

Prostate-specific antigen in vaginal fluid after exposure to known amounts of semen and after condom use: comparison of self-collected and nurse-collected samples

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BACKGROUND: Prostate-specific antigen (PSA) in vaginal fluid indicates exposure to semen, and was used to assess condom effectiveness, although validity and reliability have not been fully evaluated. Our objective was to compare PSA in self-collected samples with samples collected by a nurse. **METHODS:** We conducted two studies, each with 100 women aged 18–48 years. In the first, a nurse exposed each participant to her partner's semen (10, 100 and 1000 µl), and nurse and participant collected samples. In the second, each participant sampled before and after using two male condoms (MC) and two female condoms (FC); a nurse collected another sample afterwards. **RESULTS:** PSA concentration increased with semen exposure, but was lower in nurse-collected samples. Both procedures were sensitive, almost 100% after exposure to 100–1000 µl of semen. PSA detection rates with MC and FC were 13% and 28% in self-collected samples, 8% and 9% in nurse-collected samples. Concordance between sample types was 93% with the MC (95% CI: 89%; 96%), 78% with the FC (95% CI: 72%; 84%). PSA decay between sampling times may explain higher values in self-collected samples. **CONCLUSIONS:** PSA is a highly sensitive surrogate endpoint for condom effectiveness studies. Self-collected and nurse-collected samples are equivalent, but sample collection timing is critical.

Keywords: condoms; semen exposure; PSA; women

Introduction

The male latex condom (MC) and the polyurethane female condom (FC) are the only contraceptive methods that offer dual protection against unintended pregnancy and reproductive tract infections (RTIs), including infection with the human immunodeficiency virus (HIV). The MC is effective in preventing HIV and unintended pregnancy (Pinkerton and Abramson, 1997; Davis and Weller, 1999; Warner *et al.*, 2004a,b), but the extent of risk reduction afforded by MC use for other RTIs is still unclear (Pinkerton and Abramson, 1997; National Institute of Allergy and Infectious Diseases, 2001; Holmes *et al.*, 2004; Warner *et al.*, 2004a,b; 2006). Although there is adequate evidence that the FC is effective in pregnancy prevention (Farr *et al.*, 1994; Trussell, 1998) and limited evidence that it is effective against certain RTIs (Soper *et al.*, 1993; Fontanet *et al.*, 1998; Feldblum *et al.*, 2001; French *et al.*, 2003), it is still unclear whether it is as effective as the MC. There is no direct evidence that the FC is effective against HIV.

The evaluation of the effectiveness of condom use in preventing RTIs faces serious methodological challenges

(National Institute of Allergy and Infectious Diseases, 2001; Holmes *et al.*, 2004; Warner *et al.*, 2006). Studies that rely on participant self-report to measure sexual activity and condom use are prone to bias (Zenilman *et al.*, 1995). In addition, information on the infection status of partners is often unknown. Because transmission occurs only with exposure to an infected partner, and the latter can be associated with condom use, confounding may lead to underestimating condom effectiveness (Warner *et al.*, 2004a,b; 2005). The difficulties in accurately assessing condom effectiveness justify the increasing interest in developing and evaluating objective markers of condom failure that target exposure to semen as a surrogate outcome for pregnancy or disease (Mauck *et al.*, 2007).

Detection of prostate-specific antigen (PSA) in vaginal fluid is a well-established marker for exposure to semen (Graves *et al.*, 1985). A method based on self-sampling of vaginal fluid before and after intercourse, coupled with PSA testing (Lawson *et al.*, 1998; Macaluso *et al.*, 1999) has been successfully employed in clinical trials of MC and FC use as a way to

assess effectiveness (Macaluso *et al.*, 2003, 2007; Galvão *et al.*, 2005). These and similarly designed studies (Walsh *et al.*, 1999, 2003) have documented the presence of PSA in vaginal fluid after condom use in the absence of any self-reported problems such as condom breakage or slippage, raising the concern that contamination of the sampling swab by the participant, rather than unreported condom failure, may explain such findings (Steiner *et al.*, 2003).

