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Detection of two biological markers of intercourse: prostate-specific antigen and Y-chromosomal DNA

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Abstract

Background—Although biological markers of women's exposure to semen from vaginal intercourse have been developed as surrogates for risk of infection or probability of pregnancy, data on their persistence time and clearance are limited.

Study Design—During 2006–2008, 52 couples were enrolled for three 14-day cycles of abstinence from vaginal sex during which women were exposed in the clinic to a specific quantity (10, 100 or 1000 μL) of their partner's semen. Vaginal swabs were collected before and at 1, 6, 12, 24, 48, 72 and 144 h after exposure for testing for prostate-specific antigen (PSA) and Y-chromosome DNA (Yc DNA).

Results—Immediately after exposure to 1000 μL of semen, the predicted sensitivity of being PSA positive was 0.96; this decreased to 0.65, 0.44, 0.21 and 0.07 at 6, 12, 24 and 48 h, respectively. Corresponding predicted sensitivity of being Yc DNA positive was 0.72 immediately postexposure; this increased to 0.76 at 1 h postexposure and then decreased to 0.60 (at 6 h), 0.63 (at 12 h), 0.49 (at 24 h), 0.21 (at 48 h), 0.17 (at 72 h) and 0.12 (at 144 h).

Conclusions—Overall findings suggest that PSA may be more consistent as a marker of very recent exposure and that Yc DNA is more likely to be detected in the vagina after 12 h postexposure compared to PSA.

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Keywords

Clearance; Prostate-specific antigen; Semen biomarkers; Y-chromosome DNA

1. Introduction

Traditionally, biologic and behavioral studies related to sex and contraceptive efficacy rely on self-reports of sexual behavior and condom use, which are susceptible to bias [1–3]. Difficulties in accurately assessing condom and microbicide effectiveness have led to the development of objective markers of exposure to semen as surrogate outcomes for risk of infection or probability of pregnancy [4]. Prostate-specific antigen (PSA), the most widely used and extensively characterized biomarker of semen exposure, occurs at high concentrations in seminal fluid. Although PSA can occur in other body fluids, concentrations of endogenous PSA identified in vaginal fluid are too low to be misclassified as resulting from semen exposure [5,6]. The detection of PSA in vaginal fluid in concentrations ≥1 ng/mL is indicative of exposure to semen exposure within the previous 48 h [7–10]. A second biomarker involves detecting Y-chromosome DNA (Yc DNA) fragments from spermatozoa in vaginal fluid. Using a polymerase chain reaction (PCR) assay, Yc DNA has been found in vaginal swabs collected up to 15 days after unprotected intercourse, with a half-life for clearance of 3.8 days [11,12].

Each of the two biomarkers could be preferable in different research studies depending on the clearance time desired and whether the focus is on the probability of pregnancy or infection. That is, because PSA is expressed independently of spermatozoa, it remains useful as a measure of risk of infection (but not of pregnancy) from exposure to semen from vasectomized males or those with low sperm counts [13]. In contrast, Yc DNA is only detected when spermatozoa are present, which makes it a useful measure of probability of pregnancy. Sexually transmitted infection (STI) risk, though, may be missed if the swab for Yc DNA testing captures only preejaculate or seminal fluid without any spermatozoa.

Our primary objective was to characterize and compare the rates of decay of PSA and Yc DNA in vaginal fluid specimens and the corresponding sensitivity of specimens being PSA and Yc DNA positive at specific times after women were exposed to varying amounts of semen. Our secondary objective was to assess agreement between PSA and Yc DNA at these intervals. Because of the potential for wide variations in women’s baseline vaginal environment, couples' sexual practices and other factors that might compromise such comparisons, the best experimental conditions for comparing PSA and Yc DNA would involve measuring these biomarkers in the same samples of vaginal fluid. Accordingly, we measured PSA and Yc DNA from the same vaginal swab collected after known quantities of semen exposure and known timing since exposure.

2. Materials and methods

2.1. Study population

The study enrolled 52 couples who met the following inclusion criteria: >18 years of age; in a mutually monogamous, heterosexual relationship for ≥ 1 year; using effective nonbarrier method of birth control or intending to conceive; willing to abstain from intercourse during three 2-week “study cycles”; and not enrolled in a full-time undergraduate program. Additionally, female partners were experienced with tampon use and had regular menstrual periods. Couples were excluded if they reported using condoms >50% of the time in the past year; a history of sexually transmitted disease in the past 2 years; or a history of emotional, sexual or physical abuse within the relationship.

