HHS Public Access

Author manuscript

Sex Transm Dis. Author manuscript; available in PMC 2015 June 03.

Published in final edited form as:

Sex Transm Dis. 2012 December ; 39(12): 949–953. doi:10.1097/OLQ.0b013e31826e880e.

A Quantitative Glycogen Assay to Verify Use of Self-Administered Vaginal Swabs

Deborah J. Anderson, PhD[*], **Joseph A. Politch, PhD**[*], **Jeffrey Pudney, PhD**[*], **Cecilia I. Marquez, MD**[*], **Margaret C. Snead, PhD**[†], and **Christine Mauck, MD**[‡]

[*]Division of Reproductive Biology, Department of Obstetrics and Gynecology, Boston University School of Medicine, Boston, MA

[†]Centers for Disease Control and Prevention, Atlanta, GA

[‡]CONRAD, Eastern Virginia Medical School, Arlington, VA

Abstract

Background—Self-administered swabs are used to sample vaginal contents for a variety of clinical purposes including detection of sexually transmitted infections, condom breakage, and vaginal product use. The goal of this study was to determine whether a quantitative glycogen assay can be used to assess whether a swab has been exposed to the vagina to assure study compliance.

Study Design—Buccal, skin, or vaginal samples were tested to determine whether a commercial quantitative glycogen assay can differentiate vaginal specimens. In addition, archived remnant de-identified vaginal swabs from clinical trials were tested. Periodic acid–Schiff stain was used to identify glycogen-positive cells as a confirmation test.

Results—Glycogen concentrations in eluates of vaginal swabs from reproductive-aged women were significantly higher than those from unused swabs (mean ± SE, 964 ± 135 μg/mL vs. 14.7 ± 2.5 μg/mL, $P < 0.001$) and swabs exposed to buccal and finger/hand epithelia (40.3 ± 4.8 and 18.5 ± 5.4 μg/mL, $P < 0.001$). Glycogen concentrations were lower and more variable in vaginal swabs from older perimenopausal/menopausal women (mean ± SE, 235 ± 123, $P < 0.01$). Semen and sample storage longer than 1 year did not affect glycogen detection. Using a cutoff of 100 μg/mL of glycogen, 30 of 30 vaginal swabs from reproductive-aged women versus 0 of 28 control swabs were positive, for an assay sensitivity of 1 (95% confidence interval, 0.86–1) and specificity of 1 (95% confidence interval, 0.85–1). Periodic acid–Schiff stain correlated with soluble glycogen results but was less specific.

Conclusions—The quantitative glycogen assay provides a simple and inexpensive method to validate the use of self-administered swabs for sampling vaginal contents in clinical studies.

Correspondence: Deborah J. Anderson, PhD, Division of Reproductive Biology, Department of Obstetrics and Gynecology, Boston University School of Medicine, 670 Albany St, Rm 516, Boston, MA 02118. Deborah.Anderson@BMC.org.

The authors have no conflicts of interest.

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.

Self-administered vaginal swabs are used to sample vaginal contents for a range of clinical applications including tests for sexually transmitted diseases,[1–3] cervical cancer,[4] condom failure,[5–7] and vaginal microbicide use and safety.[8,9] Validation of proper collection of self-obtained vaginal samples is of critical importance for assessing subject adherence in clinical and research settings. For example, confirmation of vaginal exposure of swabs that are negative for a semen biomarker is critical in the evaluation of new female condoms.[10]

Detection of vaginal cells is important in forensic investigation, and several attempts have been made to validate tests for this purpose.[11–14] Detection of glycogen-positive cells has been used to prove vaginal penetration in sexual assault cases.[12,15–18] Glycogen serves as an energy source and is stored in adipose tissue. It is made primarily by the liver and muscle tissue, but during the reproductive period of women, high estrogen levels stimulate the vaginal epithelium to produce large amounts of glycogen.[19] Mucosal epithelial cells in the mouth, penile urethra, and rectum also synthesize glycogen, but to a much lesser extent.[15–17] Thus, glycogen provides an attractive marker for the detection of vaginal cells. Most methods for the detection of vaginal glycogen have been based on the detection of glycogen-rich vaginal cells in stained cell smears. However, this process is time-consuming and subjective, requires a high level of expertise, and has limited specificity and sensitivity. An alternative approach is to take advantage of the fact that glycogen is released from vaginal epithelial cells into vaginal secretions where it serves as an important energy source for lactobacilli.[20] Commercial quantitative colorimetric glycogen assays have been developed to detect alterations in blood glycogen concentrations caused by metabolic abnormalities associated with diabetes and several genetic glycogen storage diseases. These assays are inexpensive (<$2/test), straightforward, and objective. The purpose of the current study was to validate the use of a quantitative soluble glycogen assay to confirm that a swab has been inserted into the vagina.

