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*Original Article*

# Improving sensitivity of cervical cytology by removal of cervical secretions before sampling: a prospective study in Mexico

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**Abstract:** Sensitivity of cervical cytology is suboptimal, especially in developing countries such as Mexico, despite available guidelines aimed at improving this. When obtaining cervical samples, whether the samples are taken from the transformation zone and whether abnormal cells are missing must be considered. Cervical secretions (CS) are always present in variable proportions, and when cleaning the cervix, better samples may be obtained. In this study, we analyzed samples obtained with or without cleaning the cervix, and compared their contents in order to determine the sensitivity and specificity of these two methods. Methods: Of 500 patients who underwent cytology and colposcopy, 271 (54.2%) required a second opinion due to a diagnosis of cervical intraepithelial neoplasia (CIN). CS was removed and compared with the clean, second sample (SS) using in both liquid-based cytology. The quality of samples according to the Bethesda System, the presence of CIN, and inflammatory reactions were recorded. The sensitivity and specificity were calculated using biopsy as the gold standard. Results: The SS resulted in a higher proportion of adequate samples being obtained (97.6% vs. 44.8%), and in increased sensitivity (88.2% vs. 58.8%). CIN was detected in the SS 26% more often than in the CS (34 vs. 27 samples), whereas inflammatory reactions were noted more often in the CS (91.4% vs. 74%). Conclusion: Cervical sampling including CS results in lower sensitivity and CIN detection rates, and in more inflammatory reactions. By excluding CS from cervical samples, the sensitivity could be improved and the false negative rate could be reduced.

**Keywords:** Cervical mucus, liquid-based cytology, sampling studies, cervical cytology

## Introduction

Cervical cytology as screening method is well-known to have an average sensitivity of 60% [1]; however, the corresponding rate in Mexico is reportedly only between 40-54% [2]. The samples should be obtained by trained personnel, including gynecologists, and ideally, the sample should be taken from the transformation zone (TZ) [2, 3]. The methods for obtaining samples have been described elsewhere [4-8]. In these text books and manuals, how to use the relevant instruments, how to best prepare the sample in the slide or in the preservative fluid for liquid-based cytology (LBC), and how to obtain the sample from the cervical orifice and the TZ, receive the most attention [2-10]. The cervix in most women is coated with mucus or secretions of varying appearances, ranging from very scant to abundant. The composition of these secretions is reflected in the cytology specimens, and conventional cytology slides are usually abundant in inflammatory material. The practice guidelines from the American Society for Colposcopy and Cervical Pathology [9], The National Health Service Cancer Screening Programmes NHSCSP Publication 23, Taking Samples for Cervical Screening. A Resource Pack for Trainers. Available from URL: http://www.cancerscreening.nhs.uk/cervical/publications/nhscsp23.pdf. And the Hong Kong Society of cytology cervical cytology practice guideline group 2002 Users' Guidelines for obtaining Optimal Cervical Smear. www.cytolgy.org.hk/.../Final%20Draft2.pdf. Davey et al. [10] and the Clinical and Laboratory Standards Institute [11] only recommend that the secretion is removed gently, but do not pro-

vide any further details on how to perform the procedure. Kotaska et al. [12] and Obwegeser et al. [13] reported that cleaning the cervix with oversized swabs provided more adequate samples, whereas Hans et al. [14] did not find any differences between removing the cervical secretions (CS) or not in their comparative study. However, none of these studies analyzed the content of the CS in order to clarify whether there is a real reason to perform cervical cleaning or not. The Bethesda system (TBS) [15] states that a sample is considered inadequate if more than 75% of the cells on a slide are inflammatory cells, which would obscure the epithelial cells; and this is expected in the CS.

We have recently performed a survey in which we questioned colposcopists and gynecologists attending international colposcopy meetings (n ~ 50) on how they sample the cervix for a cytology specimen. The results showed that the CS is often removed and discarded if found in substantial quantity (unpublished data). CS is the result of exfoliated cells from the cervix and vaginal wall, and contains inflammatory cells and bacteria in addition to the mucus secreted from the endocervical glands, which are normally found in the cervical orifice. If the CS is removed with an oversized or normal-sized cotton swab, it is likely that the swab touches the cervical surface and may result in some CIN cells being removed; and these would hence not be present in the clean, second sample (SS). In this study, we compared the CS removed without touching the cervical surface to the SS to evaluate the adequacy of both samples, and to obtain and compare their sensitivity, specificity, and positive and negative predictive values.

