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Comparison of Techniques for HIV-1 RNA Detection and Quantitation in Cervicovaginal Secretions

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Abstract

Principles—HIV-1 in female genital secretions has been measured using swabs, Sno Strips (Akorn, Inc., Buffalo Grove, IL), and cervicovaginal lavage (CVL), but little is known regarding the comparability of these collection techniques.

Methods—We compared HIV-1 RNA detection and quantity in specimens obtained from HIV-1-seropositive women in Kenya using three sample collection techniques and three storage techniques and evaluated reproducibility in samples collected 5 days apart. Specimens were stored in no medium, freezing medium, or TRI Reagent (Molecular Research Center, Cincinnati, OH) for 2 to 15 months.

Results—HIV-1 RNA assays were conducted on 640 specimens from 20 antiretroviral naive women. Storage in TRI Reagent significantly enhanced detection of genital HIV-1 and yielded significantly higher mean log₁₀ RNA levels than specimens collected in either no or freezing medium. The prevalence of HIV-1 RNA detection in TRI Reagent ranged from 50% to 80% depending on collection method and was highest in cervical swabs. Mean log₁₀ HIV-1 RNA levels were 3.1 log₁₀ copies/cervical swab, 2.6 log₁₀ copies/cervical Sno Strip, 2.5 log₁₀ copies/vaginal swab, 2.4 log₁₀ copies/vaginal Sno Strip, 2.9 log₁₀ copies/ml for cervicovaginal lavage (CVL) cell pellet, and 2.1 log₁₀ copies/ml in CVL supernatant. Comparing specimens from days 1 and 6, there was significant concordance of HIV-1 RNA detection and correlation of HIV-1 RNA levels for cervical swabs, vaginal swabs, vaginal Sno Strips, and CVL cell pellets (κ , 0.5–0.9; r , 0.5–0.9), but not for cervical Sno Strips or CVL supernatants.

Conclusions—Cervical or vaginal swab, vaginal Sno Strip, and CVL collection led to reproducible measurement of genital HIV-1 RNA, despite storage for several months and international transport. Collection using swabs was simpler than Sno Strips or cervicovaginal lavage, and yielded the highest prevalence of HIV-1 RNA detection and reproducibility.

Keywords

Cervical; Vaginal; Female; Genital; HIV-1 RNA; Detection

As new interventions are developed to prevent transmission of HIV-1, accurate and reproducible techniques to measure genital HIV-1 are necessary to provide surrogate markers of intervention effect. Genital tract HIV-1 load is likely a more direct measure of infectivity than plasma viral load, but it has been difficult to measure genital HIV-1 RNA levels in a standardized manner.

A variety of techniques have been used to collect genital secretions for detection and quantitation of HIV-1. Studies of female genital HIV-1 have used swabs, filter paper Sno Strips, or cervicovaginal lavage (CVL) for specimen collection (1–8). Swabs and Sno Strips (Akorn, Inc., Buffalo Grove, IL) have been used for the separate collection of cervical and vaginal secretions, whereas CVL involves insertion of sterile saline into the vaginal vault with subsequent aspiration of lavage fluid (6,8).

Little is known about the comparability of these methods. CVL may have the advantage of increased sampling surface area, in that the lavage fluid may come into contact with both the ectocervix and much of the vaginal wall. Swab and Sno Strip collection, conversely, offer the ability to determine compartment-specific changes in shedding unique to either the endocervix or the vagina. Sno Strips wick up a standardized volume of secretions and enable estimation of HIV-1 concentration per ml of genital fluid. Swabs and lavage specimens, however, can also be used to obtain estimates of concentration using a standardized denominator (i.e., per swab or per ml of lavage fluid), and these denominators may be more relevant to issues of infectivity. As perinatal and sexual HIV-1 intervention studies are designed, it remains important to determine the optimal method for genital HIV-1 sampling. For international studies, it is particularly important to identify collection techniques that are feasible for field conditions in developing country settings and lead to sensitive, reproducible measurement of genital HIV-1 RNA, even after storage and transport.

We conducted a study to compare the prevalence, quantity, and reproducibility of HIV-1 RNA detection using three collection techniques (swabs, Sno Strips, CVL) and three storage methods (no medium, freezing medium, TriReagent) for genital specimens obtained from HIV-1 seropositive women in Nairobi, Kenya.

