Use of Sno Strip Filter-Paper Wicks for Collection of Genital-Tract Samples Allows Reproducible Determination of Human Immunodeficiency Virus Type 1 (HIV-1) RNA Viral Load with a Commercial HIV-1 Viral Load Assay

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To assess the reproducibility of measurements of cervical and vaginal human immunodeficiency virus (HIV) viral load, 92 duplicate cervical and 88 duplicate vaginal samples were collected from 13 HIV-infected women using Sno Strip filter-paper wicks. RNA was eluted from the strips, extracted, and assayed using a modified protocol for the Roche Cobas Amplicor HIV-1 Monitor assay. Pearson’s correlation coefficient (R), coefficient of determination (D), and Bland-Altman plots (BA) were used to compare paired log_{10}-transformed viral loads. Analysis of duplicate same-site samples showed good reproducibility (cervix: R = 0.72, D = 52%, BA = 89% within range; vagina: R = 0.72, D = 51%, BA = 87% within range); paired cervix/vagina measurements showed moderate correlation only (R = 0.56; D = 31.3%). Standardized sample collection and simple modification of the Roche Cobas Amplicor HIV-1 Monitor assay allows reproducible measurement of genital viral load.

Human immunodeficiency virus type 1 (HIV-1) RNA is present in genital secretions of most HIV-infected women, but reports differ on the correlation with plasma viremia (7, 9), the influence of antiretroviral therapy (7), or the presence of genital tract infections (6, 8, 16). It has been postulated that the level of virus present in the genital tract may be an important determinant of sexual (3) and vertical transmission (4). To further study these interactions, it becomes important to have a method of reproducibly quantifying HIV-1 RNA in this compartment.

Sampling techniques to collect female genital tract secretions for quantification of HIV-1 RNA have included Dacron swabs, cervicovaginal lavage, and absorption wicks (1, 12, 14). Before drawing conclusions from genital viral load measurements, it is important to ensure that such measurements are reproducible. It is important that both the collection method and assay method are reproducible. This implies a reliable volumetric collection system as well as reproducible RNA extraction and viral load measurement. The objective of this study was to assess the reproducibility of measurements of vaginal and cervical HIV-1 RNA viral load with a collection method utilizing Sno Strip filter-paper wicks and a simple modification of the Roche Cobas Amplicor HIV-1 Monitor viral load assay.

Thirteen HIV-infected women attending a university-affiliated multidisciplinary HIV clinic (Women & Family HIV Centre, Oak Tree Clinic) in Vancouver, British Columbia, Canada, were enrolled in a study of menstrual variation in plasma and genital viral load described elsewhere (13), with enrollment occurring from September 1997 through June 1998. The approval of the University of British Columbia Clinical Research Ethics Board was obtained. All patients had stable HIV infection and were on stable antiretroviral regimens for 3 months prior to the study and during the 2 months of the study. Study participants were asked to refrain from sexual intercourse, douching, or use of vaginal health products during the study. Women attended the clinic for eight weekly visits.

Data collection at enrollment included estimated time and probable mode of HIV acquisition; history of injection drug use; medical, obstetric, and menstrual history; history of genital tract infections; Papanicolaou test history; and medication use.

At the initial visit, CD4 count and complete genital tract assessment were performed, including pelvic examination with the Papanicolaou test, cervical samples for chlamydia and gonorrhea, and vaginal Gram stain. At initial and subsequent weekly follow-up visits (eight total visits), plasma viral load and duplicate cervical (CxVL) and vaginal (VgVL) viral load samples were taken. Genital samples were taken by absorption of fluid onto Sno Strip filter-paper wicks (Chavin Pharmaceuticals, Romford, United Kingdom); these are semiquantitative
filter papers that allow absorption to a calibrated level. The samples were taken by inserting a nonlubricated speculum into the vagina and identifying the cervix. Using a long forceps to hold each paper wick, the cervical samples were taken by sequentially placing individual paper wicks into the endocervix and awaiting absorption up to the shoulder of the filter papers. Following this, vaginal samples were taken by sequentially placing the individual paper wicks onto the lateral vaginal walls until fluid was absorbed up to the shoulder of the paper wick. Duplicate cervical and vaginal samples were collected at each visit; papers were air dried and then stored at -70°C.

