Techniques

ACTA CYTOLOGICA

Acta Cytologica 2011;55:364–367
DOI: 10.1159/000329488

Received: May 18, 2011
Accepted: May 18, 2011
Published online: July 22, 2011

A Comparison of Dacron versus Flocked Nylon Swabs for Anal Cytology Specimen Collection

Julia C. Gage[a] Arpita Ghosh[a] Sylvia Borgonovo[b] Stephen Follansbee[b] Nicolas Wentzensen[a] Patti E. Gravitt[c] Niels Grabe[e, f] Bernd Lahrmann[e, f] Philip E. Castle[d]

[a]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, DHHS, Bethesda, Md., [b]Division of Infectious Diseases, Department of Medicine, Kaiser Permanente San Francisco, San Francisco, Calif., [c]Departments of Epidemiology and Molecular Microbiology and Immunology, Johns Hopkins University, Baltimore, Md., and [d]American Society for Clinical Pathology, Washington, D.C., USA; [e]Section Medical Informatics, Institute of Medical Biometry and Informatics, University Hospital Heidelberg, and [f]Hamamatsu Tissue Imaging and Analysis Center, Heidelberg, Germany

Key Words
Cytology · Anal neoplasia · Human papillomavirus testing · Screening · Cytological techniques

Abstract
Objectives: We compared the performance of commonly used Dacron versus flocked nylon swabs for anal cytology. ***Study Design:*** From 23 HIV-positive men screened at Kaiser Permanente San Francisco (San Francisco, Calif., USA), 2 anal specimens were collected, 1 with each swab in random order, and placed into liquid cytology medium. Specimens were tested for cellularity by quantifying a genomic DNA *(erv-3)*. The number of cells was assessed from prepared slides by automated image analysis. Performance was compared between swabs using 2-sample t tests and standard crossover trial analysis methods accounting for period effect. ***Results:*** Flocked swabs collected slightly more *erv-3* cells than Dacron for the first sample although not significantly (p = 0.18) and a similar number of *erv-3* cells for the second sample (p = 0.85). Flocked swabs collected slightly more cells per slide than the Dacron swabs at both time periods although this was only significant in the second time period (p = 0.42 and 0.03 for first and second periods, respectively). In crossover trial analysis, flocked swabs outperformed Dacron for cell count per slide based on slide imaging (p = 0.03), but Dacron and flocked swabs performed similarly based on *erv-3* quantification (p = 0.14). ***Conclusions:*** Further studies should determine whether flocked swabs increase the representation of diagnostically important cells compared to Dacron.

Background

Anal cytology is currently recommended to screen for anal neoplasia among high-risk populations such as men who have sex with men [1]. Yet, anal cytology suffers from low clinical sensitivity for high-grade anal intraepithelial neoplasia, even in human immunodeficiency virus (HIV)-infected people who might have more easily detected, larger lesions [2–4]. Dacron swabs are most commonly used for sampling the anus, but it is possible that

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/acy

Correspondence to: Dr. Julia C. Gage
Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics
National Cancer Institute, 6120 Executive Blvd, EPS, MSC 7231
Rockville, MD 20852 (USA)
Tel. +1 301 594 7296, E-Mail gagej@mail.nih.gov