METHODS

Clinical Procedures

Nonpregnant HIV-1-seropositive women referred from a cohort study of perinatal HIV-1 transmission in Nairobi, Kenya were enrolled in a study to compare methods of genital HIV-1 detection. After obtaining informed consent, women were evaluated with a standardized questionnaire to assess medical, sexual, and obstetric history, and a physical examination was conducted. Cervical and vaginal specimens were collected to screen for sexually transmitted diseases (STDs), including cervical samples for *Neisseria gonorrhoeae* culture and *Chlamydia trachomatis* antigen, and vaginal specimens for wet mount microscopy for *Trichomonas vaginalis*. Enrollment of women with a microbiologically diagnosed STD was deferred until 1 week after completion of treatment. Screening tests for STDs were not repeated at the follow-up visit.

Specimen Collection

Speculum examination was conducted at two timepoints 5 days apart for collection of cervical and vaginal specimens for HIV-1 RNA assays. Three tear-flow indicator strips (Sno Strips) were simultaneously inserted into the cervical os and held in this position until cervical secretions wicked to the shoulder of all three Sno Strips. Following Sno Strip sampling, three cervical swab specimens were collected sequentially by gently rolling a plastic-handled Dacron swab one rotation in the cervical os. Three vaginal Sno Strips were

collected by placing Sno Strips simultaneously against the lateral vaginal wall and allowing vaginal secretions to wick to the shoulder of the Sno Strips. Three vaginal swabs were sequentially gently rolled against the lateral vaginal wall for one rotation. After collection of Sno Strips and swabs, CVL was performed. For CVL, 10-ml sterile nonbacteriostatic saline was inserted using a sterile plastic transfer pipette; saline was aimed at the cervical os and the fluid then aspirated from the posterior fornix. Blood (15 ml) was collected in heparinized tubes at the two timepoints (days 1 and 6) for plasma HIV-1 RNA levels.

Specimen Processing and Storage

Each of the three cervical and three vaginal Sno Strips and three cervical and three vaginal swabs was then inserted into one of three collection media in sterile cryovials; either no medium, in 1-ml freezing medium (70% RPMI-1640 culture medium, 20% fetal calf serum, 10% dimethyl sulfoxide, with added penicillin, streptomycin, and amphotericin B), or 1-ml TRI Reagent (Molecular Research Center, Cincinnati, OH). Cervicovaginal specimens were centrifuged and separated into supernatant and cell pellet fractions using a 0.45- μ m millipore disk filter. Supernatant and cell pellet aliquots were stored either with (2 aliquots) or without (2 aliquots) TRI Reagent. All specimens were stored at -70°C and transported on dry ice to Berkeley, California for laboratory testing.

HIV-1 RNA Assays

Quantitative HIV-1 RNA measurements in plasma and genital specimens were conducted using the Nuclisens assay (Organon, Teknika Corp, Durham, NC) in the Viral and Rickettsial Disease Laboratory of the California Department of Health Services, which is the Central Laboratory of the HIV Network for Prevention Trials (HIVNET). The lower limit of detection was 400 copies/ml. Assays were conducted between 2 to 15 months after collection.

Statistical Analyses

Prevalence of Detection and Quantity of HIV-1 RNA Using Different Collection/Storage Methods—Prevalence of HIV-1 RNA detection and mean $\log_{10}$ viral RNA levels (copies per swab, Sno Strip, or ml of lavage) were determined for each collection/storage method. McNemar tests were used to compare prevalence of HIV-1 RNA detection and nonparametric tests were conducted to compare median HIV-1 RNA levels for the different collection/storage methods.

Comparability of Methods—A κ statistic was determined for all pair-wise comparisons of genital HIV-1 RNA prevalence to assess concordance of HIV-1 RNA detection between methods. The Spearman r was determined for all pair-wise comparisons of $\log_{10}$ HIV-1 RNA levels to assess correlation between methods.

Reproducibility of Methods—Concordance of HIV-1 RNA detection at two timepoints 5 days apart was determined for each collection/storage method by computing a κ statistic. The Spearman r was determined to compare quantitative HIV-1 RNA levels at the two timepoints for each collection/storage method.