## Materials and methods

### *Subjects*

Out of 500 consecutive patients who attended our laboratory for cytology and colposcopy between June 2005 and March 2007, 271 (54.2%) required a second opinion due to a positive result from a previous cytology, colposcopy, or biopsy. Two hundred (40%) patients underwent screening in our laboratory for the first time, 26 (5.2%) patients undergoing follow-up had previous normal cytology and colposcopy results from our laboratory, and 3 patients had previously undergone loop electrical excision procedure conization for cervical intraepithelial neoplasia (CIN). The laboratory is a private practice, serving a low-risk population in Mexico City, Mexico. The age of the study cohort ranged from 17-67 years, with an average age of 33 years. Most patients were middle-class Caucasian or Hispanic women.

All patients provided informed consent, and an external ethical committee approved the study protocol.

### *Methods*

Existent cervical secretions were removed by gently rolling a cytobrush (**Figures 1**, **2**), starting in the peripheral zone of the cervix, and continuing in the area of major thickness of the mucus, and then rolling the brush with a 360-degree circular movement to the fornix; care was taken to avoid touching the surface of the cervix so that only the secretion was collected, and no cells from the epithelia. In patients with abundant CS, this procedure was repeated until the surface of the cervix was clean but still moist. The first sample, CS, was processed in liquid-based solution (LiquiPREP™; LGM International Inc., Melbourne, FL, USA). The SS was obtained from the transformation zone using a cervical cytobrush or broom depending of the nature of the TZ. In cases of atrophy or if the SQCJ was not visible in endocervical canal with a very small orifice, two devices were used: a small dental brush for the endocervix and a broom for the exocervix. Subsequently, the SS was processed, replace with liquid-based solution (LBS). All slides were independently reviewed by 2 experts (JJCV and JBP). The following parameters were recorded in all samples according to TBS: the quality of the samples, as determined by the presence of either normal or metaplastic endocervical cells; whether the samples were considered adequate samples (ASs); and the presence of inflammatory reactions, which were categorized as mild, moderate, or severe. All 271 patients referred to the laboratory for a second opinion had previous CIN detected 1 to 6 months earlier. In the majority of cases, photographs were obtained to allow evaluation and comparison with the actual colposcopy. A biopsy was performed in 140 cases, in which acetowhite imaging was seen; of these, 24 cases had a previous positive biopsy. In cases where the previous colposcopy was clearly overdiag-