METHODS

Samples Tested

Four different sets of specimens were collected and tested: (1) the Centers for Disease Control and Prevention (CDC) provided (n = 27) de-identified swab eluates (from vaginal, buccal, hand/finger, and empty swabs); (2) California Family Health Council (CFHC) provided (n = 26) archived de-identified remnant vaginal swabs from a condom clinical trial for assay development; (3) CONRAD provided de-identified vaginal swabs (n = 8) from 2 reproductive-aged women, 3 peri-menopausal women, and 3 postmenopausal women; and (4) in addition, Boston University Medical Campus (BUMC) recruited healthy nonpregnant reproductive-aged (18–40 years old) female volunteers (n = 13) to provide vaginal samples. Fresh semen samples were obtained as discarded de-identified specimens with normal semen parameters (n = 10) from BUMC Urology Department. This study was approved by the institutional review board of BUMC, and all volunteers provided written informed consent for the study.

Specimen Collection and Processing

Vaginal and control specimens were obtained using Falcon Swubes (Becton, Dickinson & Co, Franklin Lakes, NJ). Each Swube consists of a 6-in. (15.2-cm) cotton-tipped swab attached to the screw cap of a 1.6 × 15-cm clear plastic tube containing a Minipax (Multisorb Technologies Inc) desiccant. For vaginal sampling, the participants were instructed to introduce the swab into the vagina until resistance was felt, rotate it 5 times, and then remove it. Control samples were obtained by placing the swab against the inner cheek buccal mucosa or hand/finger skin and rotating 5 times. The participants were instructed to reinsert the swab into the Swube tube immediately after the sample was taken and to tighten the cap. The swabs were kept in the tubes for a minimum of 24 hours for drying. For elution of swab contents, dried swabs were transferred to Eppendorf tubes containing 1.5 mL of phosphate-buffered saline (PBS) with 0.05% Tween 20 (polysorbate 20; Sigma), vortexed for 10 seconds, and then incubated for 30 minutes at 4°C. This elution protocol was selected because it is widely used to elute vaginal swabs for prostate-specific antigen (PSA; semen) testing,[21] allowing us to evaluate the feasibility of using 1 eluate to test for both glycogen and PSA in the future. Swabs were squeezed against the side of the tube as they were removed, and 250 μL of aliquots of the eluates was pipetted into labeled cryotubes and stored at −80°C until used for testing.

Glycogen Assays

1. Quantitative colorimetric assay—Samples were thawed and centrifuged in a microfuge at 400*g* for 1 minute. The supernatants were tested for glycogen content using the EnzyChrom Glycogen Assay kit (BioAssay Systems, Hayward, CA) per manufacturer's instructions. Briefly, 10 μL of swab eluate was added to individual wells in a 96-well flat bottomed plate, followed by the addition of a single working reagent that enzymatically converts glycogen into glucose and detects glucose with a colorimetric assay read at 570-nm absorbance. For the preliminary studies, the samples were read on a spectrophotometer and were scored as greater than 200 μg/mL or less than 200 μg/mL (semiquantitative assay). For quantitative glycogen assessment, the samples were read on an automated BioTek Synergy HT plate reader, and glycogen values were calculated from a standard curve run in each assay, with a linear range from 50 to 200 μg/mL. Selected samples with glycogen values above the linear range of the standard curve (>200 μg/mL) were serially diluted in PBS/Tween and retested to determine the glycogen concentration.