RESULTS

Characteristics of Women in the Cohort

In this cohort of 20 HIV-1-seropositive women, the median age was 27 years and 8 (40%) were married. The median age at first sexual intercourse was 16 years. The median lifetime number of sexual partners was 3, and 2 women reported a history of prostitution. Five

women (25%) had a history of HIV-related symptoms (fever, cough, or diarrhea >1 month, itchy skin rash, or >5 kg weight loss). The median number of days since last intercourse was 21 and all women reported abstaining from intercourse over the 5-day period of the study. The median CD4 count was 266 cells/mm³ (range, 6–781). Six women (30%) had absolute CD4 counts <200 cells/mm³. The median plasma HIV-1 RNA load was 17,000 copies/ml (range, 320–1,100,000) and the mean log₁₀ plasma viral HIV-1 RNA load was 4.4 log₁₀ copies/ml (range, 2.5–6.0). No participants had received antiretroviral therapy.

Prevalence of Detection and Quantitation of Genital HIV-1 RNA

Genital HIV-1 RNA was detected in 17 (85%) of 20 women in one or more specimens collected on day 1. Prevalence of HIV-1 RNA detection was determined for cervical swabs, cervical Sno Strips, vaginal swabs, vaginal Sno Strips, CVL supernatant, and CVL cell pellet (Table 1).

Cervical and Vaginal Specimens—In day 1 cervical swabs, HIV-1 RNA was detected in 45% to 80% of samples depending on the storage medium (Table 1). The detection of cervical HIV-1 RNA was significantly higher in TRI Reagent than dry swabs ($p < .005$). The mean log₁₀ RNA level in cervical specimens ranged from 2.1 to 3.1 copies/swab, and was higher for TRI Reagent swabs than dry swabs ($p < .001$) or swabs in freezing medium ($p = .005$). For cervical Sno Strips, prevalence of HIV-1 RNA detection ranged from 35% to 50% for the different storage methods and did not differ significantly between them. However, RNA level ranged from 2.1 to 2.6 log₁₀ copies/Sno Strip (4.2 to 4.7 log₁₀ copies/ml genital fluid), and was significantly higher for samples stored in TRI Reagent compared with those in no media. For vaginal swabs and Sno Strips, prevalence of HIV-1 RNA detection ranged from 55% to 60% and from 40% to 55%, respectively, and did not differ significantly between the storage methods. Vaginal HIV-1 RNA levels were 2.4 to 2.7 log₁₀ copies/swab and 2.1 to 2.4 log₁₀ copies/Sno Strip (4.2–4.5 log₁₀ copies/ml genital fluid). Thus, performance of RNA assays for cervical specimens for cervical specimens was significantly improved by TRI Reagent storage, although this was not the case for vaginal specimens.

CVL Specimens—CVL cell pellet specimens had prevalence of HIV-1 RNA detection comparable to swab and Sno Strip specimens (45%–65%), with RNA levels of 2.4 to 2.9 log₁₀ copies/ml lavage. CVL cell pellet specimens stored in TRI Reagent had a significantly higher mean HIV-1 RNA level in compared to those stored in no medium. CVL supernatant specimens without TRI Reagent had no detectable HIV-1 RNA, whereas 50% of supernatant specimens with TRI Reagent had detectable HIV-1 RNA, with a mean HIV-1 RNA level of 2.1 log₁₀ copies/ml lavage.

Concordance and Correlation Between Methods—Each collection/storage method was compared with the others to determine concordance and correlation between methods. There was significant concordance of HIV-1 RNA detection for 80 (76%) of 105 pair-wise comparisons of different methods. Quantitative HIV-1 RNA levels were significantly correlated in 88 (84%) of 105 pair-wise comparisons of different methods.

Correlation with Plasma HIV-1 RNA Levels—Cervical swabs and vaginal swabs, either dry or in either of the two collection media, were all significantly correlated with plasma HIV-1 RNA levels (cervical swabs r , 0.5–0.8; $p = .02$ – $< .0001$; vaginal swabs r , 0.5–0.6; $p = .02$ – $.002$). Specimens collected using Sno Strips or lavage were not consistently correlated with plasma HIV-1 RNA levels (cervical Sno Strips r , 0.3–0.5, $p = .3$ – $.02$, vaginal Sno Strips 0.4–0.5, $p = .08$ – $.05$, CVL $r = 0.2$ – 0.4 , $p = .5$ – $.07$).