Contamination of a self-collected sample could occur if the swab tip came in direct contact with semen spilled on the participant's hands or other skin surfaces. To address this possibility, we sought to design a procedure that would not be prone to contamination, and compare samples obtained following this procedure with participant-collected samples. Sampling by a nurse in conditions similar to a gynecological exam appeared to satisfy the theoretical conditions for a gold standard, as it is easy to direct the nurse to avoid that the swab touch areas other than the vaginal cavity during sampling. On the other hand, we had no data on whether the PSA concentrations measured in samples collected by a nurse are equivalent the concentrations that would be obtained by a participant in the absence of contamination. Thus, the objectives of this investigation were (i) to assess whether PSA values obtained from self-collected vaginal samples are comparable with those obtained from nurse-collected samples in a context where contamination of the sampling swabs is not possible and (ii) to compare PSA values in self-collected and nurse-collected samples after sexual intercourse with MC and FCs, when self-collected samples may be subject to the risk of contamination.

Materials and Methods

Study population and design

The investigation was conducted at the Human Reproduction Unit, Department of Obstetrics and Gynaecology, Faculty of Medical Sciences, University of Campinas (UNICAMP), Campinas, Brazil, between 2006 and 2007. The protocol was approved by the Institutional Review Board and all participants signed an informed consent form prior to entering the study. Two studies were conducted with similar groups of clients of the same reproductive health clinic: (i) a study of semen exposure in a clinical setting, in which a woman was exposed to increasingly larger amounts of her partner's semen, and samples of vaginal fluid were collected both by the woman and by a nurse at specified times after each exposure, to evaluate the comparability of PSA values in use- and self-collected samples in the absence of contamination and (ii) a study of semen exposure during intercourse, in which a woman self-sampled vaginal fluid before and after using an MC or FC with her partner (in conditions where contamination of the samples is possible), and returned to the clinic where a nurse took an independent sample. The target number of participants in each study was 100. Clinic clients were eligible for the study if they were 18–49 years old, sexually active and engaged in a mutually monogamous relationship, had not used condoms as their primary birth control method for 1 year or longer, were willing to use both condom types, understood the study procedures and were willing to comply with them.

Study procedures

At the enrollment visit of the in-clinic semen exposure study, a nurse trained each woman on sample collection, and instructed her to avoid

sexual intercourse for at least 3 days, and return to the clinic with her partner, bringing a fresh sample of her partner's semen. Semen was obtained by the partner at home in a sterile plastic jar during the hour preceding the clinic appointment. At the clinic, the partner was tested for HIV infection using a rapid blood test (Determine® HIV 1/2, Abbott Laboratories, USA) and the semen was used only if the test was negative. The sensitivity and specificity of this HIV test are 100% and 97.5%, respectively. The semen was centrifuged at 400g to separate the seminal fluid and the spermatozoa and only the supernatant free of spermatozoa was introduced in the vagina.

The in-clinic study was conducted in ~4 h. A nurse took a baseline vaginal swab to be tested for PSA, to confirm adherence to the 3 day abstinence requirement. The nurse then deposited 10 µl of the partner's seminal fluid in the vagina using a micropipette, and after 15 min and at 3 h, first the participant and then the nurse each took a sample of vaginal fluid. Next, the nurse deposited 100 µl of semen in the vagina and 15 min later the woman and the nurse each took a sample following the same procedures. Finally, the nurse deposited 1000 µl of semen and 15 min later new samples were taken both by the woman and by a nurse. We could not exclude that the 10 µl exposure influenced the PSA measurement performed on samples taken after exposure to 100 µl, and so on (i.e. exposure carryover); we assumed, however, that the error was small, as each exposure was 10 times larger than the previous. Also, the participant always took her sample before the nurse, thus potentially reducing the amount of PSA detectable in the nurse sample.

In the at-home condom use study, women were trained by a nurse in self-sampling procedures, and were instructed to avoid sexual intercourse for at least 3 days and then use the study condoms during intercourse. Study participants were provided with a free initial supply of two FCs (Reality®, The Female Health Company, Chicago, IL, USA) or two latex pre-lubricated MCs (Elite, Blowtex, São Paulo, Brazil). The sequence of condom use (two MCs followed by two FCs or *vice versa*) was to be randomly assigned, but implementation of the protocol led systematically to distributing the MC first (see Results section). Women were instructed to collect a sample prior to intercourse and then a second sample immediately after, and to bring both samples back to the clinic together with the used condom as soon as possible, but always within 12 h after intercourse. At the clinic, a nurse took a new sample of vaginal fluid. After the participant returned the set of samples from the second condom use, the nurse provided her with two condoms of the other type. The study was completed when the participant returned to the clinic with samples from the fourth condom use, and the nurse took the final sample of vaginal fluid at the clinic.

