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Effect of topical vaginal products on the detection of prostate-specific antigen, a biomarker of semen exposure, using ABAcards☆,,☆☆

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Abstract

Background—Prostate-specific antigen (PSA) is a biomarker of recent semen exposure. There is currently only limited information on whether topical vaginal products affect PSA assays. We investigated this question using various dilutions of several vaginal products (lubricants and spermicides) and the Abacus ABAcard for PSA detection.

Study Design—Pooled semen controls and various dilutions of nonoxynol-9 (N9), carboxymethyl cellulose (CMC), Replens, Gynol 2, K-Y jelly, Astroglide, Surgilube, combined with pooled semen dilutions, were tested for PSA using the Abacus ABAcard.

Results—N9 (2% with saline) and CMC did not appear to affect the results of testing with the ABAcard, but not all semen dilutions were tested. The other products (including Replens and Gynol, which is 2% N9 with propylene glycol, K-Y, Astroglide and Surgilube) at some of the dilutions tested either affected or gave invalid results with PSA testing using the ABAcard. Both Gynol 2 and K-Y at 1:10 dilution gave false-positive results.

Conclusions—Some vaginal products affect PSA results obtained by using the semiquantitative ABAcard. In vivo confirmation is necessary to further optimize PSA detection when topical vaginal products are present.

☆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.

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Keywords

Vaginal products; PSA; Semen; Biomarker; ABAcard; Lubricants; Spermicide

1. Introduction

Prostate-specific antigen (PSA) is a biomarker used to indicate recent vaginal semen exposure [1,2]. It is a sensitive and specific method for semen detection in women, originally developed for forensic purposes [3] and more recently adapted and utilized in reproductive health studies as an indicator of recent exposure to semen from unprotected sex or incorrect condom use [1,4–6]. PSA can be used as a marker to assess self-reported condom use as well as condom effectiveness [4] and, in clinical trials of contraception or sexually transmitted infections (STIs) and HIV prevention, as an indicator of adherence to study procedures (such as avoiding recent unprotected sex).

It is important to know whether spermicides or other vaginal products can affect the detection of PSA. In a clinical study of a new contraceptive diaphragm in which PSA was measured using the Abbott AXSYM® Microparticle Enzyme Immunoassay (Abbott Laboratories, Abbott Park, IL, USA), it appeared that nonoxynol-9 (N9) may have interfered with PSA detection [5]. There are other methods available for PSA detection. One is the Abacus OneStep ABAcard (Abacus Diagnostics, West Hills, CA, USA) [6–8]. The card yields rapid and straightforward qualitative or semiquantitative results [9] and is relatively inexpensive.

Abacus OneStep ABAcard is an antigen-specific monoclonal antibody membrane assay that has a lower limit of PSA detection of 4-ng PSA/mL vaginal swab eluent [7]. Although the card was originally developed as a qualitative assay, it has been used semiquantitatively [9] (Fig. 1), and its semiquantitative results correlated well with results of the quantitative Abbott IMX assay [9]. There is only limited evidence on the effect of vaginal products on PSA testing using ABAcards. Given the lack of available information on this topic, as well as the important role that biomarkers of semen exposure can play in microbicide, HIV/STI prevention and contraception research, we undertook laboratory investigations to determine whether specific vaginal products affect PSA detection by ABAcards.

2. Methods

2.1. In vitro experiments

Table 1 describes the vaginal products (lubricants and spermicides) that were tested: N9 (formulated at Eastern Virginia Medical School laboratory as 2% N9 in saline), carboxymethyl cellulose (CMC), Replens, Gynol (2% N9 with propylene glycol), K-Y, Astroglide and Surgilube.

First, positive controls (semen alone) were prepared by serially diluting [with phosphate buffer saline (PBS)] pooled semen stock, which had been stored at −80°C at a 1:50 dilution and then thawed. PBS was used as the diluting medium rather than what is supplied by the manufacturer as it is a commonly used diluting medium. Twofold dilutions were created and

tested (without vaginal products) by the ABAcard starting with1:1600 through 1:1,638,400. Semen alone was also tested with the Abbott Architect chemiluminescent quantitative immunoassay [10], in order to quantify the PSA concentration range for each dilution and correlate with the semiquantitative results obtained from ABAcard.