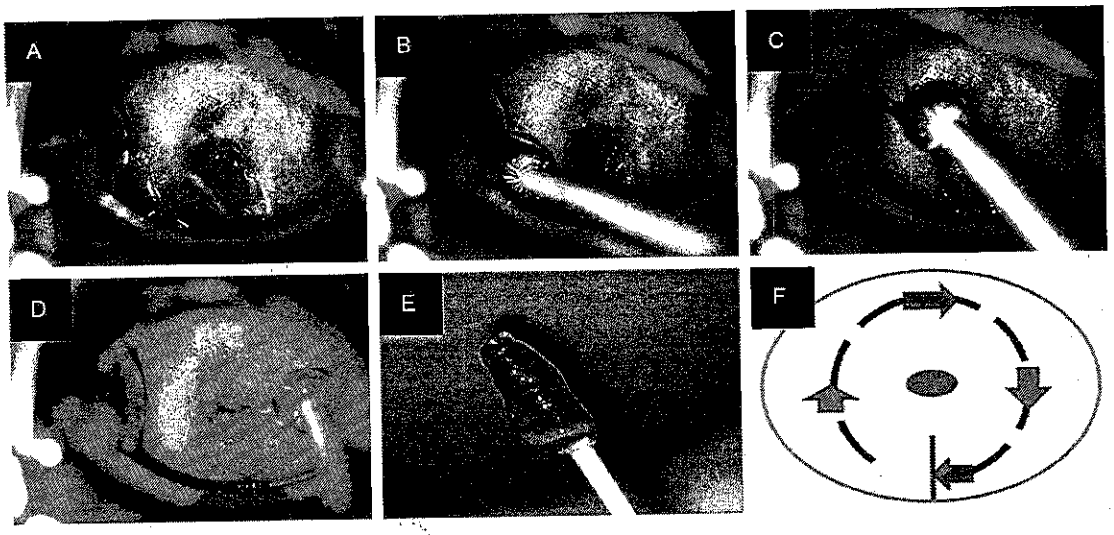


**Figure 1.** Procedure for removing the cervical mucus. A. View of the mucus on the cervical surface. B-D. The brush is rolled gently without touching the cervical surface. B. Endocervical cytobrush with the cleaned-out mucus. F. Schematic of the procedure for removing cervical mucus. E. An example of the amout of mucus retired.

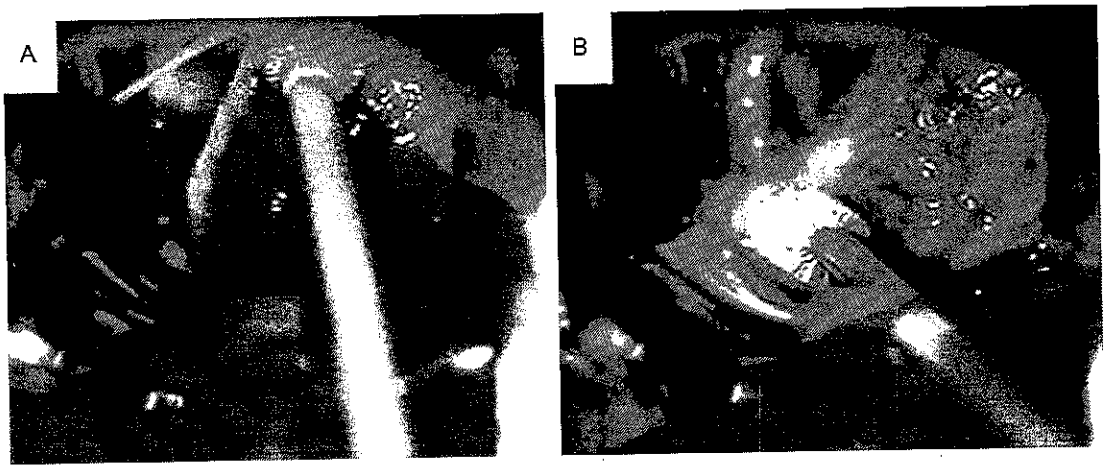


**Figure 2.** Examples of different cervix, both with large ectopia, abundant mucus, with the brush, showing how to clean it getting the mucus to the top in (A) and left in (B).

nosed, in cases with the same image of metaplasia or ectopia in the actual colposcopy, or in cases with a simultaneous previous normal cytology or biopsy report, no additional biopsies were performed. All cases with abnormal previous cytology, regardless of the colposcopy diagnosis, underwent biopsy. All biopsy samples were stained with hematoxylin and eosin as per standard protocol and analyzed by immunohistochemical staining for cyclin-dependent kinase inhibitor 2A (p16 clone J6; Cell Marke, CA, USA), using the CIN classification [16]. In cytology cases of atypical squamous cells of undetermined significance (ASC-US) or in which a high-grade squamous intraepithelial lesion could not be excluded (ASC-H), additional slides were analyzed for p16 expression to confirm or rule out CIN. The reference standard for the positive true diagnosis was the combined results of the biopsy and p16 staining, whereas a true negative diagnosis was based on negative results for CIN in the biopsy [16] and negative p16 staining [17, 18]. Cases in which biopsy was not performed were considered as true negative based on a previous and actual negative cytology or previous colposcopy results that were considered obviously mistaken.

