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Authors
Ferris, Daron G
Darragh, Teresa M
Kavuri, Sravan
et al.

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Improved anal Cytology Sampling: Tush Brush Compared With Dacron Swab

Daron G. Ferris, MD,[1,2] Teresa M. Darragh, MD,[3] Sravan Kavuri, MD,[4] Nikhil Patel, MD,[4] Jennifer L. Waller, PhD,[5] and Angela Goebel, CCRC[2]

Objective: The objective of this study was to determine the performance characteristics of the Tush brush (TB) compared with a saline moistened Dacron swab (DS) as anal cytology sampling devices.

Materials and Methods: TB and DS anal cytology tests were randomly collected from 146 patients presenting for anal cytology. High-resolution anoscopy and biopsies were obtained as indicated. Sensitivity and specificity as well as rates of satisfactory specimens were determined for each method using the areas under the receiver operating characteristic curve (AUCROC) and McNemar's test, respectively. Perceived discomfort of each device was determined using a visual analog scale and compared using a paired *t* test.

Results: The adjudicated AUCROC, sensitivity, and specificity were greater, but not significantly different, for the brush (0.63, 85.5, and 40.0, respectively) compared with the swab (0.50, 79.6, and 33.3, respectively) when the anal biopsy results were considered the criterion standard. In the 1 subject diagnosed with anal cancer, the swab cytology result was normal, but the brush result was abnormal. Specimen adequacy was 95.2% for the brush and 93.2% for the swab. Mean discomfort (visual analog scale) scores were swab 28.5 mm versus brush 35.6 mm ($p = .0003$) with both scores within the minimal to moderate discomfort range.

Conclusions: Anal cytology AUCROC, sensitivity, and specificity in detecting anal neoplasia were greater using the TB when compared with the DS. A novel anal cytology sampling device designed specifically to increase the detection of anal neoplasia would be clinically beneficial.

Key Words: anal cytology, anal cancer, anal cytology test, sampling device

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Testing cytology specimens obtained from various anatomical sites for evidence of neoplasia is a common medical practice. Analogous to cervical cancer screening, anal precancer, and cancer can be detected using cytologic techniques; patients with abnormal screening tests can be further evaluated and detected disease can be subsequently treated.[1] As such, experts recommend anal cytology testing is often used to detect anal neoplasia in high-risk men and women.[1] Results from anal cytology tests guide appropriate patient management that may help prevent or identify anal cancer.[2]

However, the current anal cytology sampling method is not standardized and results vary considerably.[3–9] Experts recommend sampling the anal transformation zone using a saline moistened Dacron swab (DS).[1] However, the generic swab is not designed specifically for this screening procedure. The swab can retrieve cells moderately well, but cells may become entrapped and poorly transferred for interpretive purposes.[10] Consequently, if specimens lack sufficient cellularity, an unsatisfactory test should be repeated. More importantly, multiple studies have demonstrated the relative inaccuracy of anal cytology tests that fail to detect disease in up to 90% of patients.[3–9] Because of swab sampling adequacy and sensitivity deficiencies, this approach may increase healthcare and patient costs, discomfort, inconvenience, and total procedural time.

Although the DS is currently recommended as the sampling device for anal cytology, there may be a need to improve the detection of anal neoplasia by using devices developed specifically for that purpose. A sampling device designed to improve test accuracy, test adequacy, quality control, and safety while minimizing unnecessary procedural discomfort would be beneficial. The purpose of this study was to determine the efficacy, specimen adequacy, and comfort of a novel anal cytology brush compared with the standard swab.

MATERIALS AND METHODS

Subject Population

A convenience sample of men and women 21 years or older presenting for anal cytology testing and/or high-resolution anoscopy (HRA) living in or near Augusta, Georgia, were asked to enroll in the study at the Georgia Cancer Center. We included individuals with a previously collected abnormal anal cytology test result, a history of high-grade HPV-associated gynecologic disease or cancer,[11] other malignancies, immunosuppressive illness,[12,13] immunosuppression due to therapeutic agents,[14–16] anorectal or perianal HPV-related disease, or men who have sex with men.[17,18] Exclusion criteria included recent anorectal surgery (≤4 months), surgical absence or severe stenosis of the anus, HPV vaccination, severe anal pain, concomitant anorectal infection, pregnancy, and unwillingness to participate. All subjects read and signed an institutional review board–approved informed consent document before participating. The Augusta University Institutional Review Board (#611652) approved the study.

Study Design

Patients scheduled for an anal cytology test were asked by the provider to participate in the study. Interested patients read and signed an institutional review board–approved informed consent document that described the study and their potential involvement. Pertinent disease-specific demographic information was collected from each subject. These data included basic demographic information, medical history, sexual orientation and behavior, immune status, gynecologic history, medications, tobacco use, as well as anal neoplasia screening, diagnosis, and treatment history.