Sampling device and procedures

The sampling device and procedures were the same as employed in previous studies and were designed to minimize the risk of contaminating the sample with semen from sources other than the vagina (Macaluso *et al.*, 1999; 2003). The sampling device is an 8 inch (20 cm) long swab with a 3/8 inch (~1 cm) rayon tip that slides out of a cardboard cylinder 4 inch (10 cm) long and 0.5 inch (~1.2 cm) in diameter. After inserting the cardboard cylinder with the tip of the swab hidden in the tube, the swab is gently sliced into the vagina by pushing the stem of the swab, maintaining the tube stationary, until resistance is felt. The swab is rotated five times to capture the vaginal fluid and retracted inside the tube before removing the tube from the vagina. In training the participant, the nurse emphasized the high sensitivity of the test for semen and that it was imperative that the tip of the swab be kept inside the cardboard tube until inserted and then retracted into the tube before removing the sampling device from the vagina to avoid the contamination of the swab.

To collect samples of vaginal fluid, the nurse first used a bivalve speculum to expose the vagina, and then collected the sample with a similar swab and precautions used by the participant. The nurse was directed to take extreme care in avoiding exposure of the swab tip to any surface other than the vaginal cavity of the participant. The swabs were packaged in sealed plastic bags with desiccant because PSA is stable in dry samples for several months (Armbruster, 1993).

Laboratory methods

The laboratory personnel processed and tested each sample without knowledge of exposure and sampling conditions (i.e. whether the sample was taken by the nurse or by the woman, after which amount of semen exposure or time after exposure, or before or after using a condom). Dried swabs were stored at 4°C until tested. The sample was extracted in 5 ml of 0.9% saline for 15 min. Fluid was expressed from the swab by rotating and pressing it against the side of the extraction tube, resulting in ~3–4 ml of eluent, which was stored at –20°C until assays. Eluents were thawed and tested with a PSA Immulite chemiluminescent immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay and inter-assay coefficients of variation were 6.1% and 9.3%, respectively. Because the upper limit of detection of the Immulite assay system is 150 ng/ml, we used dilutions of the eluent to determine the exact PSA concentration when the value of the undiluted sample exceeded 150 ng/ml. Because in previous studies that employed these methods, the swab was extracted using 3 ml saline, yielding a more concentrated eluent than in our procedure, all PSA values were multiplied by 5/3.

Data analysis

In both studies, the objective of the analysis was to compare PSA results from self-collected samples with those obtained from the samples collected by the nurse. In the in-clinic exposure studies, exposure to measured amounts of semen provided the appropriate gold standard for assessing the sensitivity of PSA detection in self- and nurse-collected samples. In addition, we evaluated concordance between PSA results in self-collected and nurse-collected samples.

In the in-clinic exposure study, we began by evaluating baseline samples, and excluded all information from women whose baseline sample had a PSA concentration >1 ng/ml, as this suggested recent semen exposure. Next, we evaluated the PSA values in the samples collected after exposure. In addition to evaluating the PSA concentration as a continuous variable, we classified PSA concentrations into categories that were employed in previous studies (Armbruster, 1993; Galvão *et al.*, 2005), as follows: (i) non-exposed (≤ 1 ng/ml); (ii) low (>1, <22 ng/ml); (iii) moderate (22–99 ng/ml) and (iv) high (≥ 100 ng/ml). In condom effectiveness studies that have employed similar sampling and assay conditions, PSA results ≤ 1 ng/ml are incompatible with recent exposure to semen (Macaluso *et al.*, 1999; 2003). In addition, PSA values <22 ng/ml may be due to low semen exposure levels, but may also be due to random variability in the difference between pre- and post-coital levels, whereas post-coital PSA levels above 22 ng/ml following pre-coital levels of <1 ng/ml are statistically incompatible with self-sampling variability. [In a previous study, 22 ng/ml was the 95th percentile of the difference between any two samples taken in rapid succession by the same woman 24 h after exposure to 1 ml of semen (Macaluso *et al.*, 2003).] The categories described above suggest two thresholds for defining a PSA-positive result: 1 and 22 ng/ml. We employed both thresholds to compute two alternative estimates of the sensitivity of the sampling and assay procedures. We used all four semi-quantitative categories to evaluate agreement between self-collected and nurse-collected samples.