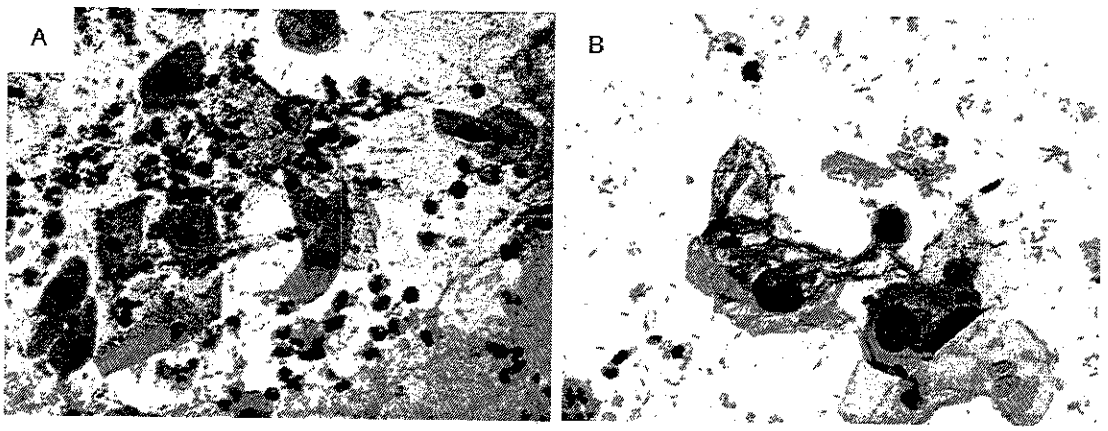


**Figure 3.** Dysplastic cells of the same patient, in (A) from the Cs, with more inflammatory cells, and the nucleus of the atypical cells are note clearly seen. In (B) the atypia is more clearly defined, less inflammatory cells on SS. (40×).

**Table 1.** Sample characteristic and kind of inflammation detected in the histophatologycal analyze in CS and SS respectively

| | | Cervical secretion (CS) | Second sample (SS) | *P*-value |
|---|---|---|---|---|
| Sample characteristic | Adequate | 224 (44.8%) | 488 (97.6%) | 0.0001 |
| | Not adequate | 276 (55.2%) | 12 (2.4%) | |
| Inflammation | Mild | 43 (8.6%) | 130 (26%) | 0.0001 |
| | Moderate | 280 (56%) | 369 (73.8%) | |
| | Severe | 177 (35.4%) | 1 (0.2%) | |

All values are presented as n (%).

**Table 2.** Frequencies of histophatologycal diagnosis in CS and SS procedures

| | Cervical secretion (CS) N = 500 | Second sample (SS) N = 500 | Biopsy N = 140 |
|---|---|---|---|
| Normal | 473 (94.6) | 466 (93.2) | - |
| Metaplasia | - | - | 106 (75.6) |
| CIN1 | 16 (3.2) | 21 (4.2) | 25 (17.8) |
| CIN2 | 2 (0.4) | 6 (1.2) | 5 (3.6) |
| CIN3 | 2 (0.4) | 2 (0.4) | 2 (1.5) |
| CxCa | 1 (0.2) | 1 (0.2) | 2 (1.5) |
| ASC-US | 6 (1.2) | 3 (0.6) | - |
| ASC-H | - | 1 (0.2) | - |

All values are presented as n (%). Abbreviations: CIN, cervical intraepithelial neoplasia; CxCa, cervical cancer; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells in which a high-grade squamous intraepithelial lesion could not be excluded.

*Statistical analysis*

The results are expressed in percentages and frequencies for all parameters. The sensitivity, specificity, positive and negative prognostic values, and their 95% confidence intervals were obtained using SPSS v.19 (SPSS Inc., Chicago, IL, USA) after adjusting for a prevalence of CIN of 6%. The statistical analyses were performed using the Chi square test, Fisher's exact test, or Phi coefficient. A *P*-value < 0.05 was considered statistical significant.

