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Does tenofovir gel or do other microbicide products affect detection of biomarkers of semen exposure in vitro?*, **, *

Margaret C. Snead^{a,*}, Athena P. Kourtis^a, Johan H. Melendez^b, Carolyn M. Black^c, Christine K. Mauck^d, Ana Penman-Aguilar^a, Dorothy M. Chaney^b, Maria F. Gallo^a, Denise J. Jamieson^a, Maurizio Macaluso^e, and Gustavo F. Doncel^d

^aThe Division of Reproductive Health, National Center for Chronic Disease Prevention and Health Promotion, Baltimore, MD, USA

^bJohns Hopkins University School of Medicine, Baltimore, MD, USA

^cThe Division of Scientific Resources, National Center for Emerging and zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

^dCONRAD, Eastern Virginia Medical School (EVMS), Arlington, VA, USA

^eDivision of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Abstract

Objectives—There is currently no information on whether products evaluated in HIV microbicide trials affect the detection of the semen biomarkers prostate-specific antigen (PSA) or Y chromosome DNA.

Study Design—We tested (in vitro) dilutions of tenofovir (TFV), UC781 and the hydroxyethylcellulose (HEC) placebo gels using the Abacus ABACard and the quantitative (Abbott Architect total PSA) assays for PSA and Y chromosome DNA by real-time polymerase chain reaction.

Results—TFV gel and the HEC placebo adversely affected PSA detection using the ABACard but not the Abbott Architect total PSA assay. UC781 adversely affected both the ABACard and Abbott Architect total PSA assays. While there were some quantitative changes in the magnitude of the signal, none of the products affected positivity of the Y chromosome assay.

Conclusions—The presence of TFV or HEC gels did not affect quantitative PSA or Y chromosome detection in vitro. Confirmation of these findings is recommended using specimens obtained following use of these gels in vivo.

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**The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

*Use of trade names is for identification only and does not imply endorsement by the US Department of Health and Human Services.

*Corresponding author: 4770 Buford Highway, Mailstop F-74, Atlanta, GA 30341, USA, Tel.: +1 770 488 6303, fax: +1 770 488 6391, msnead@cdc.gov (M.C. Snead).

Implications—Researchers should consider the potential for specific microbicides or any products to affect the particular assay used for semen biomarker detection. The ABACard assay for PSA detection should not be used with TFV UC781, or HEC.

Keywords

Biomarkers; Semen; Microbicides; Gels; Prostate-specific antigen; Y chromosome; Tenofovir; UC781; Hydroxyethylcellulose; HEC placebo

1. Introduction

Biomarkers of sexual behavior have been identified as critically needed for microbicide trials by many researchers (<http://www.mtnstophiv.org>) [1–4]. The use of semen biomarkers to detect or confirm recent sexual behavior and potential exposure to HIV is desirable in clinical trials evaluating topical microbicides such as tenofovir (TFV) gel. Prostate-specific antigen (PSA) and Y chromosome DNA have been used as biomarkers of semen exposure in forensic settings [5–7], and more recently, in reproductive health research, as indicators of semen exposure from unprotected sex or incorrect condom use [8–12]. For example, Y chromosome DNA has been used in studies evaluating teen's self-reported condom use [10], and PSA has been used to validate self-reports of recent sexual activity [11] and as an indicator of condom failure [12].

While both PSA and Y chromosome DNA are indicators of semen exposure, these two markers have unique characteristics, which researchers must take into consideration when choosing the appropriate marker for their study. PSA reliably indicates very recent semen exposure (up to 24 h), while Y chromosome DNA can reliably detect semen 12 h post exposure up to 1–2 weeks [13]. To our knowledge, there is no information as to whether use of microbicides or the hydroxyethylcellulose (HEC) placebo may affect the performance of the PSA or Y chromosome DNA assays for detection of such markers.

Given the important role that biomarkers of semen exposure can play in microbicide trials and future contraception research, as well as the lack of information on this topic, we undertook a series of laboratory investigations to determine whether TFV gel, UC781 or the HEC placebo affect PSA or Y chromosome DNA detection in vitro. TFV is an antiretroviral (nucleotide reverse transcriptase inhibitor) microbicide and has demonstrated efficacy when used as a gel vaginally for preexposure prophylaxis for HIV infection [14]. UC781 is an antiretroviral (nonnucleoside reverse transcriptase inhibitor) gel, which was evaluated as a microbicide in clinical trials for the prevention of sexual transmission of HIV [15]. Although UC781 is no longer in development as a single microbicide gel product, there are efforts under way to develop a combined UC781 and TFV microbicide product, which may make it relevant in the future. The HEC placebo is a gel that contains no active microbicide; it has been adopted as the placebo in many clinical trials of microbicides (Microbicides Trial Network, available at: <http://www.mtnstophiv.org/studies>, accessed 5/25/2012)) [16,17]. TFV's gel base is identical to HEC (2.5% hydroxyethylcellulose) (Table 1).