2. Detection of glycogen-positive cells—Cell pellets from swab eluate samples were processed for detection of glycogen-positive epithelial cells using the classical periodic acid–Schiff (PAS) stain.[17] Cells were resuspended in 20 μL of PBS/Tween, and drops of suspended cells were applied to individual spots of Teflon-coated 8-well microscope slides (Roboz Surgical Instruments, Washington, DC), dried, and fixed in absolute acetone. Slides were processed through the PAS stain for detection of cells containing accumulations of polysaccharides (i.e., glycogen), which appear bright red.[17]

Data Analysis

Data were analyzed by 1-way analysis of variance. Significant analysis of variance was followed by pairwise post hoc testing by Bonferroni multiple comparison test. Differences were considered statistically significant when $P < 0.05$. StatView (version 5.0.1; SAS Institute, Cary, NC) and Prism (version 3.0; GraphPad Software, Inc, La Jolla, CA) statistical software were used to perform the statistical computations. Sensitivity and specificity calculations were performed using a statistics Web site available through Vassar College (http://faculty.vassar.edu/lowry/clin1.html).

RESULTS

Detection of Glycogen in Vaginal and Control Swab Eluates: Preliminary Studies

Initially, buccal, hand/finger, and vaginal swab eluates from 3 healthy female volunteers (recruited at BUMC) were evaluated. All vaginal samples contained greater than 200 μg/mL of glycogen (above assay range), whereas all controls contained less than 200 μg/mL of glycogen by semiquantitative analysis. All 3 vaginal samples contained numerous PAS-positive epithelial cells, whereas the control samples had few, if any, PAS-positive cells (Fig. 1). After these encouraging results, a series of 27 coded vaginal and control swab eluates (provided by the CDC) were tested using both the quantitative glycogen assay and PAS test. The 10 vaginal swab samples were successfully differentiated from the 17 control swabs (7 never used, 5 used in mouth [buccal], and 5 used on hand/finger skin). All 10 vaginal swab eluates contained greater than 200 μg/mL of glycogen and numerous PAS (glycogen)-positive cells. In contrast, all negative control samples had less than 200 μg/mL of glycogen, and only 1 sample (buccal) had numerous PAS (glycogen)-positive cells.

Detection of Glycogen in Archived Vaginal Swabs From a Condom Efficacy Trial

A total of 26 archived de-identified remnant vaginal swabs (provided by the CFHC) from samples collected before and after condom uses as part of a condom efficacy trial were tested for glycogen to determine if the assay works for aged (>1 year) archived specimens. Eluates from all of these samples contained greater than 200 μg/mL of glycogen (data not shown).

Glycogen Concentrations in Vaginal Swab Eluates From Women Representing Different Hormonal Conditions and Negative Controls

Eighteen women (from BUMC and CONRAD) provided self-administered vaginal swabs for further validation of the glycogen quantitation assay. Twelve were nonpregnant female volunteers between the ages of 18 and 40 years, and 6 were perimenopausal/postmenopausal women. Vaginal swab eluates from all 18 women contained detectable glycogen concentrations (>50 μg/mL). Glycogen concentrations were significantly higher in vaginal swab eluates from the younger women (mean ± SE, 876.6 ± 146.4 μg/mL) than from the perimenopausal/post-menopausal women (235.3 ± 112.6 μg/mL; $P < 0.01$). Two of the women reported using hormonal contraception; their glycogen values were 1110 and 987 μg/mL. Five of the reproductive-aged women also provided buccal (n = 5) and hand/finger (n = 5) swabs to serve as negative controls. Glycogen levels were below assay linear

detection range (<50 μg/mL) in eluates from all but 1 of the negative control swabs (buccal: mean ± SE, 40.3 ± 4.7 μg/mL; median, 45 μg/mL; range, 24–50 μg/mL; hand/finger: mean ± SE, 18.6 ± 5.4 μg/mL; median, 22 μg/mL; range, 3–31 μg/mL). Thirteen eluates from empty swabs were also tested for glycogen in the quantitative assay and were all below detection range (mean ± SE, 14.8 ± 2.5 μg/mL; median, 14 μg/mL; range, 1.3–31) (Fig. 2). All vaginal swab eluates had numerous glycogen-positive cells by PAS stain; 2 of the buccal swabs had faintly positive cells, whereas all the other negative controls were negative in the PAS assay.