## Results

Of the 500 cases, ASs were found in 48.8% (224/500) of CS samples, compared to in 97.6% (488/500) of the SS ($P = 0.0001$). The inflammatory reaction was moderate-to-severe in 92.4% (457/500) and 74% (370/500) of CS and SS, respectively ($P = 0.0001$ **Table 1**). CIN was detected in 5.4% (27/500) of CS compared to in 6.8% (34/500) of SS ($P = 0.0001$; **Table 2**). All CIN-positive cases detected in the CS samples and SS were confirmed by biopsy and p16 immunohistochemical staining, and were considered true positive cases. One positive case detected in the CS was not detected in the SS. This case

**Table 3.** Comparison of sensitivity and specificity of CS and SS procedures

| | Sensitivity (95% CI) | Specificity (95% CI) | Positive predictive value | Negative predictive value | Positive predictive value* | Negative predictive value* |
|---|---|---|---|---|---|---|
| CS | 58.8% (0.588 ± 0.165) | 99.7% (0.997 ± 5.07e-3) | 95.2% | 97% | 79% | 96% |
| SS | 88.2% (0.882 ± 0.108) | 100% (1 ± 0) | 100% | 99.1% | 100% | 95% |

*Adjusted to cervical intraepithelial neoplasia prevalence in Mexico (6%). Abbreviations: CS, cervical secretion; SS, second sample; CI, confidence interval.

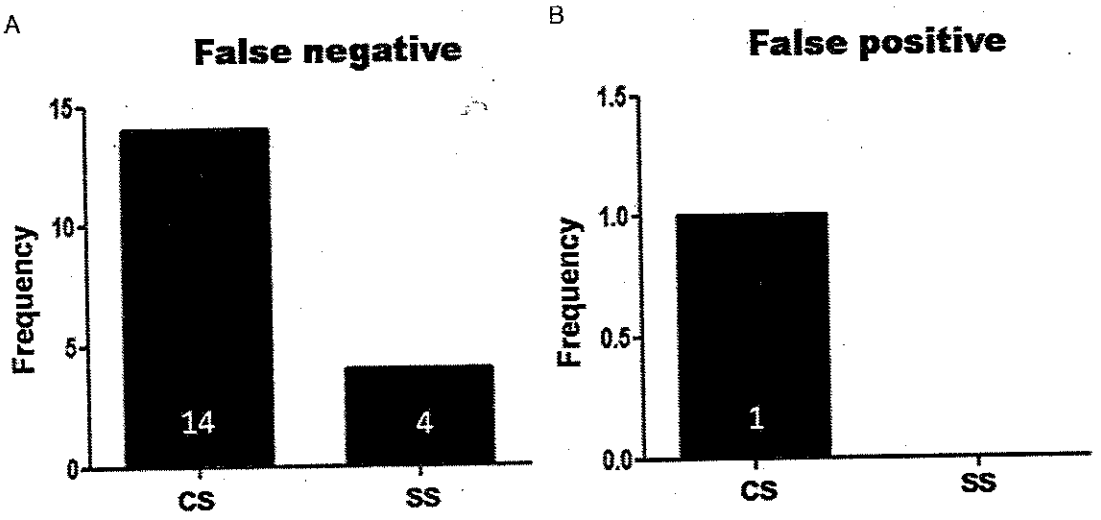


**Figure 4.** A. False negative frequency in the cervical secretion (CS) samples and clean, second samples (SS). B. False positive frequency in CS samples. There were no false positive results in the SS.

was diagnosed as vaginal condyloma, and the biopsy showed characteristic papillary and koilocytic changes. The types of diagnosed CIN were divided as shown in **Table 2**. The sensitivities for CS and SS were 58.8% and 88.2%, respectively; and the corresponding specificities were 99.7% and 100%, respectively. The positive predictive values for the CS and SS were 95% and 100%, respectively; and the negative predictive values for CS and SS were 97.07% and 99.14%, respectively (**Table 3**). The false negative rate was lower in the SS (**Figure 4**).

## Discussion

According to the TBS classification [15], our results demonstrate that a clean, second sample resulted in 117% (224 vs. 488 cases) more ASs compared to samples obtained without cleaning the cervix, with 7 more cases of CIN detected in the SS, as confirmed with biopsy. Moreover, a higher rate of inflammatory reaction was found to be present in the CS compared to the SS, whereas more epithelial cells with clear nuclear details were present in the SS compared with the CS (**Figure 2**), likely owing to the fact that these are living cells that are attached to the cervical surface, and if these are actively removed, their cytological characteristics will be more well-preserved at the time of fixation (**Figure 3**).