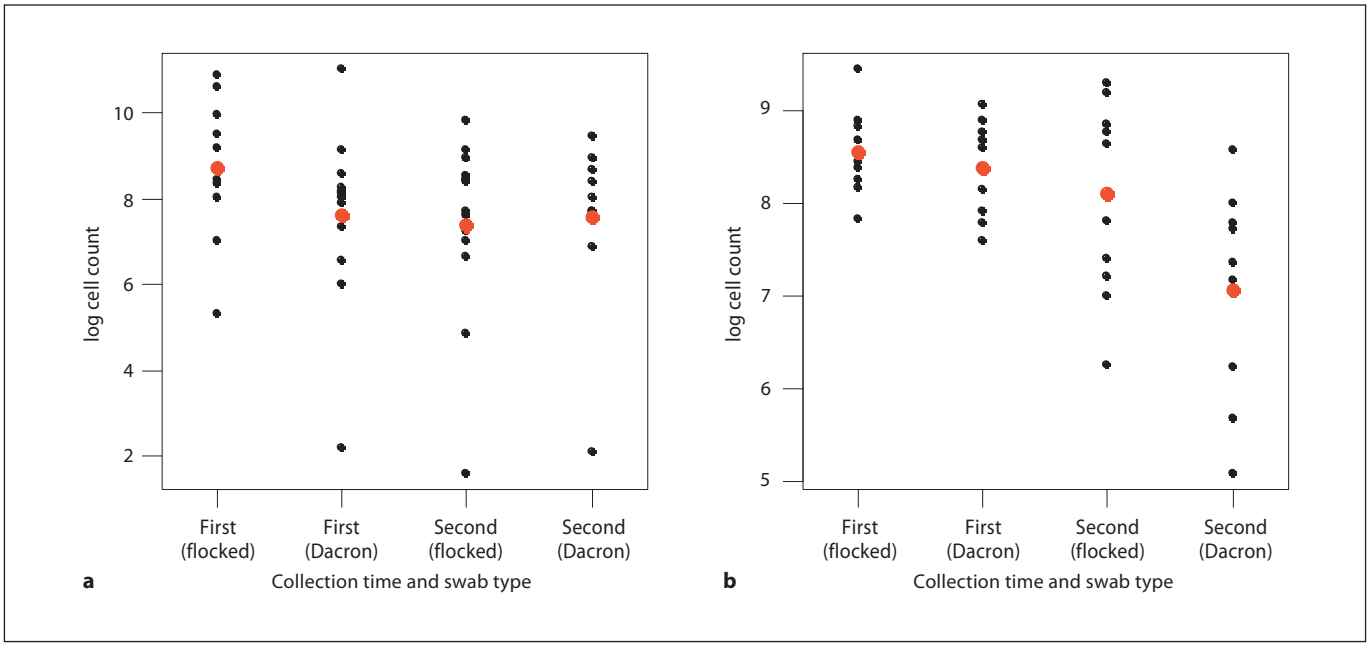

Fig. 1. Log-transformed values of cell counts together with means based on *erv-3* cell quantification per milliliter of Preservcyt (**a**) and cell count per slide based on slide imaging (**b**) by type (Dacron or flocked) and collection time (first or second) of swabs. Larger circles indicate mean values.

flocked nylon-tipped swabs may have improved sensitivity because of increased cellular yields. Specifically, flocked nylon swabs have a high surface area and, unlike the woven surface of Dacron swabs, their surface fibers are oriented perpendicularly to the tip so cells are more easily eluted. Although there are no published directed comparisons of the two materials, a previous study of flocked nylon swabs demonstrated an increased sensitivity for cervical detection of human papillomavirus (HPV) and *Chlamydia trachomatis* DNA compared with rayon-tipped swabs [5], which are quite similar to Dacron.

Material and Methods

To examine the best sampling device for collecting anal cells prior to initiating a screening study, we compared the performance of the Dacron and flocked nylon swabs in a pilot sample of 23 HIV-positive men undergoing anal cancer screening by digital rectal examinations and high-resolution anoscopy at Kaiser Permanente San Francisco (San Francisco, Calif., USA). At each examination, 2 anal specimens were sequentially collected from the anal canal. The order of collection was previously assigned by randomization to control for possible differences in quality of cell collection at the first versus second swab. Specifically, the clinician (S.B.) gently inserted the wetted swab into the anal canal until it reached the distal rectal vault (5–6 cm from the anal verge) and then withdrew the swab with rotation and lateral pressure over 15–20 s to ensure the cells were collected from the anal transformation zone. Specimens were placed into Preservcyt medium (Cytyc Corporation, now Hologic, Marlborough, Mass., USA) and irrevocably anonymized with an ID label that was not linked to the patient. All participants provided signed informed consent, and the research was approved by both Kaiser Permanente and National Cancer Institute human subjects review boards.