Effects of Human Semen and Seminal Plasma on the Glycogen Assay

Glycogen assessments were performed with semen and soluble semen components, which can be present in vaginal secretions and swab eluates. To determine whether semen contains amounts of glycogen that could contribute to a false-positive vaginal swab result, eluates from swabs that were dipped into fresh whole human semen, dried, and extracted according to the vaginal swab elution protocol were tested in the glycogen assay. These samples all had low glycogen levels (n = 5; mean ± SE, 32.4 ± 10.7 μg/mL; median/range, 31/6–68 μg/mL) (Fig. 2), suggesting that a semen specimen would not be scored as positive in the glycogen assay. To determine whether soluble seminal factors that could be present in eluates from vaginal swabs collected after intercourse interfere with glycogen detection contributing to false-negative results, 10 μL of seminal plasma was added to vaginal swab eluates or directly to glycogen standards. Figure 3 shows that soluble semen factors do not interfere with glycogen detection in the quantitative assay. In other experiments, whole semen did not affect glycogen detection when added to vaginal swabs before elution (data not shown).

Sensitivity/Specificity of the Quantitative Glycogen and PAS Assays for Detection of Vaginal Swabs

Combined vaginal swab eluate data from 30 reproductive-aged women and data from 28 control swabs were used to determine the sensitivity and specificity for detection of vaginal samples using different cutoffs in the quantitative glycogen assay. Vaginal swab data used for this determination were from the following: (*a*) 3 healthy female volunteers recruited from BUMC used for assay development, (*b*) 12 of the 18 women from the prospectively recruited group (BUMC/CONRAD), and (*c*) 15 others (de-identified eluates from CDC and remnant specimens from CFHC as described earlier). Control samples represented various types of nonvaginal sources: 5 buccal, 5 hand/finger, 5 semen, and 13 empty swabs. At a cutoff of 50 μg/mL, 30 of 30 vaginal samples and 2 of 28 control samples were positive for a sensitivity of 1 (95% confidence interval [CI], 0.86–1) and specificity of 0.93 (95% CI, 0.75–0.99). At an assay cutoff of 100 μg/mL, 30 of 30 vaginal versus 0 of 28 control samples were positive for a sensitivity of 1 (95% CI, 0.86–1) and specificity of 1 (95% CI, 0.85–1). At an assay cutoff of 200 μg/mL, 29 of 30 vaginal versus 0 of 28 control samples were positive for a sensitivity of 0.97 (95% CI, 0.81–1) and a specificity of 1 (95% CI, 0.85–1).

The PAS stain was performed on cell smears from 22 of the vaginal swab eluates and 27 controls (10 buccal, 10 hand/finger, and 7 never-used swabs). All 22 of the vaginal samples, 3 of 10 buccal samples, and none of the other 17 control samples had glycogen-positive cells

in the PAS assay, for a sensitivity of 1 (95% CI, 0.81–1) and a specificity of 0.86 (95% CI, 0.64–0.96) (Table 1).

DISCUSSION

The vaginal environment contains a number of factors that could potentially be used as markers for validating swab sample collection. Properly used vaginal swabs should contain exfoliated cells, bacterial flora and a broad array of proteins, polysaccharides, and low-molecular-weight molecules. Recent proteomic studies have identified more than 1000 proteins in cervicovaginal secretions, 136 of which have been classified as core proteins, found in high abundance in most subjects regardless of physiological status.[22] Among the most abundant core proteins are those involved in immune defense such as antimicrobial peptides, cytokines, and chemokines. Several of these have been quantified in vaginal swab samples,[9] but their concentrations can vary widely, even in swabs carefully collected by trained medical staff, and specificity is low because these factors are also abundant in other types of mucosal samples such as saliva[23] and semen.[24] Concentrations of other potential markers in cervicovaginal secretions, such as mucins, are influenced by female reproductive hormones (reviewed in Gipson[25]). The presence of characteristic vaginal flora species such as *Lactobacilli* spp. offer another possible marker for swab use validation, but vaginal flora profiles vary considerable between women[26]; for example, women with bacterial vaginosis have little or no lactobacilli in their vaginal secretions.[27]