2.2. Study design

After an initial enrollment visit, during which female partners were trained on self-collection of vaginal swabs, each couple contributed three 14-day study cycles of abstinence. Study cycles were scheduled to begin just after the female partner completed her menstrual cycle. On day 8 of each study cycle, male partners self-collected semen samples a maximum of 1 h before female partners presented to the study clinic for semen exposure. At the clinic, a clinician used an artificial insemination catheter to deposit a specific quantity (10, 100 or 1000 μL depending on the study cycle) of the male partner’s semen into the woman’s vagina. These quantities were selected to be consistent with past research [9,10]. The highest quantity used was about one third of a typical volume of ejaculate [14], while the lower semen amounts might be similar to the magnitude of an exposure from a condom malfunction. In most cases, the order of exposures was sequential from lowest to highest. We used an intrauterine catheter (MedGyn Products, Inc.; Addison, IL, USA) without a speculum to introduce the semen sample into the vagina without making any special effort for its even distribution. Participants did not ambulate before the first swab collection (immediately after exposure), but resumed usual activities before subsequent swab collection. Female partners self-collected vaginal fluid specimens using a double-headed swab (Starswab II; Starplex Scientific, Cleveland, TN, USA) before and immediately after semen exposure and at 1, 6, 12, 24, 48, 72 and 144 h postexposure.

Participants completed detailed daily diaries during the 14-day study cycles. Diaries asked about vaginal intercourse, condom use, oral sex, anal sex, use of sex toys and use of vaginal products. Participants were counseled extensively on the importance of providing valid self-reports, and they were advised that they would still receive the modest study reimbursement for study participation even if they reported deviating from study procedures such as abstinence.

Institutional review boards at The Johns Hopkins University (JHU) School of Medicine and US Centers for Disease Control and Prevention (CDC) approved the study.

2.3. Laboratory methods

Immediately following sample collection, all swabs were placed in a resealable bag with two dessicants, and swabs were stored at room temperature until processing. The double-headed

swabs were rehydrated in 1 mL of phosphate-buffered saline (PBS) for 20 min. Following rehydration, the samples were centrifuged for 5 min at 8000 rpm, and all of the supernatant except for 100 μL was removed and frozen for later PSA testing. The remaining pellet was processed for Yc DNA at JHU using a differential extraction protocol and was tested by real-time PCR on the LightCycler v1.2 system (Roche Diagnostics, Indianapolis, IN, USA) as previously described [15]. The semen was not processed. In the first cycle, presence of sperm was verified by microscopy, but we did not perform semen analysis or sperm counts.

Frozen specimens packaged on dry ice were sent via overnight delivery to the CDC laboratory. Upon their receipt, specimens were stored at −80°C until testing, at which point they were allowed to sit at room temperature (68°C to 77°C) until visibly thawed (approximately 10 min). A technician vortexed the thawed specimens (in cryotubes) for 3–5 s and pipetted 200 μL of the specimen into a sample cup to test with the Abbott Architect Total PSA assay (Abbott Diagnostics, Abbott Park, IL, USA). Samples exceeding the upper limit of quantification of the assay (100 ng of PSA per mL) were diluted with PBS and retested in order to quantify the concentrations. The lower limit of detection for Yc DNA is 10 copies, and there is no upper limit because the assay is not saturable.

2.4. Statistical analysis

We calculated and compared the sensitivity of vaginal fluid specimens being PSA and Yc DNA positive at specific times over the course of 6 days after women were exposed to varying amounts of their partner's semen. The natural logarithm transformation was used for all longitudinal modeling of quantitative values of PSA and Yc DNA. To create categorical outcomes for the two biomarkers, a positive PSA result was defined as ≥1 ng/mL [10] and a positive Yc DNA result was defined as >0 (detection of any Yc DNA).

Quantitative values of each biomarker were plotted against scheduled time for self-sampling following semen exposure (separately for the three deposited amounts of semen) using a linear mixed-effects model to account for repeated measurements. Each plot was depicted with a fitted curve and, to help visually assess the fit of the curve, a nonparametric smoothed spline curve. Models were fitted for each biomarker and each quantity of semen for the 0–6-day interval following exposure to semen (144-h models). Additionally, because nonstudy semen exposures were considered more likely to occur later in the study cycle (when couples might have more difficulty continuing to abstain from vaginal sex), models also were constructed for the shorter interval of 0–3 days postexposure (72-h models). Although initial PSA and Yc DNA concentrations were determined for each cycle, these data were not used in the calculation of the decay curves.