In the at-home condom use study, the objectives of the analysis were to compare the percentage of sexual intercourses with positive PSA after using an MC or FC, and to compare the results between self-collected samples with samples collected by a nurse. For each condom use, the pre-coital sample taken by the participant served as the baseline. Thus, we interpreted a PSA-positive (≥ 1 ng/ml) pre-coital sample as indicating that exposure to semen occurred before the present condom use, and excluded both pre- and post-coital results from the analysis. Next, we evaluated the PSA concentrations in the post-coital samples taken by the participant immediately after sex, and by the nurse at the clinic, and classified these values using the categories described above for the in-clinic exposure study. Means and their 95% confidence intervals (CI) were the measures employed to evaluate the PSA concentration in samples as a continuous variable, and comparisons between means employed standard normal distribution assumptions. Because of the skewed distribution of PSA values, some analyses employed log-transformed values. Estimation of the sensitivity of the sampling and assay procedures and comparisons of categorized PSA values from self- and nurse-collected samples entailed computing proportions and their exact binomial 95% CIs. Hypothesis testing about differences in proportions employed χ^2 statistics. When appropriate, chance-adjusted agreement was measured using Cohen's kappa statistic, and Bowker's test of symmetry was used to determine whether discordant results were randomly distributed, i.e. whether there were as many instances in which the PSA value was higher in the self-collected sample than in the nurse-collected sample as there were instances in which it was higher in the nurse-collected sample, and whether the differences were as extreme.

Results

Recruitment continued in each study until the target number of 100 women completed the protocol. A total of 137 eligible women were invited to participate in the in-clinic exposure study. Of these, 32 refused (23 because the partner could not to leave the job to attend the clinic and 9 because the partner refused to donate semen) and 105 agreed to participate. Of these, five did not return to the clinic for their visit, and the target number of 100 women completed the protocol. All male partners were tested for HIV, and all were negative. A total of 145 eligible women were invited to participate in the at-home condom use study (women who participated in one study were not eligible for participation in the other). Of these, 17 refused (15 because the partner did not want to use condoms and 2 because she did not want to use FCs) and 128 agreed to participate. Of these, 18 never returned any condoms, 10 used fewer than four condoms, and the target number of 100 completed the protocol. The age of women who participated was (mean $\pm$ SD) 33.4 ± 0.7 years (range 18–46) in the in-clinic exposure study and 31.6 ± 0.7 years (range 21–48) in the at-home condom use study. All women were living with a stable partner.

Seven women in the in-clinic exposure study had a baseline PSA value ≥ 1 ng/ml and were excluded from the analysis. The mean PSA values ($\pm$ SE) of the partners' semen before and after centrifugation were $13\,293 \pm 816$ and 8423 ± 1183 ng/ml, respectively. There was a direct relationship between the amount of semen deposited in the vagina and the PSA value in self-collected and nurse-collected samples (Table I). The mean PSA concentration in the eluent from

Table I. PSA (ng/ml) in vaginal fluid samples taken by the user and by a nurse, after exposure to known amounts of semen, by amount and time since exposure, and sensitivity estimates (% with 95% confidence intervals) for two thresholds of detection.

Amount and time since exposure	Self-collected			Nurse-collected		
	Mean $\pm$ SE	Sens. (>1 ng/ml) (95% CI)	Sens. (>22 ng/ml) (95% CI)	Mean $\pm$ SE	Sens. (>1 ng/ml) (95% CI)	Sens. (>22 ng/ml) (95% CI)
10 μ l 15 min	282 $\pm$ 51	83 (75; 90)	66 (56; 75)	167 $\pm$ 29	91 (84; 96)	69 (59; 78)
10 μ l 3 h	29.8 $\pm$ 8.2	73 (63; 81)	31 (22; 41)	31.3 $\pm$ 14.4	67 (57; 76)	23 (15; 32)
100 μ l 15 min	5114 $\pm$ 824	99 (95; 100)	96 (90; 99)	1424 $\pm$ 193	100 (97; 100)	100 (97; 100)
1000 μ l 15 min	11 529 $\pm$ 1184	100 (97; 100)	100 (97; 100)	5525 $\pm$ 922	100 (97; 100)	100 (97; 100)

samples collected by the participant increased from 282 ng/ml (95% CI: 182; 382) 15 min after exposure to 10 μ l of the partner's semen, to 11 529/ml (95% CI: 9208; 13 850) 15 min after exposure to 1000 μ l. This relation was also evident for samples collected by the nurse, but the PSA values were systematically lower than those measured in self-collected samples. The mean difference in PSA values between self- and nurse-collected samples increased from 115 ng/ml (95% CI: 27; 203) after exposure to 10 μ l of semen to 5735 ng/ml (95% CI: 2911; 8559) after exposure to 1000 μ l.