Obwegeser et al. [13] reported that cleaning the cervix with a cotton swab prior to sample collection may be responsible for the similar results obtained by conventional cytology and LBS. Kotaska et al. [12] specifically analyzed the difference between cleaning or not cleaning the cervix with an oversized cotton swab in the same population by comparing recent samples in which the cervix was cleaned, with their previous historical cytology results and with the average rates of cytology diagnoses in British Columbia, Canada. They found that after the cervix had been swabbed, more ASs were obtained but less CIN cases were detected, and concluded that this was likely attributable to the age of the participants, as well as the possibility that some cases of CIN had cleared

when the second study was being conducted. On the other hand, another study by Hans et al. [14] compared the results of cleaning or not cleaning the cervical surface, and did not detect any differences. Accordingly, the authors concluded that the cervix should be wiped at the discretion of the clinician.

To date, no analysis on what the mucus contains from the cervix has been performed. Removing the secretions without touching the cervix has been shown to preserve important diagnostic cells in the cervix, which may be removed using a swab, especially if the lesion is small. Based on our findings, removing the CS is a key feature to ensure higher detection rates of CIN. If the sensitivity, which is currently estimated to be approximately 60% [1], is directly related to the quality of the sample, it could be raised to more than 80% by cleaning the cervix as described herein; this is especially important in developing countries where the sensitivity is reported to be even lower than 60% [2].

In our study, colposcopy was used to detect small lesions, which were confirmed by cytology and diagnosed by biopsy. Some cases appeared very clean even if the surface was covered with abundant transparent mucous; and hence, at first sight, some gynecologists or colposcopists may decide not to clean the surface. However, when the cervix is cleaned using a cytobrush, the abundance of the mucus becomes apparent, and these CS samples were found to have less epithelial cells and more inflammatory reactions than the samples obtained from the cleaned cervix. If not cleaned, the mucus may prevent the instrument from obtaining cells from the cervical surface.

The results of the present study raise an important question: could the sensitivity of cytology, especially for false-negative cases, be due to CS being the only sampled material? Koss [19] answers this question by referring to some very early papers, including Sedlis et al. [20] and Shulman et al. [21] who examined two samples obtained simultaneously and studied using the same cytology procedure. These studies found that at least 33% and 50% of cells were not present on one of the samples for carcinoma in situ and slight-to-moderate dysplasia, respectively, and the false negative rate was estimated as 25%. Taking two simultaneous samples would reduce the risk of obtaining false negative results by 50%, but this is time consuming and was not recommended by the authors. Both samples were taken in the same fashion, using a wooden-tipped spatula, and the different results were attributed to the first slide being more exfoliated and containing more superficial cells, whereas "deeper" cells could be found in the second sample; however, no mention was made for the rate of inflammatory reactions in these two samples. If the cervix is adequately visualized and the sample is taken from the surface of the cervix or from the cervical oz, then why are 40% of samples considered inadequate? This could be justified in part by the presence of an atrophic cervix, a small TZ, or by the squamocolumnar junction not being visible. In our study, the only positive case detected in the CS not detected in the SS was a lesion in the vaginal wall that was not accessible to the instrument for sampling the cervix in the SS. Conversely, the CS was in contact with the vaginal wall and contained diagnostic cells in this case, and the biopsy found a typical condyloma with koilocytes. This condition is rare and was detected from a thorough inspection of the vagina when sampling the cervix.

In conclusion, it is currently not clear for the physicians and personnel who are involved in sampling the cervix what the optimal way to handle CS is. Based on our results herein, we believe that cervical samples containing CS are not optimal, as the rate of ASs is low. On the other hand, samples obtained after cervical cleaning were found to be superior, with the sensitivity for CIN detection being 50% higher than for CS. Thus, gently removing the CS without touching the cervical surface should be recommended in any procedure for obtaining a cervical cytology sample.

## Disclosure of conflict of interest

None.

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