Plasma viral load was quantified using the Roche Cobas Amplicor HIV-1 Monitor standard viral load assay (version 1.5; Roche Diagnostic Systems, Branchburg, NJ) with a lower limit of detection of 400 copies/ml.

The laboratory method used to extract the HIV-1 RNA in the genital tract specimens was as follows. Sno Strip filter-paper wicks were cut into pieces in a sterile fashion, and fragments were placed into individual 2-ml polystyrene microtubes. Roche Monitor quantitation standard (Roche Diagnostic Systems, Branchburg, NJ) reagent (100 μl) was added to one vial (9 ml) of lysis buffer, also from the Roche Monitor kit. Then 600 μl of this mixture was added to each sample microtube. In order to elute as much HIV RNA as possible from the Sno Strip filter-paper wick, the sample microtubes were incubated at room temperature on a shaker for 45 min at 800 rpm. After incubation, the lysis buffer mixture was removed from the sample microtube, leaving only the Sno Strip filter-paper wick, and placed into another corresponding 2-ml microtube. Isopropanol (525 μl to match the lysis buffer mixture volume) was added to each sample microtube, which was then vortexed.

From this point on, all samples were processed according to Roche Cobas Amplicor HIV-1 Monitor package insert instructions. Controls were prepared according to the Roche package insert instructions. The duplicate cervical and vaginal samples were measured on different assay runs, conducted at the provincial reference laboratory for HIV viral load (Diagnostic Virology and Reference Laboratory, University of British Columbia). Viral load was measured using the standard Roche HIV-1 Monitor assay (lower limit of detection, 400 copies/ml). The Roche Ultrasensitive assay could not be used because the ultracentrifugation prestep required by this procedure could not be carried out on the original sample (fluid absorbed by a Sno Strip filter-paper wick). Since the input volume of sample in the Sno Strip filter-paper wick was measured at a mean of 8.3 μl, compared with the normal sample input volume of 200 μl, the actual lower limit of detection in this assay was 9,638 copies/ml.

For statistical analysis, measurements (copy numbers) of cervical and vaginal viral loads were log transformed (base 10). Undetectable viral loads were assigned a copy number of 1 and a corresponding log-transformed value of 0. Duplicate measurements of cervical and vaginal viral loads were averaged. Pearson's correlation coefficient, coefficient of determination, and Bland-Altman (BA) plots were used to compare paired log₁₀-transformed viral loads as well as the averages of the cervical and vaginal viral loads from the same patient.

Thirteen women were followed over 8 weeks; six women did not complete measurements at every cycle point. None of the women had evidence of a current sexually transmitted infection at the initial visit. Most of the women had moderate immune compromise, as shown by absolute CD4 count (mean 109/liter; range, 100 to 750 10⁹/liter).

HIV-1 plasma viral load at the initial visit was detectable in 10 of 13 women (median, 24,000 copies/ml; range, <400 to 75,000 copies/ml). More than 70% of the women were undergoing dual or triple antiretroviral therapy, consistent with patterns of care during the study period.

Ninety-two pairs of cervical viral load (CxVL) measurements were performed (Fig. 1, Table 1). Pearson's correlation coefficient for the 92 CxVL pairs was 0.72; the coefficient of determination was 51.9%. The Bland-Altman plot for the CxVL pairs showed that only 10 of 92 (10.9%) were out of range (mean of both values ± 2 standard deviations) (Fig. 2).

Eighty-eight pairs of vaginal viral load (VgVL) measurements were performed (Fig. 3, Table 2). Pearson's correlation coefficient for the 88 VgVL pairs was 0.72; the coefficient of determination was 51.2%. The Bland-Altman plot for the VgVL pairs showed that only 11 of 88 (12.5%) fell outside the range (mean of both values ± 2 standard deviations) (Fig. 4).