Next, each of the vaginal products was mixed with semen. Each was diluted in PBS at 1:10 and 1:40 dilutions (Surgilube was also diluted at 1:20) and then added to the series of semen dilutions in equal volumes so that the final semen dilutions were twofold, ranging from 1:1600 through 1:1,638,400. For each of the samples tested with the ABAcard, 200 μL of an extract was placed in the test device, and all test results read at 10 min. Results were considered invalid if there was no control line visible within 10 min.

Last, negative controls, that is, products that had been mixed with PBS only (no semen and, therefore, expected to give negative PSA results) were also tested with the ABAcards. All controls were tested on the same day as the products.

3. Results

The effects of the vaginal products on PSA detection by ABAcard are presented in Table 2. N9 and CMC, at the semen dilutions tested, did not appear to affect PSA detection. Replens at the 1:10 dilution may have interfered slightly, with a weakly positive PSA result at the 1:102,400 semen dilution instead of the positive result that would have been expected based on the results of the semen-alone “positive control” and a negative result at the 1:204,800 dilution instead of the expected weakly positive one. However, at the 1:40 dilution, no effect was seen. Both Gynol and K-Y at 1:10 dilution had positive results at all semen dilutions, as well as false-positive results in the absence of semen; at the 1:40, dilution there was no effect of the products on PSA detection. All dilutions of Astroglide yielded invalid results. Surgilube produced invalid results at the 1:10 dilution but not at the 1:20 and 1:40 dilutions, which showed higher than expected results at the 1:204,800 semen dilution.

4. Discussion

The lower-than-expected results seen with Replens at a 1:10 dilution, if seen in a clinical trial, would give a woman with a vaginal concentration of PSA that was really 5.6–7.6 ng/mL, an apparent concentration of 3.0–3.9, and a woman with an actual concentration of 3.0–3.9 ng/mL, an apparent concentration of 2.0 ng/mL or less. The higher-than-expected results seen with Surgilube at 1:20 and 1:40 would mean that a woman with an actual concentration of 3.0–3.9 ng/mL would have an apparent concentration of 5.6–7.6 ng/mL. None of these differences would be likely to greatly affect interpretation of trial results if using quantitative PSA assays since cutoffs of 3 ng/mL or 1 ng/mL [11,12] are commonly used to indicate presence or absence of semen [1,13], particularly since the proportion of samples with low PSA has been small in previous studies [11,12], but is a concern when using the ABAcards since their lower limit of detection is 4 ng/mL [7,9]. The false-positive results seen with Gynol and K-Y at 1:10 dilutions are of more concern, since a woman with no semen exposure at all would appear to have a concentration of 5.6–7.6 ng/mL.

Importantly, other than the lower-than-expected results seen with Replens at 1:10, there were no false-negatives or other indications that products inhibited detection of PSA. The invalid results produced by all dilutions of Astroglide and the 1:10 concentration of Surgilube were probably due to the viscosity of the products. These results were considered invalid because there was no control line visible within 10 min. It is unclear whether clinical specimens obtained from women who had used these products would give the same results as the in vitro experiments. Products in the experiment that either gave invalid results or false-positive results (such as K-Y, Gynol, Astroglide or Surgilube) especially need to be further evaluated when the ABAcard is being used to assess PSA in vaginal fluids. If further evaluations confirm in vivo the effects of these products on ABAcard results, then other PSA assays should be considered in populations where such product use is common.

The evidence on whether vaginal products or other substances affect PSA detection is limited and the results contradictory. Currently, the World Health Organization and general guidelines for forensic examinations recommend not using lubricants on specula during pelvic examination because they may affect forensic analysis [14]. Maher et al. [15] found an association between N9 and false-positive PSA results when using the biofilm membrane test. Sutton et al. [16] found that N9 and other substances (such as human milk, urine, feces and blood) potentially affected an Enzyme Linked Immunosorbent Assay (ELISA) for PSA detection, and using a similar assay, Johnson and Kobuwski [17] found false-negative results when samples were contaminated with common household detergents. In contrast, Stowell et al. [18] found no effect of menstrual blood, male or female saliva or female urine but did find that PSA recovery was affected by the presence of certain proteins found in body fluids using a sandwich ELISA. Melendez et al. [19] found that while some topical products (such as Silicorel, a silicone-based lubricant) did not affect the Abbott Architect's ability to detect PSA, other products (such as Replens and Gynol) seemed to inhibit detection (with lower-than-expected results), but the addition of K-Y jelly showed higher-than-expected results.