Subjects were then placed in the left lateral decubitus position and the perianal region was inspected. Thereafter, each sampling device was used in a randomly assigned order to collect an anal cytology test from the anorectal transformation zone.

[1]Department of Obstetrics and Gynecology, Augusta University, Augusta, GA; [2]Georgia Cancer Center, Augusta University, Augusta, GA; [3]Department of Pathology, University of California San Francisco, San Francisco, CA; [4]Department of Pathology, Augusta University, Augusta, GA; and [5]Department of Population Health Sciences, Division of Biostatistics and Data Science, Augusta University, Augusta, GA

Correspondence to: Daron G. Ferris, MD, Department of Obstetrics and Gynecology, Medical College of Georgia, Georgia Cancer Center, HH-1013, Augusta University, Augusta, GA 30912. E-mail: dferris@augusta.edu

D.G.F. is the inventor of the Tush Brush and Augusta University holds patent rights. The other authors have declared they have no conflicts of interest.

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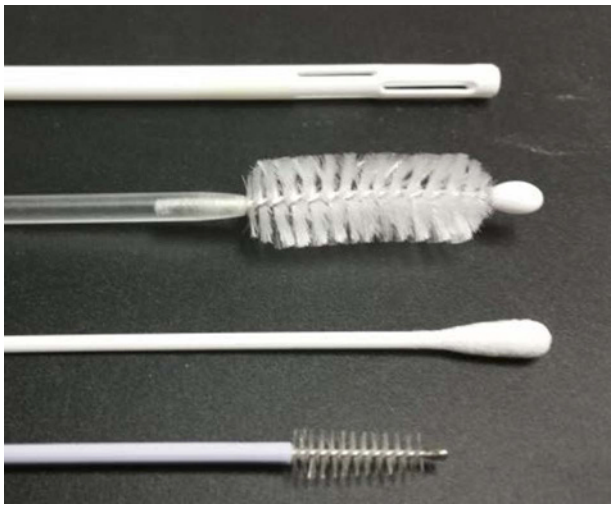

FIGURE 1. Cytology transfer mechanism, TB, DS, and Cytobrush TM Plus. The TB has a cellular transfer mechanism designed to safely and effectively remove the cellular specimen from the sampling region and minimize hazardous exposure to the user. The extended linear span and soft elongated bristles of the TB satisfactorily sample the entire anorectal transformation zone with a single 360-degree rotation after insertion. The bidirectional contoured tip facilitates easy, safe insertion, and removal. A generic DS used for anal cytology testing. A Cytobrush TM Plus, approved for sampling only the endocervical canal in women, shown as a comparison.

Subjects were initially blinded to the sampling devices. The saline moistened DS was inserted into the anal canal until the rectum was reached (4–5 cm) and then rotated, exerting lateral pressure on the anal canal, and using simultaneous to-and-fro motion several times, then withdrawn.[19] An Ayre spatula was used to scrape cells from the swab into PreservCyt solution (Hologic, Marlborough, MA). The Tush brush (TB) was inserted into the anal canal until the nylon bristles were no longer observed, rotated 360 degrees, and removed. The cellular transfer mechanism was used to dislodge cells from the brush into PreservCyt solution. Each specimen bottle was labeled with a coded subject specific number to insure anonymous samples and method of sampling. Liquid-based cytological specimens (ThinPrep) were processed according to manufacturer's instructions, stained with Pap stain and interpreted using the Bethesda System.[20] Pathologists were blinded to device types. Specimen adequacy was assessed by examining for the presence of nucleated squamous cells (>1–2 per high power field) and reported as satisfactory or unsatisfactory.[1] A 3-member pathology group adjudicated test results according to accepted practice.

Immediately after the use of each anal cytology test collection device, subjects made a mark on a visual analog scale (VAS) indicating perceived level of discomfort (scale 0–100 mm with 0 no discomfort and 100 severe) for each device. Afterward, investigators determined the VAS scores for each device by measuring the distance from the zero end of the scale to the subject's mark and entered these measurements into the database. Thereafter, subjects were unblinded as to the sampling devices used and completed a short questionnaire concerning their responses to each device.