The PSA concentration in vaginal fluid declines rapidly after exposure: samples collected by the participants 3 h after exposure to 10 μ l of semen yielded PSA concentrations (mean; 30 ng/ml; 95% CI: 14; 46) that were on average one order of magnitude lower than those of the samples they had collected 15 min after exposure. This relation was also evident in nurse-collected samples. However, at 3 h, there was no difference in PSA concentrations between self- and nurse-collected samples (mean difference: -1.5 ng/ml, 95% CI: -34 ; 18). Despite the systematic difference between PSA results in the self- and nurse-collected samples, the sensitivity of the two procedures was similar, and statistically indistinguishable when using either threshold. The data indicate that the overall sensitivity to detect exposure to 10 μ l was reasonably high: 83% (95% CI: 75; 90) and 91% (95% CI: 84; 96) in self- and nurse-collected samples, respectively, if all results >1 ng/ml were considered PSA-positive (Table I). However, sensitivity estimates varied depending on the PSA threshold used. The sensitivity to detect exposure to 10 μ l was lower if the more stringent threshold of 22 ng/ml was used: 73% (95% CI: 63; 81) and 67% (95% CI: 57; 76) in self- and nurse-collected samples, respectively (Table I).

Sensitivity was much higher after exposure to 100 μ l of semen, and was between 96% and 100% for both sampling methods and at either threshold after exposure to 1000 μ l. The statistical precision of these estimates was also very high: the lower 95% confidence boundary of the sensitivity estimates after exposure to 100–1000 μ l ranged from 90% to 97% (Table I).

Analysis of the concordance of categorized PSA values indicated a moderate to high degree of agreement between self-collected and nurse-collected samples, which was dependent on the amount of exposure: the proportion of paired samples that were classified in the same PSA category increased from 39% (95% CI: 29%; 49%) for samples collected 15 min after exposure to 10 μ l of semen, to 98% (95% CI: 93%; 100%) for samples collected 15 min after exposure to 1000 μ l of semen (Table II). In fact, whereas about one-third of the samples collected 15 min after exposure to 10 μ l of semen would be classified as PSA-negative or of uncertain significance (<22 ng/ml), very few samples collected by either the woman or the nurse after exposure to 100 or 1000 μ l of semen fall in these categories. The kappa statistic values decreased from 0.46 (95% CI: 0.34; 0.58) to virtually zero for the same sets of samples, reflecting the lack of variability in the categorized PSA values after exposure to 100 and 1000 μ l of semen. The kappa statistic compares the observed agreement with that which is expected on the basis of the marginal distributions: as the amount of exposure increased, the proportion of samples yielding high PSA values increased to almost 100%, so that the very few discordant pairs of PSA values have an inordinately high influence in the calculations. The symmetry test was significant only for samples collected after exposure to 10 μ l of semen, suggesting that the

Table II. Concordance of categorized PSA concentrations in self-collected samples and samples collected by a nurse (categories in ng/ml: ≤ 1 ; >1 ; <22 ; 22–99; ≥ 100), by exposure amount (μ l of semen) and time since exposure.

	Exposure amount and time since exposure			
	10 μ l, 15 min	10 μ l, 3 h	100 μ l, 15 min	1000 μ l, 15 min
Concordant	46	55	82	91
Self-collected $>$ Nurse-collected	24	31	4	2
Nurse-collected $>$ Self-collected	23	10	7	0
Total samples	93	93	93	93
Percent concordant (95% CI)	39 (29, 49)	59 (49, 69)	88 (80, 94)	98 (93 100)
Kappa (95% CI)	0.46 (0.34, 0.58)	0.50 (0.37, 0.62)	0.07 (-0.14 , 0.27)	NC
Bowker's symmetry test (<i>P</i> -value)	0.06	0.04	NS	NS

NC, not calculated; NS, not significant.

Table III. PSA in vaginal samples taken by the user and a nurse, before and after sexual intercourse with MC and FC.

	MC		FC	
	N	%	N	%
PSA detected in the sample taken before coitus	10	5	20	10
Post-coital samples included in analysis	190	95	180	90
Self-collected post-coital samples with PSA >1 ng/ml	25	13	51	28
PSA concentration (ng/ml), mean $\pm$ SE*	4177 $\pm$ 2118		1852 $\pm$ 1044	
Nurse-collected post-coital samples with PSA >1 ng/ml	16	8	16	9
PSA concentration (ng/ml), mean $\pm$ SE*	3014 $\pm$ 1541		3732 $\pm$ 2350	

*Computed only among post-coital samples with PSA >1 ng/ml.

systematically higher PSA values measured in self-collected samples did not have a major effect on categorized PSA values for exposures to 100 and 1000 μ l of semen (Table II).

