The Optimal Technique for Obtaining a Papanicolaou Smear with the Cervex-Brush

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Background. The Cervex-Brush (Rovers, The Netherlands; Unimar, Wilton, Conn) is a cervical cytologic sampling device used to simultaneously collect endocervical and ectocervical cells. The optimal method for using this device to collect cervical epithelial cells for a Papanicolaou smear has not been described in the medical literature.

Methods. Using the Cervex-Brush, Papanicolaou smears were collected from 516 women in a prospective randomized study to evaluate five different clockwise rotation techniques: 180° rotation of the brush, 360°, 720°, 1080°, and 1800°. The Bethesda System of classifying Papanicolaou smears was used to report cytologic results. Endocervical cell presence as an indicator of smear adequacy was quantitated from 0 to 4+.

Results. As the number of rotations was increased, the number of endocervical cells collected also increased ($P < .05$). Abnormal epithelial cells were found in 22.3% of the smears obtained by rotating the brush 1080° and 1800°. This was compared with abnormal cells found in 8.2%, 4.5%, and 11.5% of the smears collected by rotating the brush 180°, 360°, and 720°, respectively ($P < .001$). Two smears that identified high-grade squamous intraepithelial lesions were collected using the 1800° rotation. Unsatisfactory smears were obtained in 39 (7.5%) of the 516 smears. The percentage of slides containing hemorrhagic artifact was greatest (16.4%) in the 1800° rotation group, but the likelihood of the Papanicolaou smear being considered unsatisfactory was not increased ($P < .05$).

Conclusions. Using a clockwise 1800° rotation of the Cervex-Brush to obtain a Papanicolaou smear maximized the collection of endocervical cells, detected the greatest percentage of abnormal cells, and did not statistically exceed the acceptable limit for hemorrhagic artifact.

Key words. Papanicolaou smear; cytology; cervix dysplasia; cervix neoplasms. *J Fam Pract 1992; 34:276–280.*

The purpose of the Papanicolaou smear is to identify premalignant or malignant disease occurring in the transformation zone of the cervix. Adequate sampling of cervical epithelial cells from the transformation zone is imperative for accurate cytopathologic interpretation.[1–3] Papanicolaou smear collections have been accomplished using various combinations of the traditional Ayre spatula, the Cytobrush, and cotton-tipped applicators, with differing results.[3–7] The appropriate use of a well-designed cervical cytologic collection device may potentially reduce the complications of incomplete sampling. The Cervex-Brush (Rovers, The Netherlands; Unimar, Inc, Wilton, Conn) allows simultaneous collection of both endocervical and ectocervical cells with a single device. The design of the Cervex-Brush is similar to that of a small paintbrush with longer central bristles, which are placed into the cervical os for the collection of endocervical cells (Figure 1). After collection of the Papanicolaou smear, the brush's contents are "painted" onto a single slide. The consistent placement of endocervical cells on the center of the slide and ectocervical cells on the periphery may contribute to a more accurate cytologic interpretation.

To date, the best technique for obtaining a Papanicolaou smear with this device has not been described. The optimal number of brush revolutions on the cervical transformation zone would yield an adequate number of epithelial cells, minimize hemorrhagic artifact, and maximize sensitivity to abnormal cells. The purpose of this investigation was to critically evaluate the results obtained by using five different sampling device rotation techniques to determine the optimal technique for obtaining a Papanicolaou smear with the Cervex-Brush.

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Figure 1. The Cervex-Brush. The long central bristles sample cells of the endocervical canal, and the shorter, lateral bristles collect cells from the ectocervix.

Methods

Between July 1990 and March 1991, 520 women between 15 and 93 years of age were recruited from three sites: the Medical College of Georgia Student Health Service and the Medical College of Georgia Family Practice Center, both in Augusta, and Memorial Medical Center Family Practice Center in Savannah, Georgia. Women with scheduled appointments for annual Papanicolaou smears who were eligible for participation were asked to volunteer. The inclusion criteria were that the subject be female, 15 years of age or older, and had requested a Papanicolaou smear. The exclusion criteria were surgical absence of the uterine cervix, menses, and douching within the previous 24 hours.