Sensitivity analyses were conducted to evaluate the effect of each of the following: (a) participants who failed to provide at least 1000 μL of semen for the study cycle requiring this amount (n=4 couples); (b) study cycles in which participants reported failure to adhere to abstinence from vaginal sex (n=1 couple for the 10-μL cycle, n=2 couples for the 100-μL cycle, n=4 couples for the 1000-μL cycle); (c) study cycles that were characterized by positive biomarker values (Yc DNA and/or PSA) for swabs collected immediately before semen exposure (n=7 couples for the 10-μL cycle, n=8 couples for the 100-μL cycle, n=11 couples for the 1000-μL cycle) and (d) observations with missing values for the exact time

since exposure (n=1 couple for the 10-μL cycle, n=2 couples for the 100-μL cycle, n=2 couples for the 1000-μL cycle). To determine the effect of differing amounts of semen deposited, a continuous covariate for the actual amount was added to the 1000-μL mixed-effects models. For failure to adhere to abstinence, we both excluded observations accompanied by self-reported vaginal sex and included them in main models with a binary covariate indicating self-reported vaginal sex. Similarly, for study cycles with positive values for Yc DNA or PSA immediately before semen exposure, we both excluded these cycles and included them in main models with a binary covariate indicating positivity for either biomarker. Finally, to address the issue of missing values for self-reported time since exposure, we added a binary covariate indicating this, and we fit a model that used self-reported time of swab collection when they were available and imputed prescribed collection times when these data were not available.

We calculated the predicted sensitivities for positive PSA and Yc DNA results (and the difference between the two) at each time point with 95% confidence intervals (CIs) using generalized linear mixed-effects models with a logit link function.

3. Results

The decay in biomarker values over time since exposure to semen is presented in Fig. 1 [log(PSA)], Fig. 2 [log(Yc DNA)] and Table 1. Both the PSA and Yc DNA data were heavily skewed, especially for time points closest to exposure. The fitted postexposure baseline values of biomarker concentration (i.e., the intercepts of the fitted curves) tended to increase with the amount of semen deposited; however, postexposure baseline values for the Yc DNA models were similar for the 100-μL and 1000-μL exposures. For both biomarkers, regardless of truncation, the rates of decay tended to be lower in the 10-μL models and similar in the 100-μL and 1000-μL models. The curves better fit the data in the 72-h models, as evident by the differences in the smoothed curves, as compared to the 144-h models, which were influenced by outliers (e.g., see dispersion of the PSA data for 100 μL at 144 h postexposure).

Predicted sensitivities for positive PSA and Yc DNA results at each time point are presented in Table 2 and depicted in Fig. 3. For 1000 μL, the largest amount of exposure in this study, the sensitivity of being PSA positive, which began at 0.96 immediately after exposure, decreased to 0.44, 0.21 and 0.07 within 12, 24 and 48 h, respectively. At 48 h, which has been considered to constitute the end of the window for PSA positivity, the highest sensitivity detected was 0.07. At 144 h, the sensitivity of being PSA positive was 0.09, which represented an unexpected increase from 0.07 at 72 h.

The sensitivity of being Yc DNA positive with a 1000-μL exposure, which was 0.72 immediately post-exposure, increased after 1 h to 0.76, after which it fluctuated and then decreased to 0.49, 0.21, 0.17, and 0.12 at 24, 48, 72 and 144 h, respectively. At 144 h, which is typically considered to be within the window for Yc DNA positivity, the highest sensitivity that is consistent with our results was 0.12.

Immediately after exposure, the two biomarkers had similar sensitivities of detecting semen in the 10-μL semen cycle and slightly higher sensitivities of detecting PSA than Yc DNA in the 100- and 1000-μL semen cycles (Table 2). From 1 to 12 h after exposure in the three cycles, the sensitivities of detecting semen were either similar or higher for Yc DNA in all three cycles. For 24–48 h after exposure, the sensitivity of detecting semen for Yc DNA was significantly higher for all three cycles. By 72 and 144 h, the detected semen levels were detected at slightly higher frequency for Yc DNA, but this difference was not statistically significant.

3.1. Sensitivity analyses

Among the 1000-μL semen cycles, the decay curve fitting was not appreciably influenced by the few cases in which the exposure volume was actually less than 1000 μL (all parameter changes were <10%).

The covariate for self-reported vaginal sex during the study was statistically significant for the two models for PSA after exposure to 1000 μL of semen; however, lack of significant interaction with time since exposure indicated that the rate of decay was unaffected. When we removed self-reported exposures from the models, no parameters changed >10%, other than the postexposure baseline values, which increased in the 144-h PSA models after 1000 μL of semen exposure and in the 72- and 144-h Yc DNA models after 10 μL of semen exposure.

The covariate for being preexposure positive was significant only in the 144-h model after 10 μL of semen exposure for PSA; again, lack of significant interaction with time since exposure showed that the rate of decay was not significantly affected. Nevertheless, when we removed all preexposure positive study cycles from the models, the rate of decay in the 144-h model after 100 μL of semen exposure for PSA became more extreme and that of the 144-h model after 10 μL of semen exposure for Yc DNA became less extreme. Nearly all fitted postexposure baseline values decreased by >10% when we excluded preexposure positive cycles, which, by definition, began with higher values of PSA and/or Yc DNA.

Covariates representing missing values for self-reported time since exposure were nonsignificant in all models, and the fitted curves that used self-reported swab collection times were not appreciably different from those that included imputed times.