Protein stains have been successfully applied to identify used applicators in a vaginal microbicide study[28,29] but will not work well with swabs because cotton absorbs protein stain. We selected glycogen detection because high glycogen levels are fairly specific for vaginal secretions. The glycogen detection assay worked very well for our study. Soluble glycogen at levels higher than 100 μg/mL was detected in vaginal swab eluates from all reproductive-aged women, whereas eluates from control swabs (empty, buccal, hand/finger, semen) all had glycogen concentrations higher than 100 μg/mL. Likewise, the PAS cell glycogen stain positively identified all of the vaginal swab samples and was negative or weakly positive for the control samples.

A potential caveat not addressed in this study is the possibility that bacterial vaginosis, sexually transmitted infections, or use of hormonal contraceptives or certain vaginal products can affect glycogen levels or detection of glycogen in vaginal swabs. A recent study suggests that glycogen levels in vaginal secretions are significantly lower in women with BV,[30] and another study that used a protein stain to detect vaginal applicator use in a vaginal microbicide gel trial showed that the presence of gel in the vagina interfered with the detection of protein on the applicators.[31] More studies are needed to determine whether the glycogen test can be broadly applied, especially in clinical studies in different geographical locations. Further research should also be conducted to validate the use of the glycogen test with other types of swabs used for vaginal sampling such as Dacron, rayon, and nylon-flocked swabs.

This glycogen test is promising and is currently being used to verify exposure to the vagina of swabs that tested negative for PSA in a study of a new female condom (C. Mauck,

personal communication). Without the glycogen test, a PSA-negative swab that had not been exposed to the vagina for whatever reason would be categorized as providing evidence that the female condom had worked as a successful barrier, biasing results. By using the glycogen test, such swabs would be removed from the analysis of condom performance. A similar approach could be used to validate vaginal swab use in studies in which PSA is used as a biomarker for unprotected intercourse. The low cost, ease of use, and stability of samples support the feasibility of using this glycogen test in low-resource settings.

Acknowledgments

We would like to acknowledge the technical support from Ryan Palapaz and Caitlin Blaskewicz and valuable advice and support from Ron Frezieres, Terri Walsh, Maria Gallo, Carolyn Black, Teresa Brown, Marianne Callahan, and Jill Schwartz.

Support by the United States Agency for International Development (USAID) under USAID Cooperative Agreement GPO-A-00-08-00005-00 with CONRAD. The views expressed in this article are not necessarily those of USAID.

References

1. Rose SB, Lawton BA, Bromhead C, et al. Self-obtained vaginal swabs for PCR chlamydia testing: A practical alternative. Aust N Z J Obstet Gynaecol. 2007; 47:415–418. [PubMed: 17877602]
2. van Dommelen L, Dukers-Muijrers N, van Tiel FH, et al. Evaluation of one-sample testing of self-obtained vaginal swabs and first catch urine samples separately and in combination for the detection of *Chlamydia trachomatis* by two amplified DNA assays in women visiting a sexually transmitted disease clinic. Sex Transm Dis. 2011; 38:533–535. [PubMed: 21217415]
3. Xu F, Stoner BP, Taylor SN, et al. Use of home-obtained vaginal swabs to facilitate rescreening for *Chlamydia trachomatis* infections: Two randomized controlled trials. Obstet Gynecol. 2011; 118:231–239. [PubMed: 21775837]
4. Balasubramanian A, Kulasingam SL, Baer A, et al. Accuracy and cost-effectiveness of cervical cancer screening by high-risk human papillomavirus DNA testing of self-collected vaginal samples. J Low Genit Tract Dis. 2010; 14:185–195. [PubMed: 20592553]
5. Bahamondes L, Diaz J, Marchi NM, et al. 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. Hum Reprod. 2008; 23:2444–2451. [PubMed: 18664473]
6. Duerr A, Gallo MF, Warner L, et al. Assessing male condom failure and incorrect use. Sex Transm Dis. 2011; 38:580–586. [PubMed: 21278626]
7. Macaluso M, Lawson ML, Hortin G, et al. Efficacy of the female condom as a barrier to semen during intercourse. Am J Epidemiol. 2003; 157:289–297. [PubMed: 12578798]
8. Abdool Karim Q, Abdool Karim SS, Frohlich JA, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science. 2010; 329:1168–1174. [PubMed: 20643915]
9. Dezzutti CS, Hendrix CW, Marrazzo JM, et al. Performance of swabs, lavage, and diluents to quantify biomarkers of female genital tract soluble mucosal mediators. PLoS One. 2011; 6:e23136. [PubMed: 21858008]
10. Mauck CK, Weaver MA, Schwartz JL, et al. Critical next steps for female condom research—Report from a workshop. Contraception. 2009; 79:339–344. [PubMed: 19341844]
11. Gaensslen, RE. Identification of menstrual blood/vaginal secretions. In: Gaensslen, RE., editor. Sourcebook in Forensic Serology, Immunology, and Biochemistry. Vol. 121–122. Washington, DC: National Institute of Justice; 1983. p. 168-129.
12. Hausmann R, Pregler C, Schellmann B. The value of the Lugol's iodine staining technique for the identification of vaginal epithelial cells. Int J Legal Med. 1994; 106:298–301. [PubMed: 7524629]