As clinically indicated, after a digital anorectal examination and insertion of a lubricated anoscope, HRA was performed (Leisegang Colposcope; Cooper Surgical, Trumbull, CT) by a single experienced provider to visualize the anorectal transformation zone and detect anal neoplasias. Anal biopsies were obtained from the areas representing the most severe HRA changes and lesions with different morphologic appearances were sampled. No

TABLE 1. Descriptive Statistics

Variable	Level	
Sex	Female	49 (33.6)
	Male	97 (66.4)
Race	American Indian	1 (0.7)
	Asian	1 (0.7)
	Black	85 (58.2)
	Multiracial	3 (2.1)
	White	56 (38.6)
Age, mean (SD)		43.8 (12.8)
First cytology sampling device	Tush brush	73 (50.0)
	Dacron swab	73 (50.0)
Histology	AIN 1	22 (15.1)
	AIN 2	11 (7.5)
	AIN 3	21 (14.3)
	Cancer	1 (0.7)
	Normal	6 (4.1)
	Inadequate sample	1 (0.7)
	Not done	84 (57.5)
HRA	Normal	36 (24.7)
	Low grade	32 (21.9)
	High grade	25 (17.1)
	Cancer	1 (0.7)
	Condyloma	26 (17.8)
	Not done	26 (17.8)
Positive histology or HRA	Positive	84 (70.0)
	Negative	36 (30.0)
	Not done	26
Tush brush cytology	ASC-H	2 (1.4)
	ASCUS	22 (15.6)
	HSIL	16 (11.0)
	LSIL	55 (39.0)
	Normal	49 (34.8)
	Missing data	5
Tush brush abnormal cytology	Abnormal	92 (65.3)
	Normal	49 (34.7)
	Missing	5
Dacron swab cytology	ASCH	5 (3.7)
	ASCUS	23 (16.8)
	HSIL	16 (11.0)
	LSIL	50 (36.5)
	Normal	44 (32.1)
	Missing data	9
Dacron swab abnormal cytology	Abnormal	93 (67.9)
	Normal	44 (32.1)
	Missing	9
Tush brush sample adequacy	Adequate	139 (95.2)
	Not adequate	7 (4.8)
Dacron swab sample adequacy	Adequate	136 (93.2)
	Not adequate	10 (6.8)

Data are presented as *n* (%), unless otherwise indicated. Total number = 146.

ASC-H indicates atypical squamous cells, cannot exclude high grade; ASCUS, atypical squamous cells of undetermined significance; HSIL, high grade squamous intraepithelial lesion; LSIL, low grade squamous intraepithelial lesion.

TABLE 2. Positive Cytology or Sample Adequacy Controlling for Order of Sampling Methods

Outcome	Sampling order	Dacron swab	Tush brush		κ (95% CI) [McNemar *p*]		
			Abnormal	Normal	Brush 1st	Swab 1st	Overall
Adjudicated final diagnosis							
Positive cytology	Brush 1st	Abnormal	37 (58.7)	5 (7.9)	0.76 (0.59 to 0.93) [0.2568]		0.71 (0.58 to 0.83) [0.3349]
		Normal	2 (3.2)	19 (30.2)			
	Swab 1st	Abnormal	43 (61.4)	7 (10.0)		0.63 (0.43 to 0.83) [0.3657]	
		Normal	4 (5.7)	16 (22.9)			
			Tush brush		McNemar's test *p*		
			Adequate	Not adequate	Brush 1st	Swab 1st	Overall
Sample adequacy	Brush 1st	Adequate	62 (84.9)	2 (2.7)	0.26 (−0.08 to 0.58) [0.0956]		−0.02 (−0.05 to 0.01) [0.1118]
		Not adequate	7 (9.6)	2 (2.7)			
	Swab 1st	Adequate	69 (94.5)	3 (4.1)		−0.02 (−0.05 to 0.01) [0.3173]	
		Not adequate	1 (1.4)	0 (0.0)			

random biopsies were obtained if the HRA examination was normal. Histologic specimens were labeled and submitted separately in 10% formalin for processing. The tissue was sectioned, glass slides prepared, and then stained using standard hematoxylin and eosin stains. Histology was reported using the LAST/anal intraepithelial neoplasia (AIN) classification systems. All cytological and histological specimens were processed at a central laboratory (Augusta University), then analyzed, and adjudicated at 2 locations by cytopathologists (Augusta University and University of California, San Francisco, California).

Instruments

The 2 anal cytology sampling devices were used in random order during the trial. A saline moistened DS (Baxter Healthcare Corporation, McGraw Park, IL) consists of a Dacron tip and a plastic shaft. It was used in conjunction with saline to moisten the Dacron tip as indicated previously. The TB, a novel anal cytology sampling device, was designed to be used only for screening men and women for anal neoplasias (see Figure 1). The brush sampling region consists of soft bidirectional tapered nylon bristles and a smooth rounded tip to facilitate introduction. The span of the brush sampling region is designed to obtain a cellular sample from the entire anorectal transformation zone if inserted until the brush is no longer visible externally. Hence, no to-and-fro motion is required. The length of the bristles permits a single rotation on its axis without the need for pressure to be exerted on the lateral anal canal walls. Once sampling has been completed, the cellular transfer mechanism is used to safely and effectively remove the cellular specimen from the brush sampling region. The cellular transfer mechanism is essentially a tube with distally positioned fenestrations that slides over the brush sampling region to dislodge cells into liquid cytology transport media.