2. Methods

2.1. PSA detection

There are several methods available for PSA detection. For our investigation, we used the following two PSA assays; the Abacus One Step ABACard [18] is a rapid qualitative or semiquantitative assay with a lower limit of PSA detection of 4 ng/ml [3,18,19]. The total PSA assay [20] used on the Abbott Architect system is a chemiluminescent immunoassay that yields quantitative results [20], with a readable range of 0 to 100 ng/mL.

2.2. Y chromosome PCR assay

Real-time polymerase chain reaction (PCR) was used for detection of Y chromosome DNA as previously described [21]. The Y chromosome assay offers a qualitative indication of the presence of Y chromosome DNA (positive at >0 ng/mL); the assay also yields quantitative results that have been shown to decrease with time since exposure (detectable up to 14 days) [5–7]. The specificity of the Yc PCR assay for sperm-derived Yc sequences is based on a series of centrifugation steps to separate semen components (as well as other cells) from sperm cells. Briefly, semen samples with and without gels were centrifuged for 3 min at 12,500 rpm, and 100 μ L of the supernatant was removed. The sample was extracted for DNA using a multistep extraction protocol. First, a proteinase-K solution (10 mg/mL) was added to the specimens to lyse nonsperm cells and incubated for 30 min at 56°C. The specimen was then centrifuged for 3 min at 12,500 rpm, and DNA from the cell pellet (containing sperm cells and product, if present) was extracted. Two hundred microliters of a proteinase-K (10 mg/mL)/DTT (40 mmol) solution were added to the specimen, vortexed and incubated in a sonicator bath at 56°C for 30 min. The resulting sample was used for PCR-based detection of Yc DNA sequences.

2.3. In vitro experiments

The following products were tested (Table 1): TFV 1% vaginal gel formulated in 2.5% hydroxyethylcellulose (Gilead Sciences), UC781 gel 0.1% formulated in methylcellulose and carbomer 974P (Cellegy Pharmaceuticals, now merged with Adami Pharmaceutical Corporation) [15] and the HEC (2.5% hydroxyethylcellulose) placebo (ReProtect) [16,17].

First, semen controls (positive controls) were prepared by serially diluting [with phosphate buffered saline (PBS)] pooled semen stock which had been stored at -80°C at a 1:50 dilution and then thawed. Twofold serial dilutions were created, and then, select dilutions were tested (without the products), using the ABACard [18] and Abbott Architect total PSA assay [20] (for PSA assessment) and real-time PCR (for Y chromosome DNA assessment) [22].

Next, each of the gels was mixed with semen to achieve final gel dilutions of 1:10, 1:20 and 1:40 (and 1:80 for Y chromosome DNA only). These dilutions were estimated to represent a plausible range for the amount of product that would be present following typical vaginal use. Specifically, each of the products was diluted in PBS and then added to the series of semen dilutions in equal volumes; the final semen dilutions were twofold 1:1600 through 1:1,638,400. Selected dilutions were tested with and without the products for each assay.

Finally, products mixed with PBS only, without semen, were tested as negative controls for the ABACard PSA assay. Even though no negative controls (no semen) were used for the total PSA and Yc DNA assays, very high semen dilutions (up to 1:409,600 for the quantitative PSA assay, and 1:12,800 for Y chromosome DNA assay) were used, with or without product. For each of the samples tested for PSA with the ABACard, 200 μ L were placed in the test device, and all test results were read at 10 min. For each of the samples tested for PSA with Abbott Architect, 200 μ L were placed in the machine's sample cup, and quantitative results were produced upon completion. For each of the samples tested for Y chromosome DNA, 5 μ L were tested by real-time PCR [22].

All PSA testing was performed by a single lab technician at the Centers for Disease Control and Prevention laboratory. The quantitative total PSA assay testing was performed in triplicate. All Y chromosome DNA real-time PCR testing was done in quadruplicate by a female technician (to avoid accidental Y chromosome contamination) at the Johns Hopkins University laboratory.