4. Discussion

In our study, when women were exposed to the larger volumes of semen (100 or 1000 μL), PSA was consistently detected immediately postexposure, frequently detected at 1 h pos-exposure, less consistently detected at later times and only rarely detected by 48 h pos-exposure. Yc DNA positivity declined more slowly but nevertheless was rarely detected by 48–144 h after exposure to 10, 100 or 1000 μL of semen. PSA was detected more often than Yc DNA immediately after exposure, Yc DNA was detected more frequently during 1–24 h after exposure, and the detection of the two biomarkers did not differ at 72 and 144 h after exposure.

For each biomarker, rates of decay were similar for 100-μL and 1000-μL exposures but not for the 10-μL exposure. We consider the 10-μL exposure to be different from higher doses in several important ways. First, 10 μL corresponds to less than a drop of semen, which easily could be missed by a vaginal swab even if specimen collection occurred immediately after exposure. Furthermore, if the sample had not dispersed within the vagina, the initial swabs could have removed most of the sample. The multiple swab collections also could have substantially decreased the volume of semen present at subsequent time points. The low predicted sensitivities of biomarker positivity following exposure to 10 μL of semen suggest that, for condom failures resulting in only minute exposures to semen, PSA and Yc DNA would be detectable only inconsistently. However, the probability of pregnancy or STI from these low levels of exposure might be unlikely [16].

The present findings differ somewhat from previous studies of the decay rates of the two biomarkers. Two controlled trials using a similar protocol of depositing specific quantities of semen from the woman's male partner into her vagina found higher sensitivities of detecting PSA immediately following exposure (100% and 82%–98% [9]) but then a quicker clearance, with PSA cleared from almost all specimens (97%–99%) collected 48 h after exposure [10]. The two prior trials used a larger (1-mL capacity) swab that has been shown in vitro to yield significantly higher mean concentrations of PSA (3–185 times higher depending on the duration of freezer storage before testing) than the swab used in the present study [17]. Also, the concentrations of PSA in the present study could have been further diluted given differences between studies in extraction processes.

An earlier study of Yc DNA, in which women were instructed to self-swab at regular intervals after having an act of unprotected sex with their male partner, found higher sensitivities of Yc DNA among nonmenstruating women: Yc DNA was detected in 87%, 18% and 13% of specimens collected at 1, 5 and 7 days following exposure [18]. Similarly, a second study, in which women self-swabbed following an unprotected act, found mean concentrations of Yc DNA that were substantially higher than those in the present study [11]. These differences in Yc DNA findings might be explained by the use of unprotected sex as the source of exposure in the earlier studies (rather than controlled applications of semen by a clinician), which might have resulted in exposure to higher quantities of semen or local changes induced by sexual activity, such as lubrication or blood flow, which then influenced biomarker persistence or detection.

To our knowledge, no other published clinical studies with known levels of semen exposure have assessed the level of agreement between the two biomarkers or have compared their rates of decay. In an earlier study, 264 women attending a reproductive health clinic in Central African Republic, who reported being sexually active, had vaginal washings collected for testing for PSA and Yc DNA [19]. The two biomarkers agreed in 214 cases (81%). In the remaining 50 cases, Yc-DNA was positive and PSA negative, which could be expected since Yc-DNA persists longer than PSA.

Interestingly, we found that the predicted sensitivities of Yc DNA positivity sometimes increased from one time point to the next (even from immediately postexposure to 1 h postexposure, a time interval which is unlikely to have been influenced by new, nonstudy

semen exposures). Similarly, it was not uncommon for a Yc DNA-negative swab to be followed by a Yc DNA-positive swab within a single study cycle. Thus, assuming the absence of new exposures, women could test positive for Yc DNA after having already tested negative. Overall, the Yc DNA results appear to have more inconsistency than the PSA results, which may reflect a “hit or miss” probability of the swab encountering sperm containing the DNA. In contrast, seminal fluid might disperse more evenly or widely in vaginal fluid and, thus, be more consistently detected. Unexpected increases in Yc DNA concentrations also could have occurred if inhibitors of the PCR were present in semen and these diluted over time in the vagina.

Deviations occurred when participants were unable to generate a full 1000 μL of semen for that cycle, reported having engaged in vaginal sex during study cycles, were positive for PSA and/or Yc DNA before being exposed to semen in the clinic or failed to record time of swab collection. Our sensitivity analyses indicated that none of these factors on its own was likely to have influenced the interpretation of our findings. Nevertheless, we did not examine how these factors might have interacted to affect the study findings.