Specimens were tested for cellularity using a validated molecular assay that quantified a specific genomic DNA sequence *(erv-3)*, a human endogenous retrovirus gene which is present as 2 copies per diploid cell [6–9]. This allows direct estimation of human cell equivalents per unit volume without a concern of amplification of pseudogenes, which is common for other human gene targets. In addition, slides were prepared, and the number of cells per slide was assessed by digitizing the slides with the Hamamatsu Nanozoomer HT Scan System and applying a previously validated automated algorithm for cell detection [10]. Cellular counts were log-transformed and compared using t tests, and the order effect was taken into account using crossover analysis methods [11]. Although minimum cellularity is often used in the laboratories, no benchmark threshold has been established, and we therefore chose to compare mean values as an indicator of increased or decreased likelihood that specimens will meet a given threshold.

To measure the comparative performance of the swabs for detection of HPV DNA, the specimens were tested by linear array [12, 13].

We used 2-sided p values to determine whether flocked swabs performed better or worse than Dacron swabs.

Table 1. Means, standard errors (SE) and p values for data on log-transformed values of cell counts based on *erv-3* cell quantification and slide imaging for 23 HIV-positive men screened in a pilot study of the Anal Cancer Screening Study conducted at Kaiser Permanente, Northern California

		log *erv-3* cell quantification per milliliter of Preservcyt					log cell count per slide based on slide imaging[1]				
		n	mean	SE	2-sided 95% CI	2-sided p values	n	mean	SE	2-sided 95% CI	2-sided p values
First collection	flocked	10	8.72	0.53	0.54–2.76	0.18 (21)[2]	10	8.54	0.14	0.25–0.59	0.42 (19)[2]
	Dacron	13	7.61	0.56			11	8.38	0.14		
Second collection	flocked	13	7.39	0.60	1.69–2.02	0.85 (21)[2]	11	8.11	0.31	0.08–2.01	0.03 (19)[2]
	Dacron	10	7.56	0.65			10	7.06	0.34		
All	flocked	23	7.97	0.42	0.34–2.22	0.14 (21)[3]	21	8.31	0.18	0.15–2.28	0.03 (19)[3]
	Dacron	23	7.59	0.42			21	7.75	0.23		

CI = Confidence interval for difference in means. Figures in parentheses indicate degrees of freedom.
[1] Three slides taken from 2 patients had heavy mucus and were not readable. [2] Two-sample t test, stratified by collection time. [3] Two-sample t test, crossover analysis.

Results

Figure 1 illustrates the log-transformed cell counts based on *erv-3* quantification and slide imaging for first and second collections separately, with the means for each strata denoted by larger circles. Table 1 presents the corresponding mean values, standard errors, 95% confidence intervals and 2-sided p values. On average, flocked swabs collected more cells per milliliter of Preservcyt based on *erv-3* quantification than Dacron swabs for the first sample, although this was not statistically significant ($p = 0.18$). For the second sample, Dacron and flocked swabs collected a similar number of cells ($p = 0.85$). Using cell counts based on slide imaging, the flocked swabs collected on average more cells per slide than the Dacron swabs for both the first and second collections, although not attaining statistical significance for the first sample ($p = 0.42$ and 0.03 for first and second samples, respectively).

Using standard crossover trial analysis methods for the pooled data to take into account the period effect, we found that flocked swabs clearly outperform the Dacron swabs using cell count per slide based on slide imaging ($p = 0.03$). However, based on *erv-3* quantification per milliliter, although flocked swabs yield a higher number of cells on average than Dacron, they are not statistically significantly different ($p = 0.14$).