13. Randall B, Riis RE. Penile glycogenated epithelial cells as an indicator of recent vaginal intercourse. Am J Clin Pathol. 1985; 84:524–529. [PubMed: 4036882]
14. Randall BB, Fierro MF, Froede RC. Practice guideline for forensic pathology. Members of the Forensic Pathology Committee, College of American Pathologists. Arch Pathol Lab Med. 1998; 122:1056–1064. [PubMed: 9870853]
15. Hausmann R, Schellmann B. Forensic value of the Lugol's staining method: further studies on glycogenated epithelium in the male urinary tract. Int J Legal Med. 1994; 107:147–151. [PubMed: 7534471]
16. Jones EL Jr, Leon JA. Lugol's test reexamined again: Buccal cells. J Forensic Sci. 2004; 49:64–67. [PubMed: 14979345]
17. Randall B. Glycogenated squamous epithelial cells as a marker of foreign body penetration in sexual assault. J Forensic Sci. 1988; 33:511–514. [PubMed: 3373168]
18. Rothwell TJ, Harvey KJ. The limitations of the Lugol's iodine staining technique for the identification of vaginal epithelial cells. J Forensic Sci Soc. 1978; 18:181–184. [PubMed: 89184]
19. Farage M, Maibach H. Lifetime changes in the vulva and vagina. Arch Gynecol Obstet. 2006; 273:195–202. [PubMed: 16208476]
20. Boskey ER, Cone RA, Whaley KJ, et al. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. Hum Reprod. 2001; 16:1809–1813. [PubMed: 11527880]
21. Mauck CK. Biomarkers of semen exposure. Sex Transm Dis. 2009; 36:S81–S83. [PubMed: 19218891]
22. Zegels G, Van Raemdonck GA, Tjalma WA, et al. Use of cervicovaginal fluid for the identification of biomarkers for pathologies of the female genital tract. Proteome Sci. 2010; 8:63. [PubMed: 21143851]
23. Al-Harthi L, Wright DJ, Anderson D, et al. The impact of the ovulatory cycle on cytokine production: Evaluation of systemic, cervicovaginal, and salivary compartments. J Interferon Cytokine Res. 2000; 20:719–724. [PubMed: 10954915]
24. Politch JA, Tucker L, Bowman FP, et al. Concentrations and significance of cytokines and other immunologic factors in semen of healthy fertile men. Hum Reprod. 2007; 22:2928–2935. [PubMed: 17855405]
25. Gipson IK. Mucins of the human endocervix. Front Biosci. 2001; 6:D1245–D1255. [PubMed: 11578960]
26. Lamont RF, Sobel JD, Akins RA, et al. The vaginal microbiome: New information about genital tract flora using molecular based techniques. BJOG. 2011; 118:533–549. [PubMed: 21251190]
27. Hillier SL. Diagnostic microbiology of bacterial vaginosis. Am J Obstet Gynecol. 1993; 169:455–459. [PubMed: 8357044]
28. Wallace A, Thorn M, Maguire RA, et al. Assay for establishing whether microbicide applicators have been exposed to the vagina. Sex Transm Dis. 2004; 31:465–468. [PubMed: 15273578]
29. Wallace AR, Teitelbaum A, Wan L, et al. Determining the feasibility of utilizing the microbicide applicator compliance assay for use in clinical trials. Contraception. 2007; 76:53–56. [PubMed: 17586138]
30. Mirmonsef P, Gilbert D, Veazey RS, et al. A comparison of lower genital tract glycogen and lactic acid levels in women and macaques: Implications for HIV and SIV susceptibility. AIDS Res Hum Retroviruses. 2012; 28:76–81. [PubMed: 21595610]
31. Austin MN, Rabe LK, Hillier SL. Limitations of the dye-based method for determining vaginal applicator use in microbicide trials. Sex Transm Dis. 2009; 36:368–371. [PubMed: 19556930]