Statistical Analysis

All statistical analysis was performed using SAS 9.4 and statistical significance was assessed using a significance level of 0.05. Descriptive statistics, frequencies, and percentages for categorical variables as well as means and standard deviations for continuous variables, were calculated A κ statistic and corresponding 95% CI, McNemar's test or Bowker's test of symmetry accounting for the order of the 2 sample collection methods was used to examine agreement and differences in adequacy of the sample (satisfactory or unsatisfactory), and abnormal cytology dichotomized (abnormal: atypical squamous cells, cannot exclude high grade/ atypical squamous cells of undetermined significance/ low grade squamous intraepithelial lesion/ high grade squamous intraepithelial lesion cancer vs normal) between the swab and the brush. Sensitivity and specificity were determined for both the swab and brush dichotomized cytology with histology (normal vs abnormal) considered the true diagnosis, a clinical diagnosis using HRA (positive or negative) considered the true diagnosis, or with the combination of histology and HRA (either abnormal vs both

TABLE 3. Sensitivity (%), Specificity (%), and AUCROC of Anal Cytology Test Devices by Histologic, Clinical, and Combined Endpoints

Disease endpoint	Anal cytology test device						AUCROC comparison *p*
	Dacron swab			Tush brush			
	Se	Sp	AUCROC (95% CI)	Se	Sp	AUCROC (95% CI)	
Anal biopsy[a] (*n* = 60 for DS, *n* = 60 for TB)	79.6	33.3	0.50 (0.30–0.71)	85.5	40.0	0.63 (0.38–0.87)	0.5430
HRA[b] (*n* = 113 for DS, *n* = 119 for TB)	78.8	57.6	0.68 (0.58–0.77)	78.6	60.0	0.71 (0.61–0.80)	0.3989
Biopsy and HRA[c] (*n* = 114 for DS, *n* = 120 for TB)	79.3	59.4	0.69 (0.59–0.79)	79.0	61.8	0.72 (0.62–0.82)	0.3915

[a]Histology diagnosis from anal biopsy.

[b]Clinical diagnosis by HRA (no biopsy).

[c]Histology and clinical diagnoses combined.

DS indicates dacron swab; Se, sensitivity; Sp, specificity; TB, tush brush.

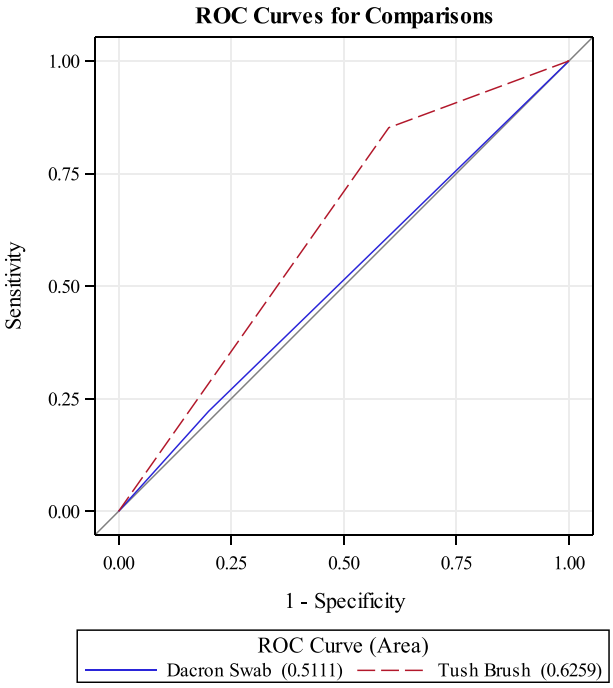

FIGURE 2. The AUCROC of adjudicated DS or TB cytology results by histology results.

normal). Logistic regression was used to calculate the areas under the receiver operating characteristic curve (AUCROC) for the swab and for the brush and the AUCROC were compared.[21] To examine differences in patient perception of discomfort between the swab and the brush, a cross-over design paired *t* test that accounted for the ordering of the 2 sampling methods was used. A paired *t* test was used to examine differences between the swab and the brush methods for questionnaire items.