The study design evaluated five different Papanicolaou smear sampling device rotation techniques. A clockwise direction was used for rotations of 180°, 360°, 720°, 1080°, and 1800°. Subjects were initially randomly assigned to one of the first three rotation techniques; however, a significant number of preliminary Papanicolaou smear reports were interpreted as inadequate. Therefore, further randomization into the last two rotation groups was determined to be necessary and ethically prudent. If the initial smear was determined to be inadequate, the physician chose another standard method to obtain a repeat Papanicolaou smear.

All Papanicolaou smears were evaluated at a private laboratory according to established protocol by one cytotechnologist and one pathologist to control for variability of interpretation.[8] Both were blinded to the degree of rotation used to obtain each smear. Papanicolaou smears were accompanied by a requisition form listing demographic data, possible risk factors, and previous cytologic results. Results were classified by the cytotechnologist with regard to the number of endocervical cells collected. If no endocervical cells were obtained, the smear was given a score of 0; if 1 to 25 cells were collected, 1+; if 25 to 75 cells were collected, 2+; if 75 to 100 cells were collected, 3+; and if more than 100 endocervical cells were collected, 4+. If endocervical cells, endocervical mucus, or squamous metaplastic cells were present, the smear was considered satisfactory for interpretation. For a smear to be considered satisfactory also required that there be no obstructive hemorrhage, excessive exudate or inflammation, or cellular drying artifact. Cytotechnologist or pathologist interpretations were noted on the requisition and returned to the principal investigator and the physician who obtained the Papanicolaou smear. The Bethesda System[9] of interpreting cervical cytology was used to classify and report cytologic results. Smears were reported as "within normal limits," "infection," "inflammation," or "epithelial cell abnormalities indicative of atypia or squamous intraepithelial lesions" (SIL).

The chi-square test was used to calculate differences between rotation techniques, reported Papanicolaou smear results, and patient demographic data, except in the case of an expected cell count of less than five for which Fisher's exact test was used. Data were analyzed by the SAS system.[10]

Results

Data were collected on 520 subjects. Three subjects were excluded after Papanicolaou smear collection because of a previous hysterectomy, and another one was excluded because of the absence of demographic data. Therefore, data for analysis were available for 516 subjects. The average age of subjects was 32.4 years (age range 15 to 93 years). Subjects were randomly assigned to one of five groups, for each of which a different sampling device rotation technique was used (Table 1).

More than half of the 180° and 360° rotations yielded a smear containing no endocervical cells (51.3% and 54.4%, respectively). The percentage of Papanicolaou smears collected using the 720° technique in which no endocervical cells were obtained was 39.1%, while the percentage in which no endocervical cells were obtained using the 1080° technique was 37.3%. Papanicolaou smears with no endocervical cells were obtained in only 26.5% of the 1800° rotation group. As the degree of rotation was increased, the percentage of smears in which endocervical cells were not obtained decreased, and the

Table 1. Distribution of Abnormal, Hemorrhagic, and Satisfactory Papanicolaou Smears for Cytologic Sampling Device, By Degree of Rotation

Degree of Rotation	Number of Rotations	Papanicolaou Smears Collected	Papanicolaou Smear Result		
			Abnormal* %	Hemorrhagic %	Satisfactory† %
180°	½	78	8.2	9.0	89.7
360°	1	91	4.5	12.1	94.5
720°	2	105	11.5	11.4	95.2
1080°	3	120	22.3	11.7	92.5
1800°	5	122	22.3	16.4	90.2
Total		516	14.9	12.4	92.4

**Abnormal denotes Papanicolaou smears reported other than "within normal limits" by Bethesda System criteria.*
†Satisfactory denotes presence of endocervical cells, squamous metaplastic cells, or endocervical mucus, and absence of obstructive hemorrhage, excessive exudate or inflammation, and cellular drying artifact.

number of endocervical cells that were collected increased (Figure 2).

The five rotation techniques also differed in the number of normal vs abnormal Papanicolaou smears. As the degree of rotation was increased, the yield of cytologically abnormal smears also increased (Table 1). The difference between normal and abnormal cytologic classification by degree of rotation was statistically significant ($P < .001$). The smears obtained by using 180° and 360° rotations yielded abnormalities consisting of infection, inflammation, or atypia (Table 2). Only the smears obtained by using 720°, 1080°, and 1800° rotations detected cytologic characteristics of premalignant squamous intraepithelial lesions. Notably, the only two Papanicolaou smears classified as high-grade SIL were collected using the 1800° rotation.