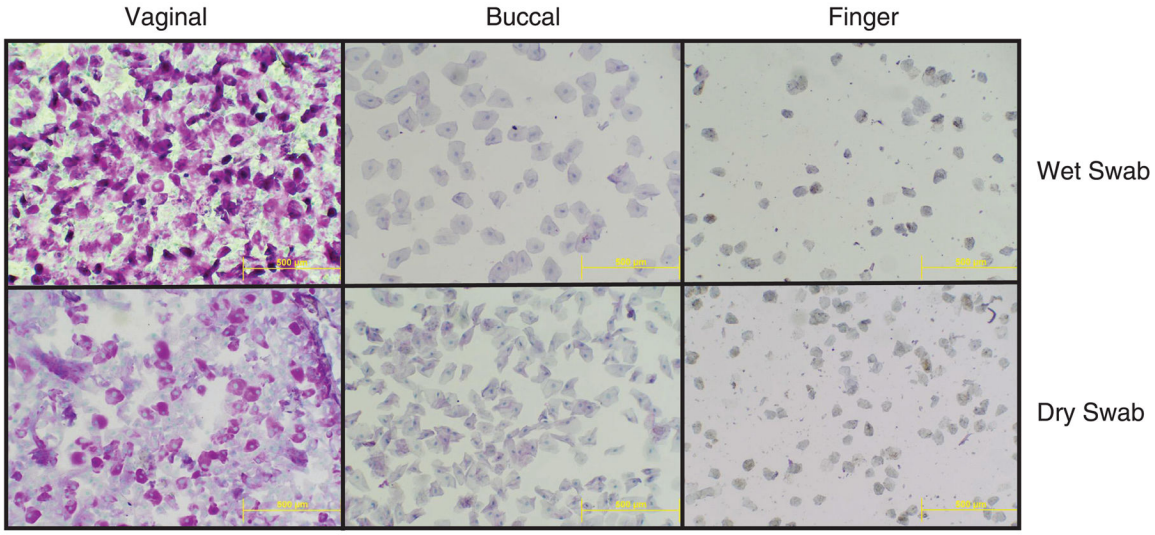

Figure 1.

Periodic acid–Schiff stain of cells eluted from fresh/wet swabs versus stored/dry swabs used to sample vaginal secretions, buccal secretions, and finger skin. Glycogen-positive cells appear reddish-purple.