RESULTS

Of 150 patients approached to participate in the study, 1 declined and 2 were excluded for anal douching less than 24 hours before sampling. We enrolled 147 subjects and 1 study subject withdrew before cytology collection. Subjects were 66.4% male, 58.2% were black, and the mean (SD) age was 43.8 (12.8) years (see Table 1). Of the subjects, 62 had anal or perianal biopsies collected with one being inadequate. Of the 61 with adequate histology, 36.1% (22/61) had AIN 1 and 34.4% (21/61) had AIN 3. One patient was diagnosed with squamous cell carcinoma 1.6% (1/61). Of the total subjects examined by HRA, 30.7% (36/120) had a normal examination and 26.6% (32/120) had an HRA impression of low grade. Of the subjects who had a histology sample taken, 88.8% had abnormal histology, 70% with HRA examinations had an abnormal clinical diagnosis based on HRA, and 71.1% had abnormal histology or HRA combined.

Adjudicated specimen adequacy rates were 95.2% for the brush and 93.2% for the swab. Abnormal cytology and sample adequacy results when controlling for device order of sampling are seen in Table 2. For sample adequacy, when the brush was used first for AU (data not shown), there was lack of symmetry with there being a higher proportion in the brush adequate and swab not adequate cross classification ($p = .03$) than in the brush not adequate and swab adequate cross classification.

The AUCROC, sensitivity, and specificity for the brush and swab were determined by histology, clinical, and combined endpoints (see Table 3). The adjudicated AUCROC, sensitivity, and specificity were greater, but not significantly different, for the brush (0.63, 85.5, and 40.0, respectively) compared with the swab (0.50, 79.6, and 33.3, respectively) when the anal biopsy results were considered the criterion standard. The AUCROC of adjudicated swab and brush cytology results compared with histology results are seen in Figure 2. In the 1 subject diagnosed with anal cancer, the swab cytology result was normal, but the brush result was abnormal HSIL (AIN 3).

Subjects' perceived discomfort from sampling by the 2 devices is seen in Table 4. The mean discomfort VAS scores obtained immediately after sampling were 29.5 mm for the swab and 37.3 mm for the brush ($p = .0003$). More subjects indicated that the swab was less uncomfortable upon removal compared with the brush ($p = .0208$). There were no significant differences between devices with respect to insertion discomfort, severe pain, device preference, irritation, and soreness experienced after sampling. Subjects considered anal cytologic test accuracy more important than comfort (1.7 [1.0]). They also disagreed that the brush was more uncomfortable than an injection (3.4 [1.3]).

DISCUSSION

This clinical trial was the first to critically examine the potential diagnostic utility of the TB a novel anal cytology sampling device. When compared directly with the currently recommended DS, the TB was better able to detect anal neoplasias. Although not statistically significant based on sample size, the positive trend in improved performance may have resulted from careful efforts to create a sampling device that conforms specifically to the intended anatomic site. Using deidentified MRI images of the anorectal

TABLE 4. Perceived Sampling Device Discomfort and Perception of Device

Variable	Dacron swab, mean (SD)	Tush brush, mean (SD)	t^a	*p*
Sampling device discomfort (VAS)[b]	29.5 (27.9)	37.3 (27.7)	−3.75	.0003
Insertion uncomfortable	2.9 (1.2)	2.7 (1.2)	1.87	.0634
Removal uncomfortable	3.3 (1.1)	3.0 (1.2)	2.34	.0208
Experienced severe pain	3.6 (1.2)	3.7 (1.1)	−0.45	.6536
More comfortable than other method	2.9 (1.3)	3.2 (1.3)	−1.38	.1685
Prefer the Dacron swab	3.0 (1.2)	2.8 (1.2)	−1.45	.1481
Irritating	3.2 (1.2)	3.1 (1.2)	0.85	.3943
Sore after use	3.5 (1.1)	3.5 (1.1)	0.62	.5354

All other remaining variables determined by Likert scale results (1–5: 1 strongly agree, 2 agree, 3 neutral, 4 disagree, 5 strongly disagree).

[a]Paired *t* tests for differences in VAS.

[b]VAS - results recorded between 0 (no discomfort) and 100 (maximum).

region, the TB was designed to comprehensively sample the anorectal transformation zone, the site of anal neoplasias. For example, the device is inserted until all the sampling brush region is beyond the anal verge. The length or span of sampling area and lateral extent of the sampling region on the brush conform to the targeted anatomical site. Consequently, in contrast with the swab, simplified, comprehensive, and standardized sampling of the anorectal transformation zone is insured. Sampling quality control is essentially built into the device by eliminating the human factor or main reason for sampling errors. In addition, use of the cellular transfer mechanism easily removes the cellular specimen from the sampling region of the brush.