Reproducibility of Methods for Detection and Quantitation of Genital HIV-1 RNA

For each collection/storage method, concordance of HIV-1 RNA detection and correlation of HIV-1 RNA levels at the two timepoints were determined. In this cohort of 20 women, plasma log₁₀ HIV-1 RNA levels at the two time points were highly correlated ($r = 0.97$; $p < .0001$). Cervical swabs collected in all 3 storage media had significant concordance ($\kappa = 0.5-0.7$; $p < .05$) and correlation ($r = 0.7-0.8$; $p < .001$) between specimens obtained 5 days apart (Table 2). Cervical Sno Strips had lower concordance ($\kappa = 0.3-0.5$; $p = .3-.09$) and correlation ($r = 0.2-0.4$; $p = .2-.03$) between measurements at the two timepoints. For vaginal swabs, the concordance ($\kappa = 0.6-0.9$; $p < .05$) and correlation ($r = 0.5-0.7$; $p < .05$) was high and statistically significant. Vaginal Sno Strips had significant concordance ($\kappa = 0.5-0.7$; $p < .05$) and correlation ($r = 0.5-0.7$; $p < .05$) for the two timepoints, in contrast to cervical Sno Strips. CVL supernatant samples did not have significant reproducibility. Cervicovaginal cell pellet specimens had significant concordance ($\kappa = 0.6-0.7$; $p < .01$) and high correlation if stored in TriReagent ($r = 0.9$; $p < .01$). Thus, cervical and vaginal swabs, vaginal Sno Strips, and cervicovaginal cell pellet samples had high reproducibility, but this was not the case for cervical Sno Strips or CVL supernatant.

DISCUSSION

In this study of 16 different techniques for sampling female genital HIV-1 RNA, collection of cervical swab specimens in TRI Reagent was the method that yielded the highest prevalence of HIV-1 RNA detection. In addition, this was a highly reproducible method for serial detection and quantitation of genital HIV-1 RNA. Quantitative levels of HIV-1 RNA per swab obtained 5 days apart were significantly correlated for both cervical and vaginal swabs in TRI Reagent. Concordance of detection of HIV-1 RNA for the two timepoints was similarly significant. Swab samples are easy to collect, store, and transport, and the high sensitivity and reproducibility of swab HIV-1 RNA measurement in our study suggests that swabs provide an ideal technique for female genital specimen collection in studies evaluating intervention effect on genital HIV-1.

Vaginal Sno Strips also provided an adequate measure of genital HIV-1 RNA in this study, and it is likely that prevalence of detection would have been higher if we had used all three Sno Strips for each assay, as described in other studies (7). Each Sno Strip wicked 8- μ l secretions, in contrast to swabs that may absorb 140 to 200 μ l (5). Vaginal Sno Strips yielded more reproducible detection and quantitation than cervical Sno Strips. This may be because cyclical cervical mucus changes affect the ability to consistently wick cervical secretions onto Sno Strips. In contrast to swab and lavage specimens, Sno Strips enable quantitation of HIV-1 RNA per ml of genital fluid, allowing direct comparison with plasma viral RNA levels; this may be useful in understanding viral dynamics between the genital and plasma compartments. In this study, for example, the median HIV-1 RNA level per ml in cervical and vaginal Sno Strip specimens was significantly higher than plasma RNA levels. Per ml levels, however, are not necessary to determine interventional effect on genital HIV-1 RNA shedding. Per swab or per lavage measurements can also be used, and they may better reflect infectivity in the context of sexual and perinatal transmission. Infectivity is likely related to the total amount of virus to which the host is exposed rather than concentration of virus in a fixed volume of secretions. Per ml levels are adjusted to reflect the amount of virus in a fixed volume. In the setting of sexual or perinatal transmission, an individual exposed to scanty secretions with a high concentration of virus may have lower viral exposure than an individual exposed to copious secretions with a lower concentration of virus. Thus, the amount of virus per swab would have more relevance to the exposure that occur during sexual intercourse or during delivery than a per ml measurement. Sno Strips require wicking up secretions, which may take more time than

swabbing in women with minimal secretions, making this technique less optimal than swab collection for large epidemiologic studies.

CVL cell pellet specimens in TRI Reagent had higher prevalence and reproducibility of HIV-1 RNA detection than lavage supernatant specimens. CVL involves more complex sampling and processing procedures than swab or Sno Strip collection. CVL has been used extensively in U.S. perinatal intervention studies, and, in contrast to Sno Strips or swabs, may have the advantage of providing enough volume to enable culture of viral isolates from genital specimens. For international perinatal intervention studies, however, swabs would be more feasible due to their relative ease of collection and processing.

