Published in final edited form as:

AIDS Behav. 2014 May ; 18(5): 820–825. doi:10.1007/s10461-014-0749-4.

Measuring adherence by visual inspection of returned empty gel applicators in the CAPRISA 004 microbicide trial

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BACKGROUND

The inability of past microbicide candidates to demonstrate effectiveness may have been due, at least partly, to poor adherence to study product rather than lack of efficacy in preventing HIV (1, 2). The lack of accurate adherence measures in past microbicide trials have made it difficult to distinguish between suboptimal product use versus lack of efficacy. Microbicide trials have usually assessed adherence by direct participant self-report of sexual behavior and use of study product either through personal interaction with study personnel and/or through audio computer-assisted self-interviewing (ACASI) technology. ACASI is thought to provide more thorough responses, in some instances (3). However, most self-reports of product use have frequently proven to overestimate actual product use when compared to more objective measures such as drug concentration data (4, 5) and thus provide a poor correlation to true adherence (6–8), particularly if used as the sole measure of adherence. Errors in adherence data generated by self –report are most likely due to recall and self-presentation bias where intentions to adhere are reported rather than actual adherence behavior(5). Obtaining an accurate assessment of adherence in microbicide trials remains a major challenge.

While the return of empty applicators, which are assumed to have been used by the study participant as prescribed, has not been standard practice in past microbicide trials, it was a useful indicator of adherence in the Carraguard trial (9) and was implemented in the CAPRISA 004 tenofovir gel trial (10). The approach of maintaining full accountability of study product through monthly returns of both used (empty) and unused (full) applicators in

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Conflict of interest

All authors report no conflicts of interest

the CAPRISA 004 trial proved to be an important predictor of adherence and correlated strongly with effectiveness (10). However, it is not known if the empty applicators were actually used, i.e., insertion of the applicator and expulsion of its contents in the vagina. The Carraguard trial's dye test on returned empty applicators is one of the methods used to assess applicator insertion and thereby the reliability of empty applicator counts (11). The dye test, which has been validated on the Micralax applicator in the Carraguard trial (9, 12, 13), is however unsuitable for the HTI polypropylene gel applicator used with 1% tenofovir gel (14). In the absence of an equivalent dye test for the HTI applicator we developed a standardized visual inspection technique to assess whether empty applicators had been used in the CAPRISA 004 tenofovir gel trial. The purpose of this study was to determine whether applicators being returned empty had indeed been used, as assessed using a standardized visual technique, by the women participating in the CAPRISA 004 trial.

METHODS

CAPRISA 004, a double-blind randomized controlled trial, assessed the safety and effectiveness of 1% tenofovir vaginal gel for the prevention of HIV acquisition in women (10). Women in this trial were requested to insert one dose of gel within 12 hours before sex and a second dose of gel as soon as possible within 12 hours after sex and no more than two doses of gel in a 24-hour period. From 22 October 2007, six months after the initiation of the trial, the women were requested to return both used and unused applicators as a means of assessing gel usage.

Custom-designed, user friendly packaging was created and provided to study participants in order to facilitate the hygienic storage and return of the empty applicators to the study clinic. The packaging was opaque and available as a detachable strip of 10 bags (Striploc), storing a single applicator per bag and 10 applicators in one strip of 10 bags. At each study visit the used and unused applicators were collected at the study pharmacy for reconciliation and new stock was made available to participants. On 11 August 2008, after a 2-month pilot program, the **v**isual **i**nspection of **r**eturned **e**mpty **a**pplicators (VIREA) was initiated and continued until completion of the trial in March 2010.

The procedure was designed to ensure that all CAPRISA 004 study gel applicators, returned by women as 'used', are subjected to VIREA in a standardised manner by a trained gel assessor. In addition to their existing duties, pharmacy staff performing the role of general assistant or pharmacy clerk (provision of administrative support and language translation services) in the study pharmacy was trained as gel assessors. The training was extended to the study pharmacists and pharmacist's assistant's, who performed applicator assessments in the absence of the gel assessor. To qualify as a VIREA assessor the pharmacy staff member had to score 100% on a VIREA proficiency test set by an independent assessor.

The two main objectives of VIREA were firstly to categorize applicators as 'appears used' or 'appears unused' by applying standard procedures and criteria for the visual inspection and secondly to identify incorrect applicator technique if gel was partially expelled from applicators.

Women were instructed not to wipe or wash applicators after use and to place each applicator after use in an individual pharmacy pre-labelled (participant identifier, visit code and date) Striploc bag. Unused gel applicators were generally returned in their original foil overwrap and carton in which they were dispensed. Any applicator returned with the foil overwrap broken was assessed by VIREA.

