

## Collection of Cervical Secretions Does Not Adversely Affect Pap Smears Taken Immediately Afterward

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**Collection of cervical secretions for local immunological assessment requires that the secretions be collected prior to the Pap smear to avoid contamination with blood. The objective of the present study was to determine whether gentle collection of cervical secretions prior to a Pap smear collection influences the quality of the Pap smear. A total of 266 women were recruited. Half of the participants were assigned to collection of cervical secretions prior to Pap smear collection with Weck-cel sponges. The remaining half had only the Pap smear collection performed. Pap smear slides were reviewed and evaluated for quality by the Bethesda System adequacy criteria without knowledge of randomization. The proportions of limited or inadequate slides in the two study groups were compared by using the Pearson chi-square test. No significant differences were observed between the two study groups when overall Pap smear quality was evaluated ( $P = 0.29$ ). Comparison of the two study groups with respect to individual adequacy criteria, including presence of air drying artifact, presence of obscuring blood, absence of metaplastic or endocervical cells from the transformation zone, scant cellularity, and presence of obscuring inflammatory cells, also revealed no significant differences between the two study groups. Results from the present study suggest that the collection of cervical secretions with Weck-cel sponges does not adversely impact the quality of subsequently obtained Pap smears.**

Among human papillomavirus (HPV) researchers, there is considerable interest in measuring local cervical immunological parameters involved in the host response to genital HPV infection. For those involved in elucidating the natural history of HPV-related cervical neoplasia, interest in the measurement of cervical immune responses stems from the belief that immunological control of HPV infection is a key deterrent to progression of HPV infection and its early cytological manifestations, low-grade squamous intraepithelial lesions, to high-grade squamous intraepithelial lesions and carcinomas (4, 8, 14, 16). For those involved in HPV vaccine development efforts, the challenge is to develop well-validated serologic (systemic) measures of protective immunological responses to HPV vaccines, which can be used in vaccine trials to measure the success of immune response induction by the vaccine formulations under evaluation (13). This requires initial studies which directly compare local and systemic measures of immune responses to validate the relevance to the cervix of the more easily measured serologic HPV responses. In turn, we need to develop methods to accurately and reproducibly measure cervical immune responses.

Measurement of cervical immune responses against HPV has been hampered, however, by the difficulty in quantitatively

and reliably collecting cervical secretions for use in immunological evaluation. To date, several collection methods have been proposed, including cervicovaginal lavages, wicks, Snotrips (Akron Inc., Abita Springs, La.), and Weck-cel sponges (Xomed Surgical Products Inc., Jacksonville, Fla.), but few data are available that systematically validate these various collection methods (1-3, 6, 11, 12, 15). An effective collection method should (i) allow quantitation of the amount collected, (ii) collect a sufficient volume for assay, (iii) avoid excessive dilution of the specimen, (iv) eliminate contamination of the sample with blood introduced by the collection instrument, and (v) allow easy and complete extraction of target proteins from the collection instrument.

To address these issues, we have initiated a series of parallel studies to evaluate Weck-cel sponges for use in collecting cervical secretions for immunological assays, including antibody and cytokine enzyme-linked immunosorbent assays. These studies are being conducted within the context of a large, 10,000-woman population-based study of HPV and cervical neoplasia that is under way in Costa Rica (7).

One of the important requirements for the collection of cervical secretions for immunological evaluation is that the collection avoid contamination with blood induced by the collection instrument itself. Consequently, secretions must be collected prior to the collection of Pap smear samples, because Pap smear collection instruments often induce abrasions and minor bleeding. The order of sample collection has raised concerns among clinicians and cytopathologists that collection of these secretions prior to Pap smear sample collection may result in the trapping of abnormal cells, which would then not be available for collection by the Pap smear instrument. This, in turn, would result in a detrimental effect on the quality of

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the Pap smear and lead to a higher percentage of inadequate Pap smear results.

The present study was a randomized trial conducted to evaluate the effect of cervical secretion collection with a Weck-cel sponge on the adequacy of Pap smear slides prepared immediately afterward. A total of 266 women were examined. Half were randomly assigned to have cervical secretions collected prior to Pap smear sample collection. The other half had only the Pap smear sample collection performed.

#### MATERIALS AND METHODS

A randomized trial was conducted among women enrolled in a National Cancer Institute-sponsored, 10,000-woman population-based natural history study of HPV and cervical neoplasia being conducted in Guanacaste, Costa Rica. Details of this large-cohort study have been presented in detail elsewhere (7). For the present study, cohort participants scheduled for a follow-up Pap smear between October 1996 and January 1997 were eligible for study participation.

A total of 266 women were approached during this period and enrolled in our study. Participants were randomly assigned either to have Weck-cel sponges collected prior to the Pap smear ( $n = 135$ ) or not ( $n = 131$ ).

All material was collected by a single study nurse (L.A.M.). Collection of cervical secretions involved gently placing the Weck-cel sponge on the cervical os and allowing the sponge to passively absorb cervical effluent for approximately 20 to 30 s as described previously (5). Two sponges were collected sequentially from each woman randomly selected to have secretions collected. Cervical cells were collected from all women with a Cervex brush (Unimar) and used to prepare Pap smear slides by standard methods, as previously described (7). Pap smear slides were evaluated for quality by expert cytotechnologists (S.V.E. and B.T.M.). Evaluation was performed without knowledge of whether cervical secretions were collected prior to the Pap smear. Pap slides were evaluated for quality by the Bethesda System adequacy criteria (9, 10). Samples were initially classified as either satisfactory, satisfactory but limited, or inadequate. Slides found to be either satisfactory but limited or inadequate were further classified as to the following: presence of air drying artifact, presence of obscuring blood, absence of metaplastic or endocervical cells from the transformation zone, scant cellularity, and presence of obscuring inflammatory cells.