3. Results

3.1. Detection of PSA by ABACard

Results of a representative experiment of PSA testing using the ABACard are shown in Table 2. Using the ABACards, TFV and the HEC placebo gave invalid results at the 1:10 dilution. TFV and the HEC placebo at 1:20 and UC781 at 1:10 dilution showed false positive results (positive at all semen dilutions and even when no semen was present). TFV and UC781 at 1:40 gave a strong positive result at a semen dilution of 1:204,800. UC781 at 1:20 and the HEC placebo at 1:40 also gave this type of result, as well as a weakly positive result at 1:409,600.

3.2. Detection of PSA by the Abbott Architect total PSA assay

Results of PSA testing using the Abbott Architect total PSA assay are presented in Figs. 1 and 2. The figures depict the mean results (and, in parentheses, standard deviations) from the three experiments. Comparisons of the means for each product dilution with the corresponding control for each semen dilution were performed using student's t test, and statistical significance is reported at the 0.05 level.

As seen in Figs. 1 and 2, TFV and the HEC placebo did not affect PSA detection using the quantitative Abbott Architect total PSA assay. Results were not different with or without the two gels at each of the dilutions tested ($p > .05$ for all comparisons). All concentrations of UC781 caused invalid results (results not shown).

3.3. Detection of Y chromosome DNA using real-time PCR

None of the products in the concentrations tested altered the qualitative indication of the presence of Y chromosome DNA by PCR (interpretation of a positive result) (Fig. 3). However, the quantitative assessment was slightly (but statistically significantly) affected in some cases. For example, the levels of Y chromosome DNA in 1:12 800 semen samples mixed with 1:40 TFV were higher than in semen controls ($p = .04$). Lower levels of Y

chromosome DNA were detected when 1:200 semen samples were mixed with 1:10 UC781 ($p=.03$) (Fig. 4). For both TFV and UC781, a tendency toward inhibition of Y chromosome detection with progressively higher concentrations was observed (even though the values were not significantly different than the semen-only control, unless as noted above), suggesting a dose-dependent effect. The HEC placebo did not affect the qualitative or quantitative detection of Y chromosome DNA ($p>.05$ for all comparisons).

4. Discussion

Our findings indicate that specific concentrations of TFV, UC781 and the HEC placebo affect PSA detection in vitro when using the ABACard (Fig. 5). The ABACard's invalid results for TFV and the HEC placebo at the 1:10 dilution are probably due to the solution being too viscous to migrate through the membrane in the 10-min interval. TFV gel is formulated with HEC as its base, which may explain the similar results. On the other hand, TFV and the HEC placebo did not affect results of the Abbott Architect assay. No results could be obtained when UC781 was present probably because of the color or other intrinsic properties of the UC781 gel, which could have affected the Abbott Architect's chemiluminescent immunoassay, rendering all quantitative PSA results invalid when this product was present. Although UC781 is no longer being developed as a microbicide [15], the results were included as an illustrative example of individual differences of topical microbicide products in their effects on the performance of two different PSA assays. Further, as efforts are in process to combine TFV with UC781 in combination microbicide gels, testing its effects could be relevant in the near future [34]. None of the microbicides tested in vitro inhibited detection of the presence of Y chromosome DNA by real-time PCR, but in some cases, the products affected the amount of Y chromosome DNA detected. In most instances, this effect was not statistically significant (p was greater than .05). But, for some concentrations of TFV (1:40) and UC781 (1:10) gels, the amount of Y chromosome DNA detected by realtime PCR was statistically significantly higher or lower than what was present in the corresponding semen control. The reasons for this are unknown. A possibility could be that, since the experimental protocol required several centrifugation steps for the proper separation of sperm cells from other semen components, the particular gels could have formed a physical barrier between the sperm cells and the other semen components during centrifugation and prevented the removal of sperm cells, resulting in higher levels of Y chromosome DNA. However, as noted above, one of the differences observed between the control and the products would not affect the interpretation of the presence of Y chromosome DNA, since its presence would be considered positive [5]; in turn, the quantitative results may not be accurate or reliable. In previous work [28] we have indeed demonstrated that other products, such as Replens, completely inhibited qualitative detection of Y chromosome sequences.

To our knowledge, no previous information is available on the effect of the specific microbicide products evaluated on PSA or Y chromosome PCR detection, either in vitro or in vivo. There is some evidence on the effect of spermicides, other products (such as detergents) and proteins from body fluids on PSA detection, using various assays, with contradictory results [23–31]. Our laboratories have also evaluated the effects of spermicides and lubricants, and results are presented elsewhere [28,31]. It is important to

know if specific products or gels affect semen biomarker detection, rather than assuming that they are all universally reliable because specific products can affect specific assays differently, ultimately affecting the objective assessment of recent semen exposure.