The swab device used to detect anal cytology abnormalities fails to detect disease in many people with anal neoplasia.[3–9,22–24] In a study that estimated the prevalence of AIN in heterosexual women with genital neoplasias, anal cytology using a moistened swab had a sensitivity of only 8% and specificity of 94% to detect AIN.[7] Using similar methodology, the sensitivity of the conventional anal cytology test in detecting all AIN was 34% for HIV+ men who have sex with men in another trial.[25] The reported anal swab unsatisfactory cytology rates vary from 9.9% to 34.4%.[26–29] Multiple clinical trials have demonstrated that a small brush (cytobrush) and plastic spatula or broom-like device (cervex brush) are vastly superior to swabs with respect to cervical cytology test efficacy and adequacy.[30–49] Moreover, there is greater variation in the rate of satisfactory cytology tests healthcare providers obtain using the swab compared with the cytobrush (14%–82% vs 75%–100%, respectively). Hence, the swab is inconsistent in properly sampling the cervical transformation zone. Based on the findings from their clinical trials comparing the swab and cytobrush, investigators have commented, "The swab is ineffective for both endocervical and ectocervical sampling"[50] and "Swabs should not be used for cervical cell sampling."[51] As a result, the cotton swab is no longer a standard of care device for cervical cytology tests. Moreover, because of design limitations, product indications, and safety issues, the current 3 devices used for cervical cytology tests cannot be used to collect an anal cytology test. For example, although used by some "off label" for anal cytology, the Cytobrush TM Plus is approved for sampling only the endocervical canal in women.

We hypothesized that, by unique product design and evidence-based data derived from trials comparing cervical cytology sampling devices, a greater number of unsatisfactory anal cytology results would be encountered using the DS. Our adjudicated findings did not indicate a statistically significant greater rate of unsatisfactory specimens collected with the swab as anticipated. However, because this determination is somewhat subjective, we did observe a greater satisfactory rate for the brush. In addition to extreme variation in test sensitivity to detect anal cancer, DS have poor test adequacy problems. Test adequacy relates to insuring a comprehensive sampling of the entire anorectal transformation zone to detect disease if present. An unsatisfactory cytology test result denotes an unreliable cytologic assessment. Unsatisfactory rates of the anal cytology test using the DS are 3% to 7% or more, which are much higher than unsatisfactory rates seen with cervical cytology testing (<1%).[4,22,51] The swab can retrieve cells moderately well from the sampling site, but subsequently, the cells become trapped within the fibers.[10] Hence, cells are poorly transferred to slides or transport media for interpretive purposes. Consequently, specimens may contain an insufficient number of cells and the test must be repeated. This sampling problem increases healthcare costs. In a small study from the National Cancer Institute, flocked nylon swabs yielded a greater anal cell count compared with DS based on slide imaging review ($p = .03$).[8] Such data support the use of a brush-like sampling device for anal cytology testing.

Although moderately severe pain may be expected before an anal cytology test, on average, only mild to moderate discomfort was documented in our study.[52] In general, subjects reported less overall discomfort with the DS. However, subjects also strongly agreed that a more accurate anal cytology test is more important than the comfort of the test. Regardless, we have considered several modifications to the TB prototype design to minimize discomfort. These modifications include a slightly larger tip, a slick surface for the tip and indentations at the base of the brush section to decrease surface adhesion with the perianal skin during rotation (sampling). Based on our observations during sampling, the latter modification will likely reduce much of the discomfort experienced by individuals.

CONCLUSIONS

The TB may represent an improvement in instrumentation to screen men and women for anal neoplasias. Results from our proof-of-principle study support the potential attributes of the TB. However, although favorable trends were clearly observed, this study was not powered adequately to determine whether these findings are statistically significant. Furthermore, it was conducted in a referral population with a greater prevalence of disease compared with that normally experienced in a high-risk screening population. A larger study of a primary screening population may be warranted to confirm these preliminary findings. If confirmed, use of the improved anal sampling device combined with appropriate management may help reduce the rate of anal cancer in high-risk individuals.

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REFERENCES

1. Darragh TM, Winkler B. Anal cancer and cervical cancer screening: key differences. *Cancer Cytopathol* 2011;119:5–19.
2. Abbas A, Yang G, Fakih M. Management of anal cancer in 2010. Part 2: current treatment standards and future directions. *Oncology* 2010;24: 417–24.
3. Fox PA, Seet JE, Stebbing J, et al. The value of anal cytology and human papillomavirus typing in the detection of anal intraepithelial neoplasia: a review of cases from an anoscopy clinic. *Sex Transm Infect* 2005;81:142–6.
4. Mathews WC, Sitapati A, Caperna J, et al. Measurement characteristics of anal cytology, histopathology, and high-resolution anoscopic visual impression in an anal dysplasia screening program. *J Acquir Immune Defic Syndr* 2004;37:1610–5.
5. Nathan M, Singh N, Garrett N, et al. Performance of anal cytology in a clinical setting when measured against histology and high-resolution anoscopy findings. *AIDS* 2010;24:373–9.
6. Panther LA, Wagner K, Proper J, et al. High resolution anoscopy findings for men who have sex with men: inaccuracy of anal cytology as a predictor of histologic high-grade anal intraepithelial neoplasia and the impact of HIV serostatus. *Clin Infect Dis* 2004;38:1490–2.
7. Santoso JT, Long M, Crigger M, et al. Anal intraepithelial neoplasia in women with genital intraepithelial neoplasia. *Obstet Gynecol* 2010;116: 578–82.
8. Gage JC, Ghosh A, Borgonovo S, et al. A comparison of dacron versus Flocked nylon swabs for anal cytology specimen collection. *Acta Cytol* 2011;55:364–7.
9. Nahas CS, da Silva Filho EV, Segurado AA, et al. Screening anal dysplasia in HIV-infected patients: is there an agreement between anal pap smear and