Although participants were counseled extensively on the importance of providing accurate self-reports (e.g., of sexual activity and swab collection times), the potential for social desirability bias and misrepresentation exists. Statistical outliers reflecting high biomarker levels at 24-, 72- and 144-h collection points led us to fit models with and without data from those times. These outliers likely represented novel semen exposures possibly resulting from unreported acts of vaginal intercourse or from other sexual exposures that would not typically be expected to result in PSA or Yc DNA positivity (e.g., use of sex toys, which some couples reported). The potential for testing positive for a semen biomarker due to sexual exposures other than vaginal sex merits additional exploration. Despite employing study procedures attempting to control the female participants' exposure to semen during the study cycles and to collect swabs in a standard manner, the study is limited by its reliance on participant adherence to study instructions.

The study also was limited by a relatively small sample size. A larger sample size likely would have yielded narrower 95% CIs surrounding our predicted sensitivities of positive biomarker results. Nevertheless, our results were sufficiently precise for us to comment on the relative advantages of the two biomarkers.

Strengths of this research include its controlled aspects in terms of the precise quantities of semen inserted and the specific intervals for specimen collection. Also, measuring the two biomarkers from the same specimen reduced the risk of observing differences attributable to variations in specimen collection procedures. Although we did not explicitly consider how individual-level factors (e.g., hormonal influences or inflammation) might affect biomarker residence in the vagina, our mixed-effects models took these into account, in large part adjusting for the wide interparticipant variability that we would expect for these factors.

Although PSA and Yc DNA are both biomarkers of semen exposure, they measure different biological phenomena (presence of seminal fluid and spermatozoa, respectively). They also have different strengths and weaknesses as indicators of recent sexual activity. Researchers

who seek to incorporate biomarkers of semen exposure into studies should consider carefully the amount of exposure they wish to detect (e.g., a study of condom failure would need to be capable of detecting small amounts of semen), rates of biomarker decay and predicted sensitivities of biomarker positivity at different time points. For example, studies evaluating the effectiveness of condoms have compared PSA concentrations detected in pre- and postcoital vaginal swabs [16,20,21]. These studies benefitted from a biomarker that is detected consistently in specimens collected immediately following potential exposure. In other studies, though, the ideal biomarker might need to be consistently and reliably detectable for several days. The biomarkers also differ in their costs, with the estimated cost per assay for Yc DNA testing being higher than that for PSA. The present study findings suggest that PSA may be more consistent as a marker of very recent exposure and that Yc DNA may have greater longevity in the vagina, although both markers appear to be unreliable indicators of exposure after 24 h, highlighting the need for a marker of semen exposure covering a longer time span.

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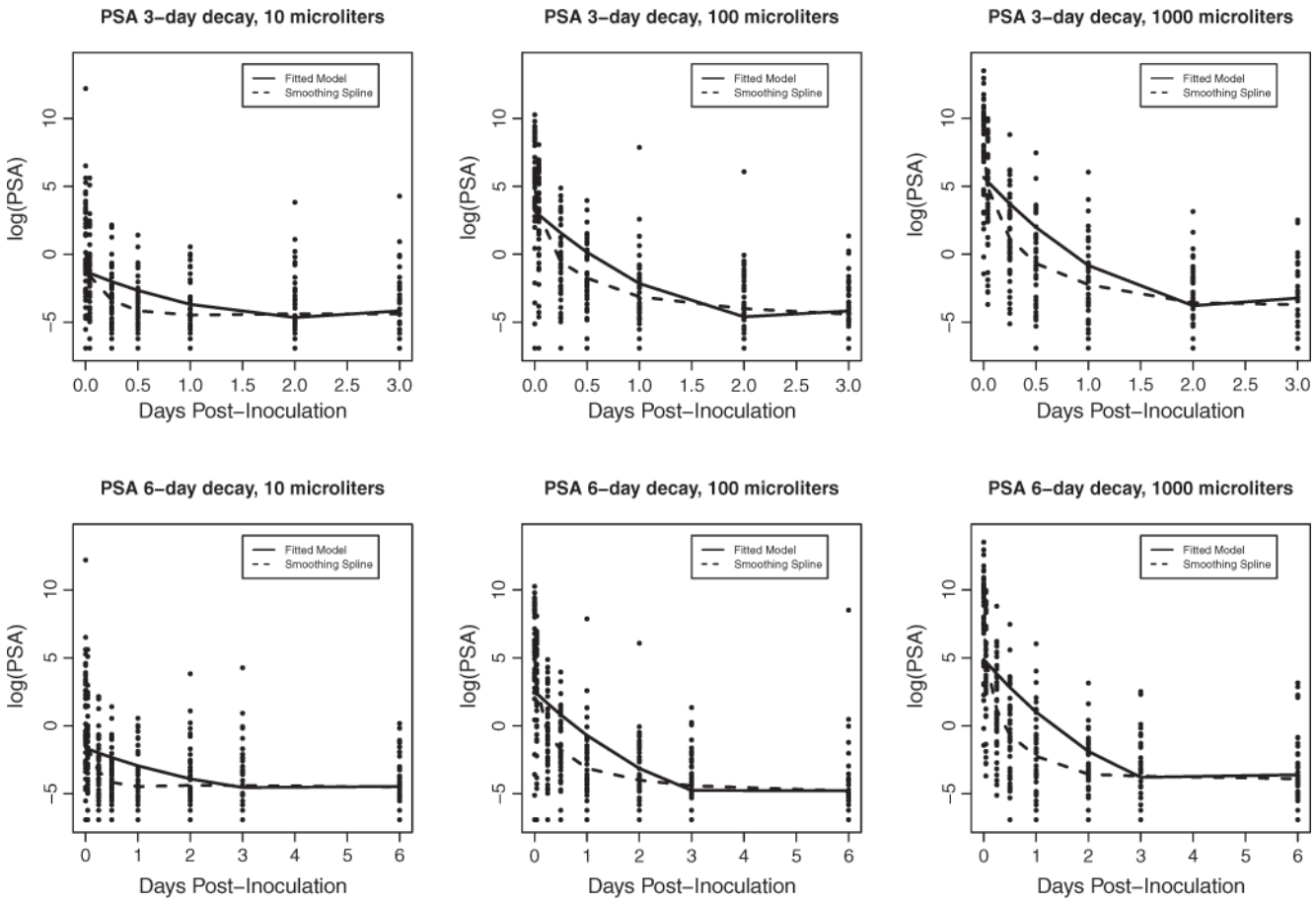