Each gel assessor was trained to apply the following standard operating procedure in assessing the appearance of the returned empty applicators:

- **i.** Each used applicator was individually removed from the packaging (Striploc bag) provided for the storage of applicators following their use. If the empty applicator was returned in a broken foil overwrap, then it was removed from the overwrap for visual inspection.
- **ii.** Each applicator was assessed to ascertain if it was a CAPRISA 004 applicator.
- **iii.** The applicator barrel was inspected for any visible residue, mucus, gel, secretions or hair. If any of these substances was visible on the barrel, besides the tip where the gel is expelled, then the applicator was categorised as ‘appears used’.
- **iv.** If in step iii the applicator barrel appeared to have no residue of any kind, then the applicator tip was inspected. If the applicator was capped, then the cap was removed and the tip inspected. If the tip was discoloured or has residue that was not gel, then the applicator was categorized as ‘appears used’.
- **v.** If in step iii the barrel was clean, and in step iv the tip was clean or had a small amount of gel as one would find if the gel was squirted out or if a small amount of ‘clean gel was visible’, then the applicator was categorized as ‘appears unused’.

The number of empty applicators returned by each study participant at each study visit was recorded. Similarly the number of empty applicators that ‘appeared used’, ‘appeared unused’ as well as the number of applicators returned unused was recorded. To reduce inter-assessor variability and enhance the reliability of the visual assessments, the assessors were provided training using a standardized curriculum and were required to pass a proficiency test before being allowed to conduct visual inspection. Two proficiency tests that were set and verified by pharmacists had to be passed before an assessor was deemed proficient.

To assess the validity of the visual assessments, two sets of returned empty applicators at each of the two study sites were randomly selected each day for a second independent assessment, which was performed without knowledge of the outcome of the first assessment. Discordance between the gel assessor’s categorizations and the independent assessment, which occurred rarely, triggered re-training of the gel assessor.

Specialty collection syringes (UNC CFAR Vaginal Specimen Aspirators) were used to obtain directly-aspirated, undiluted cervicovaginal fluid samples for tenofovir assay. After collection, samples were stored at −70 °C until analysis. At the end of the CAPRISA 004 study, samples were shipped on dry ice to the UNC Center for AIDS Research Clinical Pharmacology and Analytical Chemistry Laboratory at the University of North Carolina at

Chapel Hill. Analysis of samples was performed using an LC-MS/MS method as described previously (15).

Statistical analysis was performed using SAS® (SAS Institute Inc., Cary, NC, USA) Version 9.2. The Fisher exact test was used to compare proportions. Poisson approximations were used to calculate confidence intervals for incidence rate ratios. Cox proportional hazards regression models were used to assess predictors of HIV infection. These models were fitted using the VIREA assessment data to calculate hazard ratios while adjusting for potential confounding variables. The models utilised 'appears used' returned empty applicators as a proportion of returned empty applicators. Total 'appears used' applicator counts, which were divided by the total returned empty applicators, were fitted in the model for each woman individually. The base model included the following variables: treatment arm (active vs placebo as the reference group), age (per 5 year increase), number of sexual partners at baseline, missed visits (throughout the study) and as well as self-reported sex in the last 30 days. The results of the visual assessments were correlated with detectable tenofovir concentrations in vaginal fluid for those study visits where both were measured. It should be noted that the last gel insertion may have been days prior to the study visit, in which case, tenofovir may not be detectable in the vaginal fluid.

The CAPRISA 004 trial (NCT00441298) was approved by the University of KwaZulu-Natal's Biomedical Research Ethics Committee (E111/06), FHI's Protection of Human SubjectsCommittee (#9946) and the South African Medicines Control Council (#20060835).

RESULTS

A total of 17,031 monthly visits were completed by the 889 women (611 rural and 278 urban) enrolled in the CAPRISA 004 trial. Ninety eight of the women acquired HIV infection during study follow-up. The total number of applicators ever dispensed during the study was 181,870, of which 173,258 applicators were returned to the study pharmacy by the women at the monthly study visits. A total of 93,597 (51.5%) applicators were returned as used (returned empty) and 79,661 (43.8%) applicators were returned as unused (returned full). Of all the applicators dispensed, 8,612 (4.7%) were not returned to the study clinic and were reported by participants as lost, destroyed or discarded in the home.