The literature on the effect of contaminants or other vaginal products on PSA detection using ABAcard specifically is even more limited. Kristaly and Smith [20] did not find any effect when mixing blood and saliva with semen. Hochmeister et al. [7] similarly did not find an effect of various female body fluids, saliva or perspiration from male donors or anal swabs. Male urine was an exception, as expected, given the shared male genitourinary tract [7]. Pang and Cheung [21] did not find any effect of bodily fluids, lubricants or spermicides including N9 with PSA detection using the ABAcard. On the other hand, Bitner [22] found that spermicidal lubricants affected the Seratec semiquantitative assay (membrane strip similar to ABAcard assay) and recommended that this test not be used when testing the inside of a condom for PSA. Laffan et al. [23] evaluated body fluids and substrates (including lubricants) and found false-negative results only from caustic soda with the Seratec semiquantitative assay.

There are some limitations to our study. First, this was an in vitro experiment, and while we did our best to estimate the approximate amount of product that would potentially be present intravaginally (based on typical use and/or manufacturer's directions for use of the products), this remains an estimate. Not all of the vaginal products were tested for all of the

same semen dilution factors. PBS was used as the diluting medium rather than what is supplied by the manufacturer. Hobbs et al. [24] showed that using saline rather than that manufacturer's buffer did not affect results of the ABAcard, although it did affect semenogelin detection using the Rapid Stain Identification Series for Semen Detection assay. In addition, how the results of a controlled laboratory experiment translate to real-life situations remain uncertain. Any diluting laboratory medium could be quite different from vaginal fluids in pH or other physicochemical characteristics. In vivo factors such as phase of the menstrual cycle, vaginal inflammation or infection or hormonal contraception may also affect some of the local parameters and lead to different results. The viscosity of particular products seems to have affected migration through the antigen-specific monoclonal antibody membrane used in the ABAcard assay and, thus, affected the performance of the test. Our results are limited to PSA detection by ABAcards; the effects of lubricants and other vaginal products on other PSA assays such as the quantitative Abbott assay warrants further study.

The importance of including biomarkers of semen exposure in sexual health studies has been argued by many investigators [2,13,25,26]. PSA is an excellent biomarker of recent (up to 48h) semen exposure [4,5] and could be very useful in future research as a marker of compliance with study procedures and as a way to assess self-reported condom use and condom failure.

In conclusion, Replens, Gynol, K-Y, Astroglide and Surgilube, at some of the dilutions tested, either affected or gave invalid results with PSA testing using the ABAcard. Confirmation of the present results using actual clinical specimens will be an important next step. Future studies should consider the specific vaginal products used, such as lubricants, spermicides or microbicides, as well as the dose or amount of the product, when selecting an assay to detect PSA as a biomarker of recent semen exposure. Further considerations should also include the need to collect data on participants' product use when determining what (or if) PSA assays are to be used and the potential for misclassification. Similar considerations should apply to future use of PSA as a semen biomarker in the development of vaginal and rectal microbicides.

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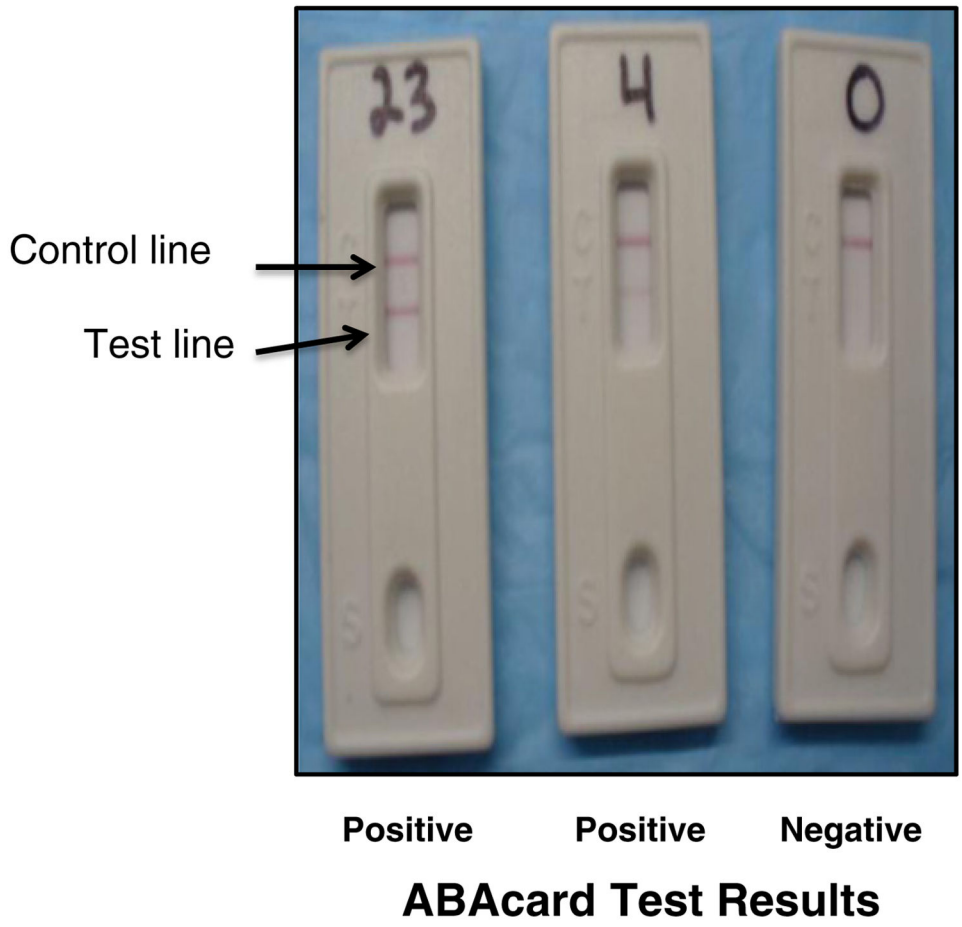