Fig. 1.

Decay of PSA by semen dose and time since exposure. *Decay curves are estimated by fitting a linear mixed-effects model and using nonparametric smoothing splines.

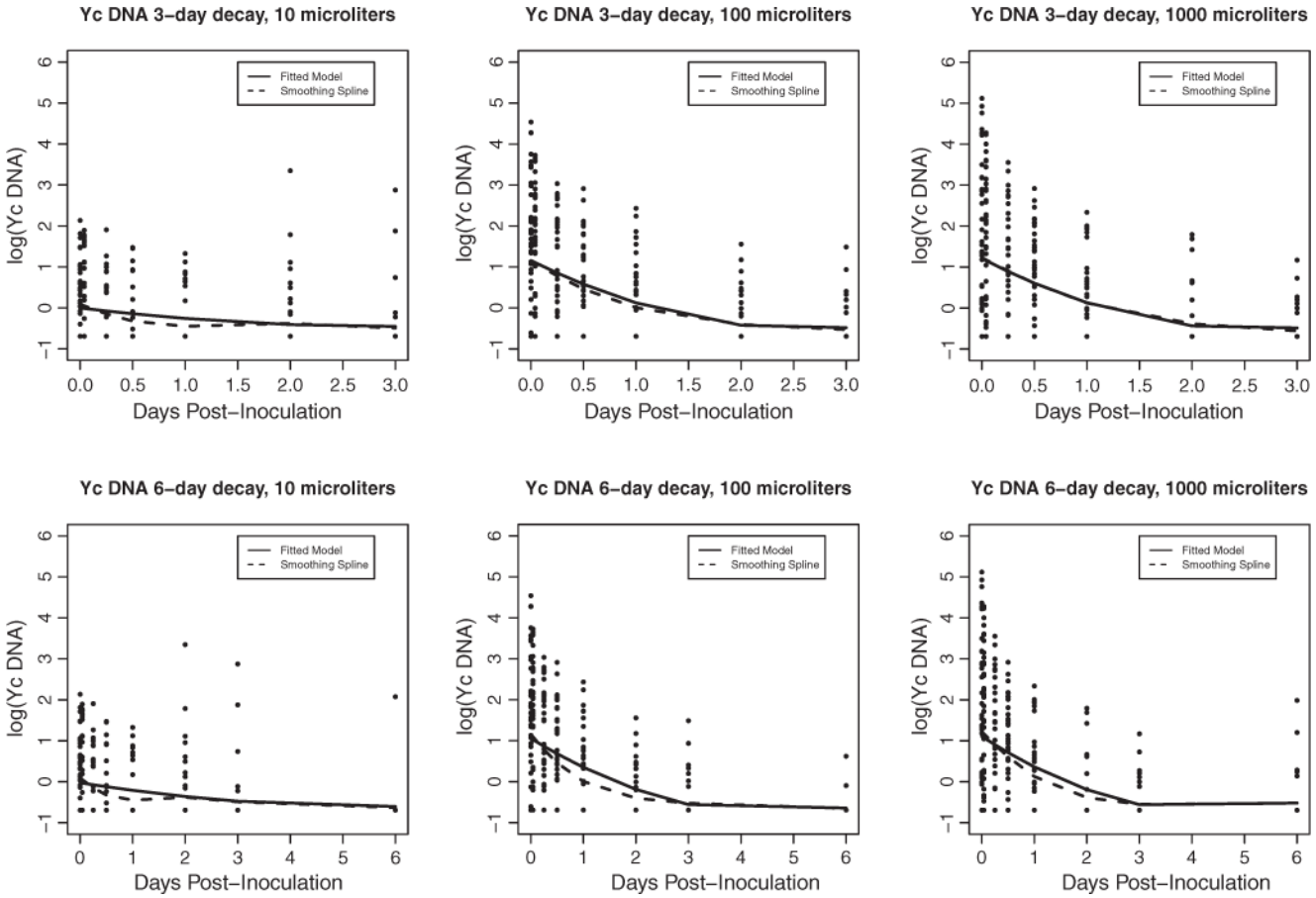

Fig. 2.

Decay of Yc DNA by semen dose and time since exposure. *Decay curves are estimated by fitting a linear mixed-effects model and using nonparametric smoothing splines.

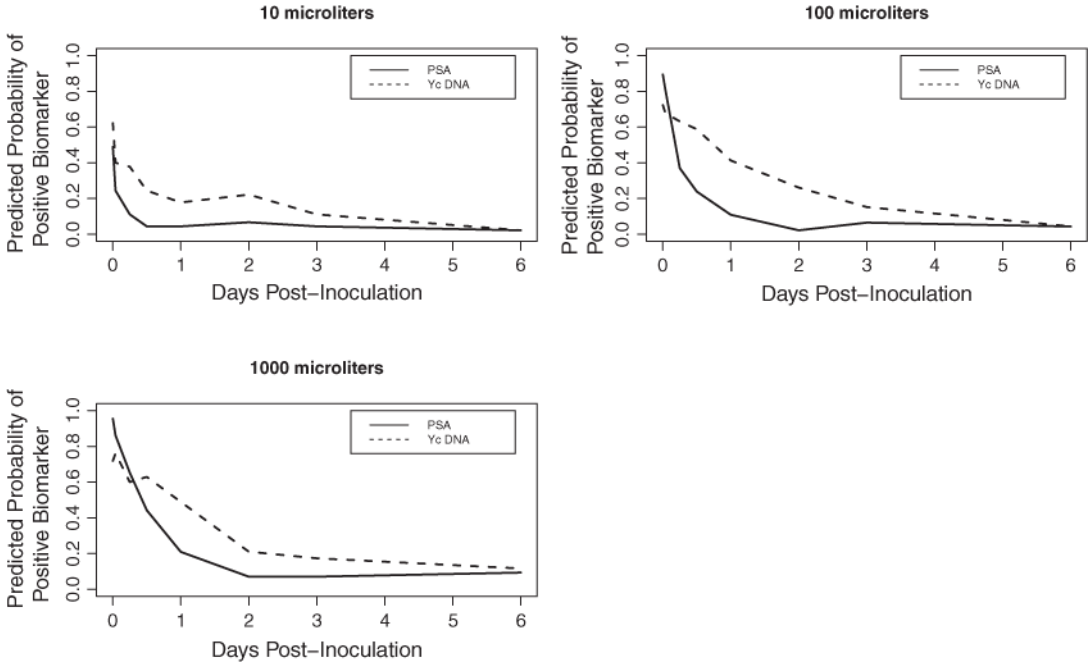

Fig. 3.
Predicted sensitivity of detecting biomarker by semen dose and time since exposure.

Table 1

Concentration of biomarker detected in vaginal swab by semen dose and time since exposure

Semen dose[a]	Time postexposure	Biomarker (ng/mL)					
		PSA			Yc DNA		
		Mean	Median	5th %ile–95th %ile	Mean	Median	5th %ile–95th %ile
10 μL	0 h	4470.14	0.68	0.00–274.36	1.56	1.13	0.00–5.54
	1 h	11.46	0.16	0.00–19.90	1.46	0.00	0.00–5.67
	6 h	0.71	0.03	0.001–7.25	0.85	0.00	0.00–2.92
	12 h	0.18	0.01	0.00–0.54	0.51	0.00	0.00–3.15
	1 day	0.13	0.01	0.00–0.92	0.41	0.00	0.00–2.41