In those smears that contained no endocervical cells, none were reported as having epithelial cell abnormalities. Of the smears that contained endocervical cells ranked quantitatively as 1+ or 2+ (n = 156), 16 (10.3%) were found to have epithelial cell abnormalities. Of those smears ranked quantitatively as 3+ or 4+ (n = 149), however, the percentage of epithelial cell abnormalities revealed was more than doubled, to 23.5% ($P = .002$). The odds ratio[11] for epithelial cell abnormalities being detected in the 3+ to 4+ group as compared with the 1+ to 2+ group was 2.69.

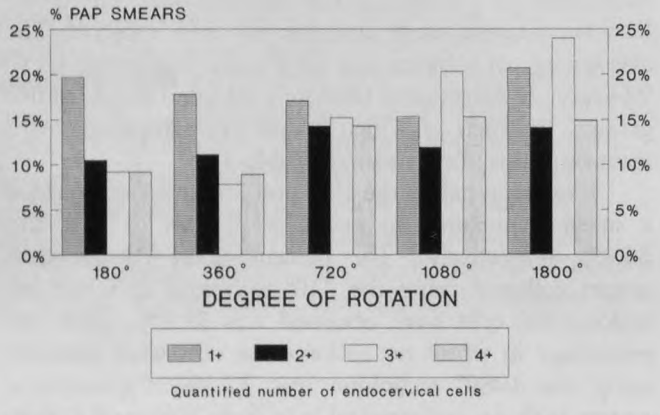

Figure 2. Distribution of the quantified number of endocervical cells obtained for the degree of cytologic sampling device rotation. Results expressed as percentage of total Papanicolaou smears for each degree of rotation, n = 305.

The presence of hemorrhagic artifact increased as the degree of sampling device rotation was increased (Table 1). However, the hemorrhagic artifact did not appear to interfere with cytologic interpretation. Although the 1800° rotation resulted in 20 smears that demonstrated hemorrhage, 86% of these hemorrhagic slides were still considered satisfactory; this can be compared with the 91% of nonhemorrhagic slides that were considered satisfactory ($P > .05$).

The 720° rotation maximized the percentage of satisfactory smears, but all five techniques produced greater than 89.7% satisfactory results (Table 1). Thus, the ability of the different rotations to produce satisfactory smears did not differ significantly ($P > .05$).

Nine of the 516 subjects were pregnant. Four (44.4%) of the smears obtained for these women were considered unsatisfactory compared with the 6.9% of smears obtained from nonpregnant subjects that were considered unsatisfactory ($P < .001$). Satisfactory smears were obtained from 87.5% of postpartum subjects, 88.9% of postmenopausal subjects, and from only 81.5% of subjects with a history of cryosurgery ($P < .05$). Endocervical cells were identified in 182 of 325 (56%) women using no contraception or nonhormonal contraception compared with 123 of 185 (67%) women who used oral contraceptive agents ($P = .02$).

Table 2. Distribution of Abnormal* Papanicolaou Smear Results (n = 73), by Degree of Rotation

Degree of Rotation	Papanicolaou Smears Collected (No.)	Abnormal Papanicolaou Smear Results		
		Infection, Inflammation, Atypia (No.)	Low-Grade SIL (No.)	High-Grade SIL (No.)
180°	78	6	—	—
360°	91	4	—	—
720°	105	12	1	
1080°	120	20	5	—
1800°	122	18	5	2
Total	516	60	11	2

**General categorization of other than within normal limits by the Bethesda System to include infection, inflammation, and epithelial cell abnormalities of atypia or squamous intraepithelial lesion (SIL).*

Discussion

The Cervex-Brush performed well in the typical populations of two family practice clinics and a student health clinic. Boon[7] reported that the Cervex-Brush performed reasonably well in The Netherlands when compared with results obtained using various combinations of the Ayre spatula, Cytobrush, Cytopick, and cotton swab. Recently Hutchinson et al[12] compared the Cervex-Brush and the Cytobrush plus a modified Ayre spatula with a control cotton swab and Ayre spatula. Cytologic atypia was detected equally with the Cervex-Brush and Cytobrush, but the Cytobrush yielded a greater percentage of endocervical cells.

The maximum number of endocervical cells collected with the Cervex-Brush were obtained using the 1800° rotation technique. Increasing the number of rotations increased the number of endocervical cells obtained. Theoretically, the increase reflects a greater circumferential and more representative columnar cell sample obtained from the endocervical canal.