We observed that TRI Reagent, a buffer with RNA-stabilizing additives, significantly enhanced sensitivity of detection and log₁₀ RNA levels in cervical swab and CVL supernatant and cell pellet samples. Our study suggests that RNA-stabilizing storage media should probably be a part of optimal genital specimen storage.

Ideally, to compare methods of collection optimally, the sequence of collection should be randomly ordered. Unfortunately, this was not possible for these techniques because CVL would adversely influence subsequent swab or Sno Strip collection. We chose to collect specimens using the technique that was least likely to affect subsequent methods first (Sno Strips) and the one most likely to affect subsequent collection methods last (lavage). This minimized the risk that one method would bias the results of another. It is possible but unlikely that the ordering of methods affected our assessment of the amount of genital HIV-1 RNA detected.

In conclusion, cervical or vaginal swabs, vaginal Sno Strips, and CVL collection of specimens obtained from women in Kenya yielded reproducible detection and quantitation of HIV-1 RNA. HIV-1 RNA was detected despite storage for up to 15 months. Cervical and vaginal swab specimens are easy to collect and yield high frequency of HIV-1 RNA detection as well as reproducible measurement of HIV-1 RNA levels. Swab collection with storage in an RNA-stabilizing buffer was the most sensitive and reproducible technique in this study. This method could be easily incorporated into international HIV-1 intervention studies to provide a useful surrogate marker for infectivity.

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TABLE 1

Detection of HIV-1 RNA and mean Log₁₀ RNA levels in genital specimens

	Cervical			Vaginal		Cervicovaginal lavage	
	Number with detectable HIV-1 RNA (%) (N = 20)	Log ₁₀ HIV-1 RNA copies/swap or Sno Strip	Number with detectable HIV-1 RNA (%) (N = 20)	Log ₁₀ HIV-1 RNA copies/swap or Sno Strip	Number with detectable HIV-1 RNA (%) (N = 20)	Log ₁₀ HIV-1 RNA copies/ml	
Swabs				Swabs		Cell pellet	
No medium	9 (45%)	2.1	12 (60%)	2.4	11 (45%)	2.4	
Freezing Medium	13 (65%) ^a	2.6 ^b	12 (60%)	2.7			
TRI Reagent	16 (80%) ^b	3.1 ^b	11 (55%)	2.5	13 (65%)	2.9 ^a	
Sno Strips				Sno Strips		Supernatant	
No medium	7 (35%)	2.0	10 (55%)	2.4	0 (0%)	0	
Freezing medium	8 (40%)	2.1	8 (40%)	2.1			
TRI Reagent	10 (50%)	2.6 ^a	10 (50%)	2.4	10 (50%) ^b	2.1 ^b	

^{a,b} Significantly higher detection or significantly higher HIV-1 RNA log₁₀ copies/method than same method (swab, Sno Strip, or lavage) at same site (cervical, vaginal or cervicovaginal) in no medium (^a*p* < .05; ^b*p* < .005).

Correlation of quantitative RNA levels and concordance of detection of genital HIV-1 RNA between day 1 and day 6 for different collection and storage methods

TABLE 2

	Cervical		Vaginal		Cervicovaginal lavage	
	Correlation (Spearman <i>r</i>)	Concordance (κ)	Correlation (Spearman <i>r</i>)	Concordance (κ)	Correlation (Spearman <i>r</i>)	Concordance (κ)
Swabs		Swabs		Cell pellet		
No medium	0.7 ^a	0.5 ^b	0.6 ^b	0.6 ^c	0.4	0.6 ^b
Freezing medium	0.7 ^a	0.6 ^c	0.5 ^b	0.8 ^a	—	—
TRI Reagent	0.8 ^a	0.7 ^a	0.7 ^a	0.9 ^a	0.9 ^a	0.7 ^a
Sno Strips		Sno Strips		Supernatant		
No medium	0.5 ^b	0.4	0.5 ^b	0.5 ^b	NA	NA
Freezing medium	0.3	0.3	0.6 ^c	0.5 ^b	—	—
TRI Reagent	0.3	0.2	0.7 ^a	0.7 ^a	0.3	0.4 ^b

^a *p* .001.

^b *p* < .05.

^c *p* < .01.