	2 days	1.18	0.01	0.00–1.24	1.06	0.00	0.00–3.04
	3 days	1.75	0.01	0.00–0.95	0.62	0.00	0.00–2.10
	6 days	0.10	0.01	0.00–0.35	0.18	0.00	0.00–0.00
100 μL	0 h	3246.14	289.07	0.01–12,437.00	13.60	3.99	0.00–72.10
	1 h	246.46	26.94	0.01–993.89	8.33	3.85	0.00–36.45
	6 h	10.84	0.58	0.01–73.23	3.96	1.26	0.00–15.40
	12 h	2.54	0.13	0.002–10.73	2.61	1.27	0.00–8.32
	1 day	57.73	0.02	0.00–3.82	1.50	0.00	0.00–6.47
	2 days	9.60	0.02	0.00–0.62	0.48	0.00	0.00–2.45
	3 days	0.17	0.01	0.00–1.07	0.28	0.00	0.00–1.50
	6 days	108.42	0.004	0.00–0.97	0.06	0.00	0.00–0.00
1000 μL	0 h	50,159.09	8061.45	6.44–292,180.00	19.51	1.75	0.00–117.00
	1 h	2730.40	252.27	0.06–9562.50	12.68	4.40	0.00–54.80
	6 h	213.04	2.33	0.02–455.78	5.78	1.72	0.00–28.30
	12 h	51.25	0.45	0.01–37.49	3.20	1.68	0.00–11.72
	1 day	12.34	0.05	0.002–24.24	1.56	0.00	0.00–7.06
	2 days	0.79	0.03	0.00–1.50	0.59	0.00	0.00–4.16
	3 days	0.66	0.01	0.00–1.12	0.26	0.00	0.00–1.31
	6 days	1.42	0.01	0.00–9.57	0.33	0.00	0.00–1.33