high-resolution anoscopy-guided biopsy? *Dis Colon Rectum* 2009;52: 1854–60.

10. Rubio CA. The false negative smear. II. The trapping effect of collecting instruments. *Obstet Gynecol* 1977;49:576–80.
11. Evans HS, Newnham A, Hodgson SV, et al. Second primary cancers after cervical intraepithelial neoplasia III and invasive cervical cancer in Southeast England. *Gynecol Oncol* 2003;90:131–6.
12. D'Souza G, Wiley DJ, Li X, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. *J Acquir Immune Defic Syndr* 2008;48:491–9.
13. Cress RD, Holly EA. Incidence of anal cancer in California: increased incidence among men in San Francisco, 1973–1999. *Prev Med* 2003;36:555–60.
14. Ogunbiyi OA, Scholefield JH, Raftery AT, et al. Prevalence of anal human papillomavirus infection and intraepithelial neoplasia in renal allograft recipients. *Br J Surg* 1994;81:365–7.
15. Hinten F, Meeuwis KA, van Rossum MM, et al. HPV-related (pre) malignancies of the female anogenital tract in renal transplant recipients. *Crit Rev Oncol Hematol* 2012;84:161–80.
16. Patel H, Silver A, Northover J. Anal cancer in renal transplant patients. *Int J Colorectal Dis* 2007;22:1–5.
17. Chin-Hong PV, Vittinghoff E, Cranston RD, et al. Age-specific prevalence of anal human papillomavirus infection in HIV-negative sexually active men who have sex with men: the EXPLORE study. *J Infect Dis* 2004;190: 2070–6.
18. Li AH, Phanuphak N, Sahasrabuddhe VV, et al. Anal squamous intraepithelial lesions among HIV positive and HIV negative men who have sex with men in Thailand. *Sex Transm Infect* 2009;85:503–7.
19. Nadal SR, Horta SHC, Calore EE, et al. How deep must the brush be introduced in the anal canal for a more effective cytological evaluation? *Rev Assoc Med Bras* 2009;56:749–51.
20. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* 2002;287: 2114–9.
21. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988;44:837–45.
22. Berry JM, Palefsky JM, Jay N, et al. Performance characteristics of anal cytology and human papillomavirus testing in patients with high-resolution anoscopy-guided biopsy of high-grade anal intraepithelial neoplasia. *Dis Colon Rectum* 2009;52:239–47.
23. Palefsky JM, Holly EA, Hogeboom CJ, et al. Anal cytology as a screening tool for anal squamous intraepithelial lesions. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;14:415–22.
24. Salit I, Tinmouth J, Lytwyn A, Chapman W, Raboud J, Shen S. Screening for HIV-associated anal cancer (TRACE study): test characteristics of cytology and oncogenic HPV testing for the detection of anal dysplasia. 23rd International Papilomavirus Conference and Clinical Workshop 2006: 388.
25. de Ruiter A, Carter P, Katz DR, et al. A comparison between cytology and histology to detect anal intraepithelial neoplasia. *Genitourin Med* 1994;70:22–5.
26. Templeton DJ, Roberts JM, Poynten IM, et al. Prevalence and predictors of unsatisfactory anal cytology tests in a cohort of gay and bisexual men in Sydney, Australia: baseline findings from the study of the prevention of anal cancer (SPANC). *Eur J Cancer Prev* 2017;26:212–6.
27. Zaccarini DJ, Khurana KK. Histopathologic and cytologic follow-up in high risk male patients with unsatisfactory anal cytology. *Patholog Res Int* 2017;2017:9780213.
28. Khattab R, McMeekin E, Taege AJ, et al. Unsatisfactory exfoliative anal cytology samples, 15-year experience with histologic, cytologic, and molecular follow-up. *Diagn Cytopathol* 2018;46:117–21.
29. Sananpanichkul P, Pittyanont S, Yuthavisuthi P, et al. Anal papanicolaou smear in women with abnormal cytology: a Thai hospital experience. *Asian Pac J Cancer Prev* 2015;16:1289–93.