The proportions of limited or inadequate samples were compared between study groups (secretions versus no secretions) by using the Pearson chi-square test. Each adequacy criterion was evaluated separately. Since the majority of the women (98.5%) were cytologically normal at the time of the follow-up visit, analyses stratified by disease status were not possible. However, analyses limited only to women with normal cytology at the time of the present study were performed and yielded identical results. Results of analyses which included all study subjects are therefore presented here.

#### RESULTS

The median age of study participants was 37 (range, 21 to 90) years, whether they were assigned to have cervical secretions collected or not. No differences between the two study groups were noted with respect to the overall quality of the Pap smears ( $P = 0.29$ ). Technically inadequate slides were observed for 2.2% ( $n = 3$ ) of the women randomized into the cervical secretion arm of the study and for 2.3% ( $n = 3$ ) of those assigned to the no-collection arm of the study. We found that the slides of 55.6% ( $n = 75$ ) of the participants assigned to the cervical secretion study arm were adequate for evaluation but limited. The comparable percentage for women randomized not to have the secretions collected was 63.4% ( $n = 83$ ). The high percentage of slides found to be evaluable but of limited quality was due, in part, to the large proportion of women in our Costa Rican cohort who have inflammation of the cervix leading to the presence of obscuring inflammation (24.8% of the 266 women in our study). Cervicitis is very common in this population.

Results from analyses which examined the two study groups with respect to individual adequacy criteria are presented in Table 1. No significant differences between the two groups were noted for any of the criteria evaluated. Compared to women who did not have cervical secretions collected, women who did were found to have a slightly lower rate of limited slides due to the absence of cells from the transformation zone

TABLE 1. Comparison of Pap smear adequacy among subjects with and without cervical secretion collection prior to pap smear performance

Adequacy criterion and classification <sup>a</sup>	No. (%) of study group members		P value <sup>d</sup>
	Secretions not collected <sup>b</sup>	Secretions collected <sup>c</sup>	
Transformation zone			0.07
Satisfactory	100 (76.3)	115 (84.2)	
Satisfactory but limited	31 (23.7)	20 (14.8)	
Inadequate	0 (0.0)	0 (0.0)	
Air-drying artifact			0.46
Satisfactory	118 (90.1)	115 (85.2)	
Satisfactory but limited	12 (9.2)	19 (14.1)	
Inadequate	1 (0.7)	1 (0.7)	
Obscuring blood			0.14
Satisfactory	112 (85.5)	103 (76.3)	
Satisfactory but limited	17 (13.0)	30 (22.2)	
Inadequate	2 (1.5)	2 (1.5)	
Scant cellularity			0.54
Satisfactory	106 (80.9)	116 (86.0)	
Satisfactory but limited	24 (18.3)	18 (13.3)	
Inadequate	1 (0.8)	1 (0.7)	
Obscuring inflammation			0.29
Satisfactory	93 (71.0)	107 (79.3)	
Satisfactory but limited	37 (28.2)	27 (20.0)	
Inadequate	1 (0.8)	1 (0.7)	

<sup>a</sup> Samples may be limited or inadequate by one or more criteria.

<sup>b</sup>  $n = 131$ .

<sup>c</sup>  $n = 135$ .

<sup>d</sup> P values were obtained by using the Pearson chi-square test.

(23.7 versus 14.8%, respectively;  $P = 0.07$ ) and a marginally higher rate of limited slides due to the presence of obscuring blood (13.0 versus 22.2%, respectively;  $P = 0.14$ ). The fact that these two effects were not statistically significant and that they went in opposite directions suggests that collection of cervical secretions did not alter the overall quality of the Pap smear. Analysis restricted to the 200 women without evidence of obscuring inflammation yielded similar results. In this subgroup, 1.9% of the women randomized to have cervical secretions collected and 2.2% of those randomized not to have secretions collected were found to have inadequate slides. The rates of satisfactory but limited slides for these two groups of women were 44.9 and 49.5%, respectively ( $P = 0.79$ ).

#### DISCUSSION

Double sampling of the cervix by aggressive cervical cell collection procedures (such as those used to obtain Pap smears) is likely have a detrimental effect on Pap smear quality. In fact, in one study of 259 women in Costa Rica in which double cervical cell sampling was performed by using random combinations of currently marketed instruments (Cytobrush plus Ayre spatula, Cervex brush, and Cellsweep), significant evidence of bleeding in Pap smears from the second collection was observed (12a). The proportion of second Pap slides (i.e., those obtained by using cells from the second collection) with evidence of bleeding ranged from 50 to 65%, depending of the collection instrument used, compared to only 16 to 38% bleed rates observed for the first Pap smear.

Based on such data, clinicians and pathologists have been

reluctant to permit any collection prior to the Pap smear. However, results from our study suggest that the collection of cervical secretions using Weck-cel sponges does not adversely affect the quality of subsequently obtained Pap smears. This finding is not surprising; collection of cervical secretions for immunological analysis is an inherently gentle procedure, given that it requires the collection of material void of contamination with blood, which could be induced by more aggressive collection procedures.

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