%ile=percentile.

[a]The 10-μL cycle includes samples from 45 couples, the 100-μL cycle includes samples from 46 couples, and the 1000-μL cycle includes samples from 43 couples.

Table 2

Predicted sensitivities of detecting biomarker[a] by semen dose and time since exposure

Semen dose[c]	Time postexposure	Biomarker				Difference (Yc DNA −PSA)	95% CI
		PSA		Yc DNA			
		Prob	95% CI	Prob	95% CI		
10 μL	0 h	0.49	0.35, 0.63	0.62	0.47, 0.75	0.13	−0.12, 0.28
	1 h	0.24	0.14, 0.39	0.40	0.27, 0.55	0.16[b]	0.02, 0.29
	6 h	0.11	0.05, 0.24	0.38	0.25, 0.53	0.27[b]	0.11, 0.42
	12 h	0.04	0.01, 0.16	0.24	0.14, 0.39	0.20[b]	0.07, 0.33
	1 day	0.04	0.01, 0.16	0.18	0.09, 0.32	0.14[b]	0.02, 0.25
	2 days	0.07	0.02, 0.19	0.22	0.12, 0.37	0.15[b]	0.03, 0.28
	3 days	0.04	0.01, 0.16	0.11	0.05, 0.24	0.07	−0.03, 0.16
	6 days	0.02	0.00, 0.15	0.02	0.00, 0.15	0.00	−0.06, 0.06
100 μL	0 h	0.89	0.77, 0.96	0.72	0.58, 0.83	−0.17[b]	−0.29, −0.05
	1 h	0.81	0.66, 0.90	0.68	0.53, 0.80	−0.13	−0.30, 0.04
	6 h	0.37	0.24, 0.52	0.63	0.48, 0.76	0.26[b]	0.09, 0.44
	12 h	0.24	0.14, 0.39	0.59	0.44, 0.72	0.35[b]	0.19, 0.51
	1 day	0.11	0.05, 0.24	0.41	0.28, 0.56	0.30[b]	0.16, 0.45
	2 days	0.02	0.00, 0.14	0.26	0.15, 0.41	0.24[b]	0.12, 0.36
	3 days	0.07	0.02, 0.19	0.15	0.07, 0.29	0.08	−0.05, 0.22
	6 days	0.04	0.01, 0.16	0.04	0.01, 0.16	0.00	−0.09, 0.09
1000 μL	0 h	0.96	0.83, 0.99	0.72	0.57, 0.83	−0.24[b]	−0.39, −0.08
	1 h	0.86	0.72, 0.94	0.76	0.61, 0.87	−0.10	−0.24, 0.05
	6 h	0.65	0.50, 0.78	0.60	0.45, 0.74	−0.05	−0.25, 0.13
	12 h	0.44	0.30, 0.59	0.63	0.48, 0.76	0.19	−0.02, 0.39
	1 day	0.21	0.11, 0.36	0.49	0.34, 0.64	0.28[b]	0.12, 0.44
	2 days	0.07	0.02, 0.20	0.21	0.11, 0.36	0.14[b]	0.01, 0.28
	3 days	0.07	0.02, 0.20	0.17	0.09, 0.32	0.10	−0.03, 0.22
	6 days	0.09	0.04, 0.23	0.12	0.05, 0.25	0.03	−0.08, 0.12

Prob=predicted sensitivity.

[a]Positive PSA result defined as ≥1 ng/mL, and positive Yc DNA result defined as >0 ng/mL (detection of any Yc DNA).

[b]Significant at p=.05 level.

[c]The 10-μL cycle includes samples from 45 couples, the 100-μL cycle includes samples from 46 couples, and the 1000-μL cycle includes samples from 43 couples.