples from consenting women during enrollment (sample order MC/ECS/eCVL), 3-month (ECS/eCVL/MC), & 6-month (eCVL/MC/ECS) visits.
- The nested study will comprise 50 samples with 3 time-point measures; this analysis includes 39 enrollment, 20 3-month, and 6 6-month samples.
- At all visits, women self-inserted the MC for >45 minutes prior to clinician removal. ECS was collected with flocked swabs inserted into the external os for 1 minute; eCVL combined an additional ECS with a 5 mL normal saline lavage.
- Samples were processed within 4 hours of collection, with mc secretions weighed then diluted at 1 gm/10-fold mL with phosphate-buffered saline.
- Matched aliquot (1 mL) sets of MC, ECS, & eCVL specimens from each woman/visit were tested for HIV RNA concentration with polymerase chain reaction using the Abbott M2000SP/RT VL assay (Abbott Diagnostics, Illinois, U.S.).

TABLE 1. Baseline characteristics of first 39 participants with genital viral load comparison by specimen collection method.

Median age in years (Interquartile Range; IQR)	
Ever pregnant (n, %)	37 (95%)
Median CD4 (cells/mm3) at screening visit (IQR)	573 (511, 810)
Genital tract infection status at screening (n, %): Trichomonas Bacterial vaginosis Chlamydia Gonorrhea Syphilis	 4 (10.3%) 6 (15.4%) 2 (5.1%) 1 (2.6%) 1 (2.6%)
Median plasma viral load in log10 copies/mL at enrollment (IQR)	3.9 (3.2, 4.4)

TABLE 2. Comparison of detection and quantity of female genital HIV RNA viral load (VL) between specimen collection methods.

Enrollment Visit (n=39)	Menstrual Cup (MC); 38	Endocervical Swab (ECS); 37	Swab-enriched lavage (eCVL); 39	p, MC vs. ECS	p, MC vs. eCVL	p, ECS vs. eCVL
Detectable genital VL (n, %)	24, 63.2	16, 43.2	17, 43.6	0.02	0.01	1.00
Median VL (IQR)*	3.0 (1.3, 3.6)	1.3 (1.3, 2.8)	1.6 (1.3, 2.9)	<0.001	<0.001	0.49
3-month Visit (n=20)	MC, n=19	ECS, n=20	eCVL, n=20			
Detectable genital VL (n, %)	13, 68.4	9, 45.0	12, 60.0	0.12	1.00	0.25
Median VL (IQR)*	3.2 (1.3, 3.7)	2.3 (1.3, 2.8)	2.4 (1.3, 3.1)	0.002	0.003	0.046

METHODS (cont.)

- Paired comparisons between methods were made with McNemar's exact tests for dichotomous outcomes. Medians of continuous measures between methods were compared with Wilcoxon matched-pairs signed-rank test.

RESULTS

- Participant characteristics are summarized in Table 1.
- Median MC specimen volume was 0.34 mL (range: 0.02-1.52 mL). MC specimens were more likely to have detectable HIV RNA at enrollment & higher HIV RNA levels among specimens with detectable HIV RNA at enrollment & 3 month visits (Table 2).
- MC samples with detectable HIV RNA had greater volumes (0.37 mL (IQR: 0.29, 0.61) n=40 vs. 0.26 mL (IQR: 0.19, 0.38) n=23; unadjusted Mann-Whitney p=0.02), but did not differ by median insertion time (both groups=95 minutes).
- MC specimens were more likely to have detectable HIV RNA than ECS or eCVL (63% vs. 43% & 44%, respectively) at enrollment & higher median HIV RNA levels than ECS or eCVL at enrollment & 3-month visits (Table 2).
- In pooled analysis of women with quantifiable HIV RNA, unadjusted median difference between MC & eCVL VL was 0.6 (IQR 0.3, 0.8; p<0.001) log10 copies/ mL, 0.8 (IQR 0.3, 1.2; p<0.001) log10 copies/mL between MC & ECS specimens, & -0.2 (IQR -0.8, 0.3; p=0.31) between ECS & eCVL.

CONCLUSIONS

Preliminary analysis suggests that MC may be a more sensitive collection method for HIV RNA detection, yielding greater quantities (generally >0.5 log10 copies/mL) than ECS or eCVL. This finding held true when MC was collected first (enrollment) and last (3-month visit). Further, MC offers a method with a known dilution factor for enhanced precision.

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ACKNOWLEDGEMENTS

We thank the clinic and study staff and patients who contributed for their contributions to the 2iudnct study. This study is funded by the Eunice Kennedy Shriver National Institute of Child Health and Development (5R01HD071804) and the Preventive Technologies Agreement from the United States Agency for International Development. The study sponsors played no role in study design; collection, management, analysis, and interpretation of data; writing of this report; and the decision to submit this report for publication.