For comparison of vaginal and cervical viral loads, only 86 sets of VgVL and CxVL samples taken from the same subject...
at the same time were available for analysis. The mean of the two VgVL was compared with the mean of the two CxVL (Fig. 5). Pearson’s correlation coefficient for 86 paired vaginal and cervical viral loads was 0.56 and the coefficient of determination was 31.3%. Twenty-two (25.6%) of set 1 and 23 (26.7%) of set 2 measurements had at least one negative viral load sample in the pair.

Paired cervical and vaginal samples taken from the same site in the same patient showed acceptable reproducibility using three different statistical tests for agreement. Although others have reported on biologic variation of HIV-1 RNA levels in the genital tract at separate sampling times (5, 12), no previous studies have reported reproducibility of duplicate samples obtained at the same time.

The paired cervical and vaginal samples taken at the same time showed only moderate correlation. Given that it is expected that there is variability in RNA level between compartments, this is a plausible variation. In addition, there was a relatively high rate of negatives in one or the other sample in the pairs. This was likely due to the low sample input volume necessitated by the use of the Sno Strip filter-paper wicks in this study; this increased the lower limit of detection of the assay from the standard 400 copies to close to 10,000 copies per ml.

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TABLE 2. Comparison of 88 repeated measurements of log-transformed vaginal viral load measurements for 13 HIV-infected women

<table>
<thead>
<tr>
<th>Descriptive measure</th>
<th>VgVL1</th>
<th>VgVL2</th>
<th>Difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.33</td>
<td>1.44</td>
<td>-0.11</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.65</td>
<td>1.67</td>
<td>1.25</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.00</td>
<td>0.00</td>
<td>-3.78</td>
</tr>
<tr>
<td>25th percentile</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.12</td>
</tr>
<tr>
<td>Median</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>75th percentile</td>
<td>2.67</td>
<td>2.88</td>
<td>0.06</td>
</tr>
<tr>
<td>Maximum</td>
<td>5.31</td>
<td>5.32</td>
<td>2.70</td>
</tr>
</tbody>
</table>

* Wilcoxon signed rank, P = 0.484.
Since the results depended upon three phases of the process—collection, RNA extraction, and HIV-1 RNA viral load measurement—we are unable to determine where the variation that occurred actually lies. In proficiency-testing studies of the Roche HIV-1 Monitor kit, the coefficient of variation has ranged from about 25% to more than 50% of viral copy number; this, of course, includes the standard RNA extraction procedure. However, our study was not designed to measure the coefficient of variation for the collection procedure. Further study is required to understand which component(s) introduces the greatest variation.

John et al. suggested that cyclical cervical mucus changes may affect the wicking of secretions during sample collection (12). In addition, other authors have reported on differences in HIV-1 RNA and DNA measurements between cervical and vaginal compartments (5, 11), and it is possible that this may illustrate an actual biological difference in viral shedding between these two sites. Interestingly, many investigations of genital tract HIV-1 RNA virus have utilized the technique of cervicovaginal lavage, which does not enable discrimination between the two compartments of the genital tract (2, 6, 7, 15). Advantages of Sno Strip filter-paper wicks include the ability to more accurately sample separate compartments in the genital tract, as well as collection of a more standardized volume of secretions in an attempt to obtain a more precise quantification of viral load. As the Sno Strip filter-paper wicks are small and nonliquid, this simplifies storage and transport. In addition, it is a gentle collection technique that is unlikely to induce bleeding, a phenomenon which has previously been suggested to affect genital HIV-1 viral RNA load (10).

The RNA elution and extraction technique presented in this study is a simple modification that enabled it to be incorporated into a commercially available quantification assay, the Roche Cobas Amplicor HIV-1 Monitor kit. Combined with sample collection by the Sno Strip filter-paper wick method, it provides good reproducibility for quantification of HIV-1 genital tract viral load. This method would allow improved ability to compare and understand future investigations of HIV-1 RNA genital tract viral load.
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REFERENCES