30. Zhao C, Domfeh AB, Austin RM. Histopathologic outcomes and clinical correlations for high-risk patients screened with anal cytology. *Acta Cytol* 2012;56:62–7.
31. Chalvardjian A, De Marchi WG, Bell V, et al. Improved endocervical sampling with the Cytobrush. *Can Med Assoc J* 1991;144:313–7.
32. Davey-Sullivan B, Gearhart J, Evers CG, et al. The Cytobrush effect on Pap smear adequacy. *Fam Pract Res J* 1991;11:57–64.
33. Deckert JJ, Staten SF, Palermo V. Improved endocervical cell yield with Cytobrush. *J Fam Pract* 1988;26:639–41.
34. Dotters DJ, Carney CN, Droegemueller W. Nylon brush improves collection of cervical cytologic specimens. *Am J Obstet Gynecol* 1988; 159:814–9.
35. Hutchinson M, Fertitta L, Goldbaum B, et al. Cervex-Brush and Cytobrush. Comparison of their ability to sample abnormal cells for cervical smears. *J Reprod Med* 1991;36:581–6.
36. Kavak ZN, Eren F, Pekin S, et al. A randomized comparison of the 3 Papanicolaou smear collection methods. *Aust N Z J Obstet Gynaecol* 1995; 35:446–9.
37. Kawaguchi K, Nogi M, Ohya M, et al. The value of the Cytobrush for obtaining cells from the uterine cervix. *Diagn Cytopathol* 1987;3:262–7.
38. Kohlberger PD, Stani J, Gitsch G, et al. Comparative evaluation of seven cell collection devices for cervical smears. *Acta Cytol* 1999;43:1023–6.
39. Koonings PP, Dickinson K, d'Ablaing G 3rd, et al. A randomized clinical trial comparing the cytobrush and cotton swab for papanicolaou smears. *Obstet Gynecol* 1992;80:241–5.
40. Kristensen GB, Jensen LK, Ejersbo D, et al. The efficiency of the cytobrush and cotton swab in obtaining endocervical cells in smears taken after conization of the cervix. *Arch Gynecol Obstet* 1989;246:207–10.
41. McCord ML, Stovall TG, Meric JL, et al. Cervical cytology: a randomized comparison of four sampling methods. *Am J Obstet Gynecol* 1992;1772–7.
42. Murata PJ, Johnson RA, McNicoll KE. Controlled evaluation of implementing the cytobrush technique to improve papanicolaou smear quality. *Obstet Gynecol* 1990;75:690–5.
43. Paraiso MF, Brady K, Helmchen R, et al. Evaluation of the endocervical cytobrush and cervex-brush in pregnant women. *Obstet Gynecol* 1994; 84:539–43.
44. Partoll LM, Javaheri G. Cervical cytology after cryosurgery, laser ablation and conization. A comparison of the cotton swab and endocervical brush. *Acta Cytol* 1993;37:876–8.
45. Pretorius RG, Sadeghi M, Fotheringham N, et al. A randomized trial of three methods of obtaining papanicolaou smears. *Obstet Gynecol* 1991; 78:831–6.
46. Rivlin ME, Woodliff JM, Bowlin RB, et al. Comparison of cytobrush and cotton swab for Papanicolaou smears in pregnancy. *J Reprod Med* 1993;38: 147–50.
47. Ruffin MT 4th, Van Noord GR. Improving the yield of endocervical elements in a Pap smear with the use of the cytology brush. *Fam Med* 1991; 23:365–9.
48. Selvaggi SM. Spatula/cytobrush vs. spatula/cotton swab detection of cervical condylomatous lesions. *J Reprod Med* 1989;34:629–33.
49. Shen JT, Nalick RH, Schlaerth JB, et al. Efficacy of cotton-tipped applicators for obtaining cells from the uterine cervix for Papanicolaou smears. *Acta Cytol* 1984;28:541–5.
50. Toffler WL, Pluedeman CK, Sinclair AE, et al. Comparative cytologic yield and quality of three Pap smear instruments. *Fam Med* 1993;25:403–7.
51. Cachay ER, Agmas W, Mathews WC. Relative accuracy of cervical and anal cytology for detection of high grade lesions by colposcope guided biopsy: a cut-point meta-analytic comparison. *PLoS One* 2012;7:e38956.
52. Blankenship SA, Debnath P, Szlachta-McGinn AW, et al. Knowledge and acceptability of anal cytology screening among women. *J Low Genit Tract Dis* 2016;20:90–6.