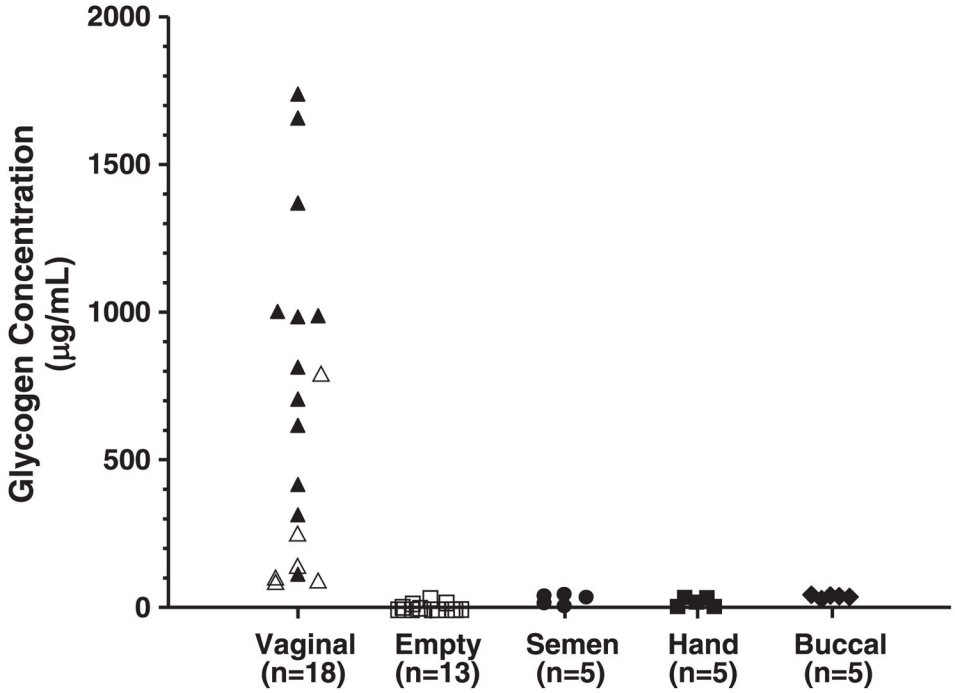

Figure 2.

Glycogen concentrations in eluates from prospectively collected vaginal versus control swabs. Black triangles represent data from reproductive-aged women; open triangles represent data from perimenopausal/postmenopausal women.

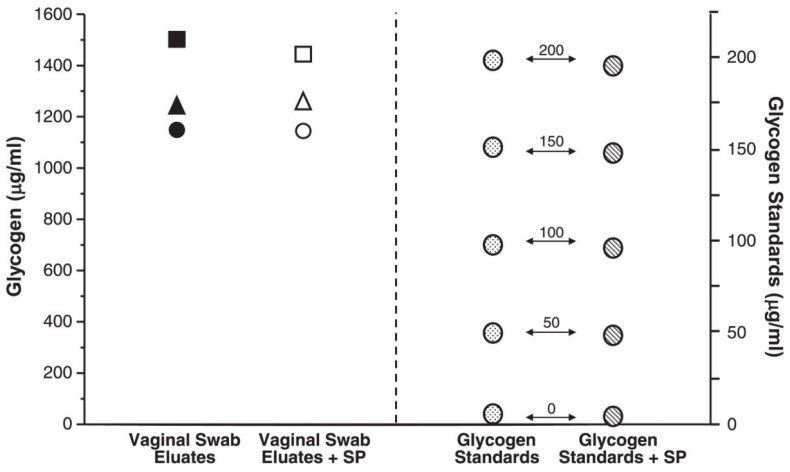

Figure 3.

Effects of semen on glycogen detection. Soluble seminal factors do not interfere with the detection of glycogen in vaginal swab eluates (n = 3) or standards in the quantitative glycogen assay. SP indicates seminal plasma.

TABLE 1

Detection of Glycogen by Quantitative Assay and PAS Stain in Various Samples

		Type of Specimen From Swab							
		Unused		Buccal		Finger/Hand		Vagina	
		Gly	PAS	Gly	PAS	Gly	PAS	Gly	PAS
BUMC (preliminary)	n = 9			3		3		3	
	Result			<200	Negative	<200	Negative	>200	All +++
CDC	n = 27	7		5		5		10	
	Result	<200	All negative	<200	1/5 +	<200	Negative	>200	All +++
CFHC	n = 26							26	
	Result							>200	ND
BUMC/CONRAD	n = 41	13		5		5		18	
	Result	<50	ND	1/5 >50	2/5 +	All <50	Negative	All >50	All +++

Gly indicates glycogen quantitative assay (in micrograms per milliliter); ND, not done.