Fig. 1.
ABAcard.

Table 1

Vaginal products included in the experiments of PSA detection by ABAcards

Product	Brand name (manufacturer)	Main ingredient	Type	Date tested
N9	N/A (formulated @ EVMS)	N9 2% in saline	Spermicide	June 2009
CMC	N/A (formulated @ EVMS)	CMC	Main ingredient for lubricants	June 2009
Replens	Replens (LDS Consumer Products)	Polycarbofil	Vaginal lubricant	September 2009
K-Y	K-Y Brand Jelly (Johnson & Johnson)	CMC	Vaginal lubricant	September 2009
Astro	Astroglide (BioFilm, Inc)	Glycerin and propylene glycol	Vaginal lubricant	September 2009
Gynol	Gynol 2 (Ortho)	N9 2% in propylene glycol	Spermicide	September 2009
Surgi	Surgilube (Fougera)	Chlorexidine gluconate and natural water soluble gums	Surgical sterile lubricant	March 2010

N/A: not applicable; EVMS: Eastern Virginia Medical School.

Table 2

In vitro effect of vaginal products on PSA detection by ABAcard

Semen alone	**Semen dilutions***						
	1: 3200	**1: 51,200**	**1: 102,400**	**1: 204,800**	**1: 409,600**	**1: 819,200**	
	+	+	+	+/−	−	−	
Product and amount							No semen, product only
N9 1:10	+	+	Nt	Nt	−	−	−
N9 1:40	+	+	Nt	Nt	−	−	−
CMC 1:10	+	+	Nt	Nt	−	−	−
CMC 1:40	+	+	Nt	Nt	−	−	
Replens 1:10	+	+	+/−	−	−	−	−
Replens 1:40	+	+	+	+/−	−	−	−
K-Y 1:10	+	+	+	+	+	+	+
K-Y 1:40	+	+	+	+/−	−	−	−
Astro 1:10	Invalid	Invalid	Invalid	Invalid	Invalid	Invalid	Invalid
Astro 1:40	Invalid	Invalid	Invalid	Invalid	Invalid	Invalid	Invalid
Gynol 1:10	+	+	+	+	+	+	+
Gynol 1:40	+	+	+	+/−	−	−	−
Surgi 1:10	Invalid	Invalid	Invalid	Invalid	Invalid	Invalid	Invalid
Surgi 1:20	+	+	+	+	−	−	−
Surgi 1:40	+	+	+	+	−	−	−

+: positive, +/−: weakly positive, −: negative, Nt: not tested, N/A: not applicable.

* Semen alone was also tested with Abbott Architect quantitative assay. The PSA concentration (ng/mL) ranges for the semen dilutions as follows: 1: 3200=90.8–>100-ng/mL PSA vaginal swab eluent; 1: 51,200=1.6–14.8 ng/mL; 1: 102,400=5.6–7.6; 1: 204,800=3.0–3.9 (corresponds to the ABAcard +/−due to 4 ng/mL lower level of detection); 1: 409,600=1.4–2.0; 1: 819,200=0.74–1.