As the VIREA assessment was implemented only from month 15 onwards, 59,800 of the 93,597 returned empty applicators were assessed through VIREA. The inspected applicators were returned by 838 (94.3%) of the 889 women in the CAPRISA 004 study; the remaining 51 were lost-to-follow-up or had seroconverted before month 15 of the trial. Of the 17 031 study visits in the trial, VIREA assessments were performed at 11 839 (69.5%) of them.

A total of 46 352 (77.5%) of the 59 800 empty applicators returned were assessed as 'appears used' by VIREA (Table 1). The median number of empty applicators returned by women at each monthly study visit was 4, though the women reported returning a median of 6 empty applicators. Similarly, women reported that 93.4% of their applicators had been used, while the VIREA outcome suggested that 77.5% of the empty applicators had been used. Taking follow-up time into account in a Cox proportional hazards regression model

(Table 2), the risk of HIV increased 2.4 fold, (95% CI: 1.4 – 4.1; p<0.001) in women with less than half of the empty returned applicators appeared ‘used’. When this regression model was adjusted for trial arm allocation, age, number of sex partners at baseline, missed visits, number of sex acts in the trial, the risk of HIV acquisition was 1.9 fold higher (95% CI: 1.1 – 3.5; p= 0.03).

Vaginal tenofovir concentrations were available for 375 study visits by women assigned to tenofovir gel (Table 3). Tenofovir was detected in only 13.5% of the women who had 50% or less of their empty applicators categorized as ‘used’ by VIREA compared to 58.3% of the women who had more than half of their empty applicators categorized as ‘used’ by VIREA (p<0.001). Note that tenofovir will only be detected in the vagina for a limited time after last use. Since some women may have inserted their last application more than a few days before the study visit, a correlation well below 100% is expected when comparing the proportion of empty applicators that ’appear used’ with detectable vaginal tenofovir concentrations.

DISCUSSION

The visual assessments used in the CAPRISA 004 trial correlated with detection of tenofovir in the vagina and with the risk of HIV acquisition. Based on the results of this procedure, 22.5% of the returned empty applicators did not have visual evidence of insertion; this is about 16% more applicators that may not have been vaginally inserted than that reported by the women. As a result, adherence estimates based on empty returned applicators without VIREA may be over-estimates. VIREA offers one approach to improve the accuracy of adherence estimates based on returned empty applicators.

While returned empty applicators assessed by VIREA may not be as accurate as measuring drug concentrations, it has the advantage of being available for both the active and placebo arms, is performed whilst the women is still in the clinic and is cheaper than drug testing. Since drug concentrations assays are expensive and run the risk of unblinding the trial if performed during follow-up, measurements of adherence based on returned empty applicators with VIREA provides a viable method of obtaining more reliable adherence estimates than self-report.

The return of empty and full applicators to the study pharmacy at each monthly study visit did not present a problem for the women in the CAPRISA 004 trial; more than 95% of dispensed applicators were returned to the study pharmacy. The well-organized pharmacy staff were able to incorporate these additional procedures rapidly into daily activities and without any negative consequences to pharmacy workflow. Although, VIREA did present initial challenges for the study pharmacy such as requiring additional staff, more detailed documentation, larger storage areas and appropriate training of staff involved; these challenges were easily overcome. Challenges were also offset by the greater reliability that can be placed on returned applicator counts, by the ability to identify difficulties that individual women were having with applicator mechanics (gel still in barrel, partially engaged plungers, excess gel in Striploc bag) that may have otherwise gone unnoticed and by the ability to assess the discrepancies with self -reported applicator use. The collection of all applicators every month sends a clear signal to the women in the study that the study staff

expects full accountability for every applicator. Moreover, the identification of women experiencing mechanical problems led to them receiving additional counseling and demonstrations to improve applicator use and gel adherence at the same clinic visit. The additional staffing costs for settings that cannot use existing pharmacy services to implement a process like VIREA is justifiable given the valuable adherence information that can be generated. Unlike late and expensive testing of drug concentrations (only possible in active arm patients) real-time assessment of product use offers an opportunity for immediate adherence support.

A limitation of VIREA is the subjective nature of the visual assessment technique; however standardizing the visual inspection process and enforcing routine checks for consistency, reduced, but did not eliminate, this shortcoming.

Beyond its potential utility to refine adherence measures when empty applicators are collected in clinical trials, there were other reasons for requesting the return of all applicators viz., to obtain more accurate estimates of actual product requirements, reduce quantity of unused tenofovir gel available to others in the community, ensure product dispensed to the participant is not subject to long periods of inappropriate temperature and storage conditions in the home by providing new supplies each month, accomplish full accountability of study product, match gel returns to reported use in behavioral assessments, and assist with the disposal challenges that may be encountered by women, particularly, in rural communities(16). Partly due to the requirement to return all applicators every month, two women in the CAPRISA 004 trial returned applicators from another microbicide trial, leading to the discovery of some women being co-enrolled in another microbicide trial (17, 18).