Of the 100 women who completed the at-home condom use study, 92 used two MCs followed by two FCs and 8 used two FCs followed by two MCs. The PSA concentration was low in most of the 400 pre-coital samples taken by the participants (90% of values <0.4 ng/ml, 75% <0.07 ng/ml), indicating good adherence to the 72 h abstinence requirement. The pre-coital PSA value was $\geq$ 1 ng/ml in 30 samples, and the exclusion of these possible protocol violations left 370 sets of post-coital samples available for analysis (190 after using an MC and 180 after using an FC). The mean lag between intercourse and self-sampling according to the participants record of the time of intercourse and the time of self-sampling in all cases was 24 min, with 90% of the samples collected within 38 min. The time of sample collection at the clinic was missing for 55 condom uses: the average lag between intercourse and nurse sampling was 2 h 20 min, with 90% of the samples collected between 1 h 20 min and 3 h 20 min.

The post-coital PSA value was higher than 1 ng/ml in 25 (13%) of the 190 samples collected by the woman at home after using an MC; the mean PSA concentration in these samples was 4177 ng/ml (95% CI: 26; 8328) (Table III). Of the corresponding 190 samples collected by the nurse at the clinic, 16 (8.4%) were PSA-positive, with a mean PSA concentration of 3014 ng/ml (95% CI: 0; 6034). The number of PSA-positive post-coital samples collected by the woman was larger after using an FC: 51 of 180, or 28%, with a mean PSA concentration of 1852 ng/ml (95% CI: 0; 3898). Of the corresponding nurse-collected samples, only 16 (8.9%) were PSA-positive, with a mean PSA concentration of 3732 ng/ml (95% CI: 0; 8338) (Table III).

The pattern of categorized PSA values showed that there was no evidence of semen exposure (PSA $\leq$ 1 ng/ml both in the self-collected and in the nurse-collected post-coital sample) after using 161 (85%) MCs, and after using 128 (71%) FCs (Table IV). Concordance between self- and nurse-collected samples was significantly higher after using an MC (88%, 95% CI: 83%; 92%) than after using an FC (78%, 95% CI: 69%; 82%). Because of the variability in the distribution of categorized PSA values, the good agreement between self- and nurse-collected samples was well reflected by the kappa statistics. The symmetry statistics indicated that self-collected samples yielded systematically higher PSA values than nurse-

collected samples after using either an MC ($P = 0.04$) or an FC ($P < 0.0001$) (Table IV).

To evaluate the hypothesis that contamination may bias PSA measurement in self-collected samples, we compare the pattern of concordance observed in Table IV with the concordance of PSA values between samples collected by the woman 15 min after exposure to 10 μ l of semen, and the corresponding samples collected by the nurse 3 h later (Table V). These data from the in-clinic exposure study cannot be influenced by contamination of the samples and well approximate the time interval between the woman's collection of a sample at home immediately after using a condom and the nurse's collection of the sample at the clinic a few hours later, as it occurred in the at-home condom use study. The data suggest that if every condom use entailed the leakage of 10 μ l of semen, the concordance between the sample collected by the woman shortly after using the condom and the sample collected later by the nurse would be much lower than observed, and highly skewed toward higher PSA concentrations in self-collected samples than in nurse-collected samples. Thus, the results of the at-home condom use study are compatible with the expected

Table IV. Concordance of PSA concentrations in self-collected samples.

	Condom type	
	Male latex condom	Female condom
Concordant	167	137
Self-collected > Nurse-collected	18	41
Nurse-collected > Self-collected	5	2
Total samples	190	180
Percent concordant (95% CI)	88 (83, 92)	76 (69, 82)
Kappa (95% CI)	0.55 (0.37, 0.73)	0.47 (0.32, 0.53)
Bowker's symmetry test (P -value)	0.04	<0.0001

Table V. Concordance of PSA concentrations (categories in ng/ml: $\leq$ 1; > 1; <22; 22–99; $\geq$ 100), 15 min (self-collected) and 3 h (nurse-collected) samples after exposure to 10 μ l of semen.

Concordant	24
Self-collected > Nurse-collected	64
Nurse-collected > Self-collected	5
Total samples	93
Percent concordant (95% CI)	26 (18, 35)
Kappa (95% CI)	0.19 (0.10, 0.28)
Bowker's symmetry test (P -value)	<0.0001

pattern of decay of PSA concentration in vaginal fluid during the time interval occurring between sample collection by the woman and by the nurse.