There are some limitations to our study. The amount or dose of a product potentially affects the assays differently. We estimated the range of product amounts that we thought would approximate the presence of products intravaginally, but we do not know if these estimates are accurate. We did not test the gel base of UC781 (methycellulose and carbomer 974P). We only tested one product at a time in the lab, but multiple products may be present in clinically obtained specimens. The characteristics of individual or multiple products theoretically can affect assays in different ways. How the results of a controlled laboratory experiment (in vitro) translate to actual clinical samples (in vivo) remains uncertain. For example, PBS, used as the diluting medium, could be quite different from vaginal fluids in pH or other physicochemical characteristics. In vivo factors such as phase of the menstrual cycle, local inflammation, vaginal infection, or hormonal contraception may affect some of the local parameters and thus lead to different results for actual clinical samples.

Future microbicide studies in which a semen biomarker is used should consider the potential for microbicides or other local products to affect the particular biomarker assay. Similar considerations should be made for rectal microbicides. Given the important role that TFV gel can play in preexposure prophylaxis against sexual HIV acquisition [1,31–33], it is particularly important to know whether its use may affect detection of such markers. While our results provide reassurance about the lack of effect of TFV gel on the quantitative Abbott Architect's total PSA assay and the qualitative findings of the real-time PCR assay for Y chromosome DNA, more studies are warranted, especially with clinically obtained specimens. Additional testing on the effects of lubricants or other products used concurrently with microbicides and confirmation of these results with in vivo studies are also needed.

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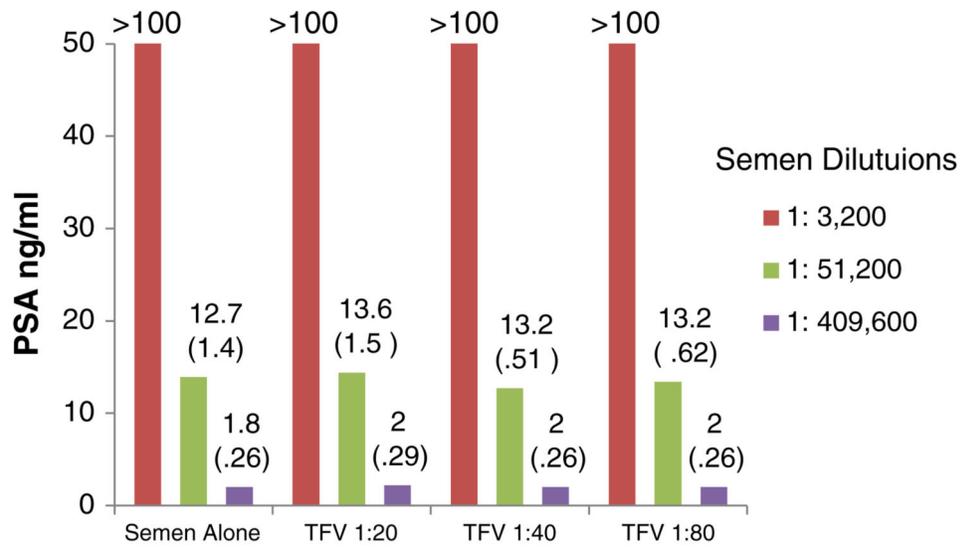


Fig. 1.

In vitro effects of TFV 1% gel on PSA detection by the Abbott Architect total PSA assay. The experiments were run in triplicate (on three different dates). The values presented in the graph are the means (in parentheses, standard deviations).