The high applicator return rate was supported by monthly reinforcement of its importance at the study pharmacy and the provision of suitable packaging to help reduce the inconvenience and potential hygiene concerns. The application of routine physical inspection of microbicides/pre-exposure prophylaxis agents currently under study, such as vaginal rings, rectally inserted applicators and the mandatory return of any unused oral or topical product, should be considered standard practice in clinical trials. Physical verification measures provide a critical adjunct to understanding adherence in the trial and identify participants with potential adherence challenges as these arise.

In conclusion, VIREA identified about 22.5% of returned applicators that may not have been inserted in the vagina. We found that VIREA was feasible and useful in improving the accuracy of the number of used applicators, which was used to calculate adherence in the CAPRISA 004 trial.

Acknowledgments

We pay tribute to the women who participated in this trial; their dedication and commitment made this study possible. The CAPRISA 004 Tenofovir Gel trial is supported by the Centre for the AIDS Program of Research in South Africa (CAPRISA), the United States Agency for International Development (USAID), Family Health International (FHI) (cooperative agreement # GPO-A-00-05-00022-00, contract # 132119), and LIFElab, a biotechnology center of the South African Department of Science and Technology. Support from CONRAD for the product manufacturing and packaging as well as support from Gilead Sciences for the Tenofovir used in the production of gel is gratefully acknowledged. We acknowledge the University of North Carolina Center for AIDS

Research (UNC CFAR grant P30 AI50410) for provision of vaginal aspirators and assay of tenofovir samples. We thank the US National Institutes for Health's Comprehensive International Program of Research on AIDS (CIPRA grant # AI51794) and the Columbia University-Southern African Fogarty AIDS International Training and Research Programme (AITRP grant # D43TW00231) for the research infrastructure and training that made this trial possible. We acknowledge Londeka Zondi, Zanele Mkhize, Cynthia Velile Ngcobo, Nolwazi Thandiwe Nzimande, Gugulethu Masinga, Bhavna Maharaj, Kershani Naidoo, Bernadene Moodley and Laura Chelini for conducting the VIREA.

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Table 1

Cumulative used applicator returns, visual assessment outcome and self-report in the last month

Applicator returns	Applicators returned as used to study pharmacy	Applicators 'appears used' by VIREA assessment	Applicators 'appears unused' by VIREA assessment	Applicators used in last 30 days (participant self -report)
Cumulative number of applicators	59 800 (100%)	46 352 (77.5%)	13 442 (22.5%)	55 874 (93.4%)
Median number of returned empty applicators in the previous month (IQR)	4 (2–6)	2 (1–5)	0	6 (4–8)

IQR = inter-quartile range; VIREA = visual inspection of returned empty applicators

Table 2

Univariate and multivariate cox proportional regression models assessing VIREA as a predictor of HIV risk

	Univariate Model		Multivariate Model	
Variable	**Hazard ratio 95% (CI)**	**p-value**	**Hazard ratio 95% (CI)**	**p-value**
Treatment arm				
Placebo	1.0		1.0	
Tenofovir	0.61 (0.37–1.01)	0.06	0.6(0.3–1.1)	0.05
Age (per 5 year increase)	0.8 (0.6–1.1)	0.17	0.8 (0.6–1.1)	0.25
Number of life time sex partners at baseline	1.0 (0.96–1.0)	0.87	1.0 (0.97–1.0)	0.99
Missed visits (throughout study)	1.0 (0.97–1.1)	0.29	1.0 (0.9–1.1)	0.91
Sex acts (actual time dependent data)	1.1 (1.0–1.1)		1.0 (1.0–1.1)	0.56
'Appears used' as a proportion of returned used				
>0.5	1.0		1.0	
≤0.5	2.4 (1.4–4.1)	0.001*	1.9 (1.1–3.5)	0.03*

Table 3

Validation of VIREA using detectable tenofovir concentrations in vaginal fluid at matching study visits

VIREA outcome	Tenofovir detected in vaginal fluid		
	No	Yes	Total study visits
≤50% 'appeared used'	32 (86.5%)	5(13.5%)	37
>50% 'appeared used'	141(41.7%)	197 (58.3%)	338
Total	173	202	375

Fisher's exact p<0.0001