Discussion

The findings of this study suggest that self-sampling of vaginal fluid was equivalent to sampling done by a nurse in obtaining information on recent exposure to semen, as measured by PSA. This is consistent with the results of studies that compared self-sampling with healthcare provider sampling with respect to the detection of *Chlamydia trachomatis* (Schachter *et al.*, 2003), Group B streptococcus (Price *et al.*, 2006; Arya *et al.*, 2008) and human papillomavirus infection (Karwalajtys *et al.*, 2006; Jones *et al.*, 2007; Safaeian *et al.*, 2007). In the present investigation, the in-clinic exposure study provided a gold standard against which to assess the sensitivity of the two sampling procedures. The results indicated that the sensitivity of PSA detection in both types of sample collected shortly after exposure to semen was close to 100%, especially after moderate–high exposure levels (100 or 1000 μ l of semen), even when the threshold of detection was high (22 ng/ml). The largest amount of semen deposited in the vagina is of the same order of magnitude as the average exposure during unprotected sexual intercourse, 3.3 ml (Roy, 1994). Exposure to the smaller amounts tested (10 and 100 μ l) may occur after a small puncture of the condom or after breakage or complete slippage, respectively.

The sensitivity of PSA detection was close to 100% after exposure to 100 μ l, but was markedly lower (66–91%) after exposure to 10 μ l, especially when the threshold of detection was set at 22 ng/ml. Uneven distribution of small amounts of semen in the vagina, yielding non-uniform PSA concentration, individual variability in the dilution of semen in vaginal fluid, variability in the sampling technique and possibly other factors may explain less accurate PSA detection after exposure to very small amounts of semen. These data confirm previous findings that PSA detection in vaginal fluid is a highly sensitive method for ascertaining recent exposure to semen and that PSA testing is very unlikely to be negative when a sample of vaginal fluid is taken shortly after exposure to even relatively modest amounts of semen (Macaluso *et al.*, 1999). The finding that the average PSA concentration in samples taken 3 h after exposure to 10 μ l was one order of magnitude lower than the concentration measured in samples collected 15 min after exposure also confirms that PSA is rapidly eliminated from the vagina (Macaluso *et al.*, 1999). Thus, in condom effectiveness studies, or in intervention studies using semen exposure as the outcome, it is important that the post-coital samples be collected as soon as possible after intercourse. Analyses based on quantitative PSA measurement or semi-quantitative categories indicate that in the in-clinic exposure study, despite similar sensitivity, self-collected samples systematically yielded higher PSA values than nurse-collected samples. Whereas we cannot exclude that, in this study, the participants were better than the nurses at collecting vaginal samples, a prudent interpretation is that the women had a higher likelihood of success in sampling from the vaginal pool because they always sampled

before the nurse. In addition, especially after exposure to such a small amount as 10 μ l, mechanical removal of semen by the swab may have decreased the amount of PSA available for sampling by the nurse. Thus, who sampled first may have been more important than who sampled, and it seems reasonable to conclude that under conditions in which contamination cannot occur, samples of vaginal fluid collected by a nurse would yield the same PSA values that would be obtained from samples collected by the participant. This conclusion provides the necessary background for interpreting the results of the at home condom use study.

The frequency of PSA detection in post-coital samples taken after MC or FC use is consistent with the observations of other studies that have employed the same methods (Macaluso *et al.*, 2003; 2007; Galvão *et al.*, 2005). The PSA (>1 ng/ml) detection rate of 28% observed in the present study with the FC is slightly higher than the 22% observed in a previous randomized trial carried out at UNICAMP (Galvão *et al.*, 2005), and the rates of 21% (Macaluso *et al.*, 2003) and 17% (Macaluso *et al.*, 2007) observed in two studies carried out at the University of Alabama at Birmingham (UAB). As in previous studies, a large proportion of PSA-positive post-coital samples had low PSA levels of uncertain significance (1–21 ng/ml). The 13% PSA detection rate observed with the MC is in close agreement with the 15% rate observed in the previous UNICAMP study (Galvão *et al.*, 2005) and the 14% rate observed in the UAB study (Macaluso *et al.*, 2007). Two other studies that have used PSA as a marker of condom failure have reported lower rates of PSA detection after MC use (Walsh *et al.*, 1999; 2003). Those studies, however, employed a less sensitive PSA assay system, and could not have documented the low and moderate levels of PSA (<100 ng/ml) observed in the present study.