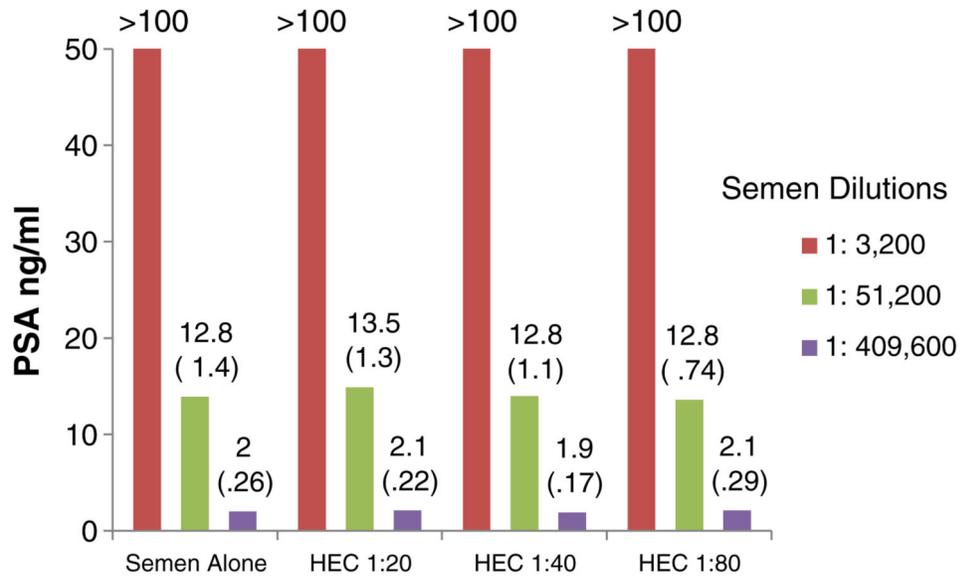


Fig. 2. In vitro effects of HEC on PSA detection by the Abbott Architect total PSA assay. The experiments were run in triplicate (on three different dates). The values presented in the graph are the means (standard deviations).

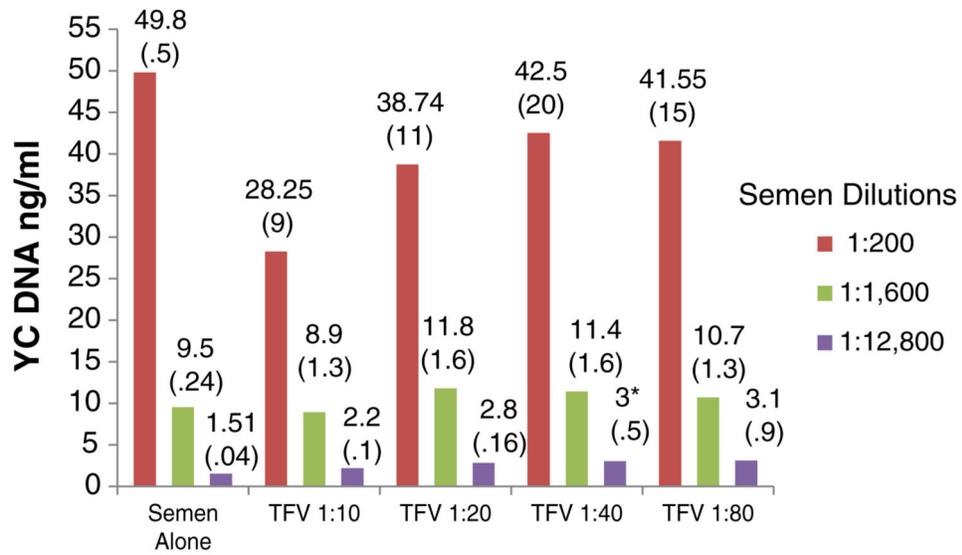


Fig. 3.

In vitro effects of TFV 1% gel on Y chromosome DNA detection by real-time PCR. Semen dilutions. The experiments were run in quadruplicate. The values presented in the graph are the means (standard deviations). Asterisks denote statistically significant difference from control ($p < .05$).