All five rotations produced high rates of satisfactory results. The 720° technique produced the maximum percentage of satisfactory smears, but statistically significant differences among the five collection techniques were not found. However, a *satisfactory* smear does not equate with an *optimal* smear. The greater the number of rotations, the greater the number of endocervical and epithelial cells (ie, the optimal results), but also the greater the degree of hemorrhage (and unsatisfactory results).

Increasing the number of rotations yields a better sampling of the transformation zone. Accurate cytologic representation of this zone and interpretation of abnormalities, if present, is important because it enables appropriate management and quality health care. For example, a smear report of inflammation should alert the physician to investigate the underlying cytology. Wilson et al[13] evaluated women with cervical cytology demonstrating inflammatory changes. Cervical intraepithelial neoplasia, or SIL, was detected in 13% of women, 25% of women had colposcopic evidence of human papilloma virus, 22.9% had one sexually transmited disease, and 75.5% had one genital infection (inclusive of vaginitis). Diagnoses based on cervical cytology frequently underestimate the severity of disease. Jones et al[14] documented that 25% of patients with a Papanicolaou smear indicating atypia actually had histologic evidence of cervical intraepithelial neoplasia. Even cytological tests in which low-grade squamous intraepithelial lesions are detected may not accurately reflect true cervical disease. Bolger and Lewis[15] colposcopically evaluated women with low-grade SIL and found that 40% had histologically proven high-grade SIL. Therefore, cytologic smears interpreted as abnormal usually have an explanation and require further evaluation to determine both the presence and true severity of disease. Consequently, those cytologic sampling methods that maximize the detection of abnormalities have value and should be adopted.

Vooijs et al[18] demonstrated a significantly greater number of epithelial cell abnormalities detected in smears with endocervical cells. The postulated positive correlation between the number of endocervical cells collected and the likelihood of cytopathology being revealed was confirmed in the present investigation. Elias et al[17] found that the relative risk of finding mild to moderate dysplasia in Papanicolaou smears containing endocervical columnar cells was 2.2 and that the relative risk of finding severe dysplasia or cancer was 4.4. Data from this study showed a strong correlation between increased numbers of endocervical cells collected and increased sensitivity to epithelial cell abnormalities.

Increased rotations also increased the number of hemorrhagic smears. Although the 1800° rotation in-

creased the percentage of smears containing hemorrhagic artifact, it did not significantly increase the number of unsatisfactory smears. The potential for greater endocervical cell recovery with brush rotations beyond 1800° might be offset by an increase in hemorrhagic artifact.

The investigation demonstrated some variation in results based on postmenopausal and pregnancy status. A slightly lower percentage of satisfactory smears was obtained in postmenopausal subjects. A significant number of false-negative smears and poor endocervical cell recovery for postmenopausal women have been noted in the past,[18–21] possibly due to stenosis of the cervical os and migration of the transformation zone into the cervical canal.[16] The narrow bristles of the Cervex-Brush easily penetrated the cervical os to collect endocervical cells and showed only a minimal decrease in efficacy in the postmenopausal population.

A decreased percentage of satisfactory Papanicolaou smears in pregnant populations has also been noted by other researchers using more traditional devices for collection of cervical smears.[1,22] Softness of the cervical epithelium and decreased endocervical cell cohesiveness are believed to contribute to substandard smears.[1] Difficulty in accessing the endocervical squamocolumnar junction during pregnancy, clinician reluctance to aggressively sample pregnant women, and the presence of the mucous plug during pregnancy may impede representative endocervical cytologic sampling.

This study was limited to the evaluation of five clockwise rotation techniques. Rotations involving simultaneous clockwise and counterclockwise rotations traumatize the thin layer of endocervical columnar cells. A brush rotation in one direction trails the arched bristles as they pass over the transformation zone. A change in direction extends the bristles momentarily before they arch in the opposite direction. The straightened sharp-ended bristles frequently penetrate the delicate columnar layer, advancing into the capillary-rich stroma and inducing hemorrhage.

In conclusion, a clockwise 1800° rotation of the Cervex-Brush maximized endocervical cell collection, detected the greatest percentage of cell abnormalities, and yet did not exceed an acceptable limit for hemorrhage. Therefore, the 1800° rotation is the optimal technique for using the Cervex-Brush and will result in a more complete and accurate portrayal of the existing cervical epithelium and the potential presence of premalignant or malignant lesions.

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See editorial comment on page 273.