In the aggregate, these results suggest that exposure to semen is more frequent during FC use than during MC use. On the other hand, the difference is mainly due to more frequent detection of PSA at low levels, which previous research indicates may be due to sampling variability or to exposure to small amounts of semen that have dubious significance for pregnancy or disease risk. The rate of moderate–high PSA ($\geq$ 22 ng/ml) detection with the FC was 12% in this study, 7% in the first UAB study (Macaluso *et al.*, 2003) and 8% and 5% in the UNICAMP and UAB trials, respectively, according to a recent analysis that compared the data from the two studies (Chen *et al.*, 2007). The corresponding rates for the MC are 6% in the present study, and 6% and 3% in the UNICAMP and UAB trials, respectively (Chen *et al.*, 2007).

We interpret the different PSA detection rates with the FC and the MC study results to indicate that vaginal exposure to very small amounts of semen occurs more commonly during use of the FC than during use of the MC, leading to a relatively high frequency of low-level PSA results, and that the PSA signal rapidly fades away after intercourse and is no longer detectable in samples collected a few hours later. The pattern of discordance between self-collected and nurse-collected samples after FC use is consistent with what would be expected after real exposure to very small amounts of semen, whose PSA signature faded away soon after intercourse.

The alternative explanation, i.e. that contamination of the sampling device is an explanation for many low PSA values, requires a more complex explanation. First, contamination must occur more often with the FC than with the MC. Although we have no data in support of this necessary assumption, we note that even if the opportunity for contamination were higher with the FC than with the MC, violation of the sampling procedure and exposure of the tip of the swab outside the vagina would have to occur at a high rate, in order to explain the large number of low-level PSA results. The self-sampling procedures are acceptable and easy to follow, and few participants report problems with sampling (Macaluso *et al.*, 2003). Thus, it seems unlikely that lack of adherence to the self-sampling instructions would have caused many false-positive low-level PSA results.

Finally, we note that comparison of the PSA results observed in the condom study with the results after exposure to measured amounts of semen clearly shows that exposure to semen is a relatively rare outcome of condom use. In fact, whereas between 79% and 88% of samples collected after exposure to 10 μ l of semen and essentially all samples collected after exposure to 100 μ l were PSA-positive, only 13% of samples collected after MC use and 28% of samples collected after FC use were PSA-positive.

Our study has some limitations. We did not randomly assign the sequence of sampling in the in-clinic exposure study, and the systematic difference in PSA results between self-collected and nurse-collected samples is confounded with the effect of the sampling sequence. Thus, it would not be appropriate to use the results of this study to conclude that self-sampling is more accurate than nurse sampling in the context of assessing semen exposure. Despite this limitation, the study provides evidence that both sampling methods are highly sensitive. In fact, even with exposure to minute amounts of semen, most samples collected within 15 min are positive for PSA. Another limitation is that the sequence of condom use was not randomized in the at-home condom use study. However, this problem is unlikely to have biased the comparison between nurse- and self-collected samples within condom type. The most severe limitation of this study was that despite the stringent requirement that the participants return to the clinic soon after intercourse, and the reasonably good adherence with the requirement, the time lag between the two samples was long enough to bias the comparison, and yielded many discrepant results, in which the self-collected sample was positive for PSA whereas the sample collected by the nurse was negative. This is problematic, because the study was designed to address the possibility that contamination may lead to false-positive PSA results in self-collected samples. On the other hand, the in-clinic exposure study provided the right framework for evaluating the discrepancies between self-collected and nurse-collected samples in the condom use study.

The study also had important strengths. To our knowledge, it is the first to compare alternative sampling strategies for assessing exposure to semen and to assess the potential impact of contamination on the frequency of low-level PSA results. Its size provided ample power to detect differences between sampling methods and between PSA detection rates after exposure to measured amounts of semen or after the use of MCs or FCs. Although its focus was methodological, the

investigation also provided good evidence on the effectiveness of the FC and the MC as barriers to semen during intercourse.

In conclusion, our study shows that the self-sampling procedures used in condom effectiveness studies that employ exposure to semen as a surrogate endpoint are highly sensitive, and that self-collected samples are comparable with nurse-collected samples with respect to the detection of exposure to semen. The findings also indicate that time of sample collection is a critical factor determining the sensitivity of the method and suggest that contamination of the sampling device with semen from sources other than vaginal fluid is unlikely to significantly bias the findings of these studies. Finally, this study confirms previous observations about the effectiveness of condoms as barriers to semen, and while showing that low-level semen exposure is more likely to occur with the FC than with the MC, it also confirms that using condoms greatly reduces the likelihood of exposure to semen resulting in unintended pregnancy or disease transmission.

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