The performance between the swabs for HPV linear array was similar (90.9% agreement, $\kappa = 0.804$ for 1 of 13 carcinogenic HPV genotypes). No difference in discomfort or bleeding was noted between the sampling devices.

Discussion

Our study is limited by small sample size and the resulting imprecision of the mean estimates. The data suggest that flocked nylon swabs might have yielded more anal cells than Dacron swabs. Greater cellular yields may increase the representation of diagnostically important cells and reduce the number of anal cytology slides judged as inadequate. Unfortunately, cytopathology reading was not possible for these specimens. The similar performance between swabs for HPV DNA testing suggests that swab type might be more important for cytology sampling compared to HPV sampling. These findings emphasize the need to further investigate the possible clinical utility of using flocked versus Dacron swabs. Although Dacron swabs present a minimal cost, consideration of the increased cost of flocked nylon swabs ($\geq$6 times more expensive) is also required.

Conclusion

Subsequent larger evaluations on optimizing anal cellular sampling are warranted using cytology outcomes as an end point.

References

1 Palefsky JM, Holly EA, Hogeboom CJ, Berry JM, Jay N, Darragh TM: Anal cytology as a screening tool for anal squamous intraepithelial lesions. J Acquir Immune Defic Syndr Hum Retrovirol 1997;14:415–422.

2 Chin-Hong PV, Berry JM, Cheng SC, et al: Comparison of patient- and clinician-collected anal cytology samples to screen for human papillomavirus-associated anal intraepithelial neoplasia in men who have sex with men. Ann Intern Med 2008;149:300–306.

3 Cranston RD, Darragh TM, Holly EA, et al: Self-collected versus clinician-collected anal cytology specimens to diagnose anal intraepithelial neoplasia in HIV-positive men. J Acquir Immune Defic Syndr 2004;36:915–920.

4 Pereira AC, Lacerda HR, Barros RC: Diagnostic methods for prevention of anal cancer and characteristics of anal lesions caused by HPV in men with HIV/AIDS. Braz J Infect Dis 2008;12:293–299.

5 Krech T, Castriciano S, Jang D, Smieja M, Enders G, Chernesky M: Detection of high risk HPV and *Chlamydia trachomatis* in vaginal and cervical samples collected with flocked nylon and wrapped rayon dual swabs transported in dry tubes. J Virol Methods 2009;162:291–293.

6 D'Souza G, Sugar E, Ruby W, Gravitt P, Gillison M: Analysis of the effect of DNA purification on detection of human papillomavirus in oral rinse samples by PCR. J Clin Microbiol 2005:43:5526–5535.

7 Fontaine J, Gravitt P, Duh LM, Lefevre J, Pourreaux K, Hankins C, Coutlee F: High level of correlation of human papillomavirus-16 DNA viral load estimates generated by three real-time PCR assays applied on genital specimens. Cancer Epidemiol Biomarkers Prev 2005:14:2200–2207.

8 Trimble CL, Piantadosi S, Gravitt P, et al: Spontaneous regression of high-grade cervical dysplasia: effects of human papillomavirus type and HLA phenotype. Clin Cancer Res 2005;11:4717–4723.

9 Yuan CC, Miley W, Waters D: A quantification of human cells using an ERV-3 real time PCR assay. J Virol Methods 2001;91:109–117.

10 Grabe N, Lahrmann B, Pommerencke T, von Knebel Doeberitz M, Reuschenbach M, Wentzensen N: A virtual microscopy system to scan, evaluate and archive biomarker enhanced cervical cytology slides. Cell Oncol 2010;32:109–119.

11 Hills M, Armitage P: The two-period crossover clinical trial. Br J Clin Pharmacol 1979; 8:7–20.

12 Gravitt PE, Peyton CL, Alessi TQ, et al: Improved amplification of genital human papillomaviruses. J Clin Microbiol 2000;38:357–361.

13 Gravitt PE, Peyton CL, Apple RJ, Wheeler CM: Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 1998;36:3020–3027.