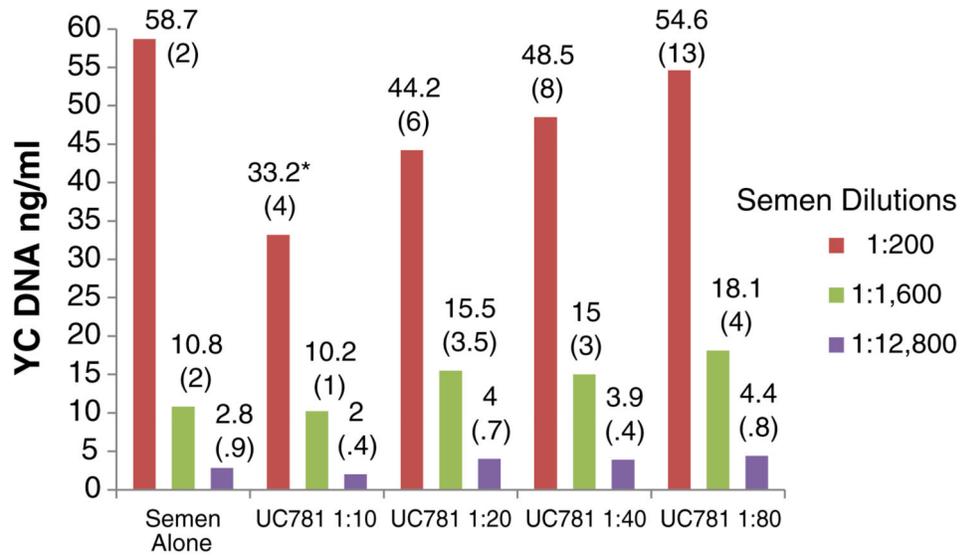


Fig. 4. In vitro effects of UC 781 on Y chromosome DNA detection by realtime PCR. The experiments were run in quadruplicate. The values presented in the graph are the means (standard deviations). Asterisks denote statistically significant difference from control ($p < .05$).

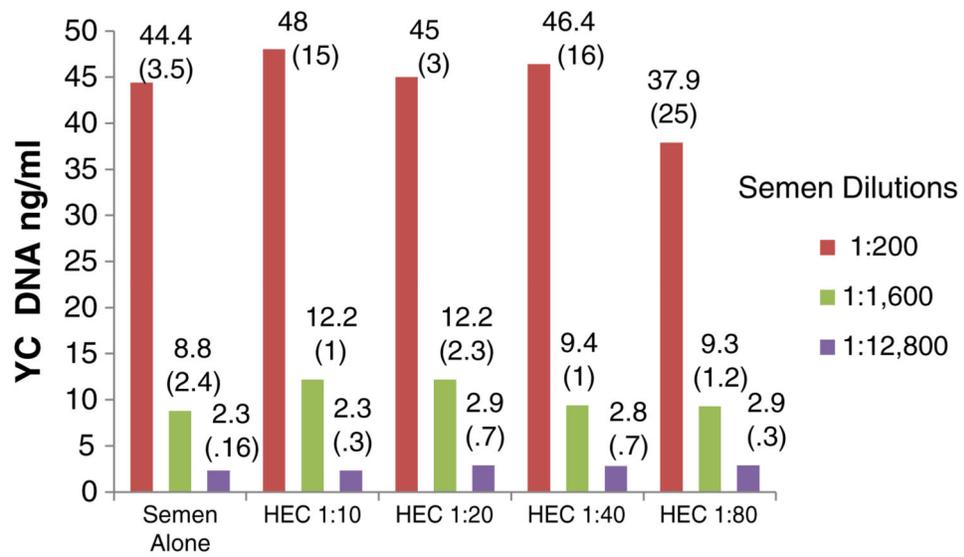


Fig. 5. In vitro effects of HEC on Y chromosome DNA detection by realtime PCR. The experiments were run in quadruplicate. The values presented in the graph are the means (standard deviations).

Table 1
Gels tested in vitro for their effects on PSA detection by the Abbott Architect and
ABAcad assays and Y chromosome DNA detection by real-time PCR

Product	Brand name (manufacturer)	Main ingredient	Type
TFV	Gilead Sciences	1% TFV with 2.5% hydroxyethylcellulose	Microbicide
UC781	Cellegy Pharmaceuticals	0.1% in methycellulose and carbomer 974P	Microbicide
HEC	ReProtect	2.5% hydroxyethylcellulose	Placebo

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Table 2
In vitro effects of TFV 1% gel, UC 781, and the HEC placebo on PSA detection by ABA card

Semen dilutions								
	1: 3,200	1: 51,200	1:102,400	1:204,800	1: 409,600	1: 819,200	No semen*	
Semen alone*	+	+	+	+/-	-	-	n/a	
TFV 1:10	i	i	i	i	i	i	i	i
TFV 1:20	+	+	+	+	+	+	+	+
TFV 1:20	+	+	+	+	+	+	+	+
TFV 1:40	+	+	+	+	-	-	-	-
UC 781 1:10	+	+	+	+	+	+	+	+
UC 781 1:20	+	+	+	+	+/-	-	-	-
UC 781 1:40	+	+	+	+	-	-	-	-
HEC 1:10	i	i	i	i	i	i	i	i
HEC 1:20	+	+	+	+	+	+	+	+
HEC 1:40	+	+	+	+	+/-	-	-	-

+, positive; +/-, weakly positive; -, negative; i, invalid; n/a, not applicable.

* Positive control: semen alone. Semen dilutions were tested without mixing with products. Negative control: no semen/product only. Products were tested without mixing with semen.