

# Comparison of Spatula and Nonspatula Methods for Cervical Sampling

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A comparison between nonspatula (cotton swab and Cytobrush) cervical sampling methods and spatula (wooden Ayre spatula and plastic extended-tip Szalay Cyto-Spatula) sampling methods was made in 109 cases. Based on the presence of endocervical cells, there were statistically significant qualitative differences between the nonspatula methods as well as between the spatula methods, but not between the Cytobrush and Cyto-Spatula smears or the cotton swab and Ayrespatula smears. In all kinds of inflammatory lesions, the spatula samples were more accurate and diagnostic than the nonspatula ones. In all cases of cervical intraepithelial neoplasia and in most cases of squamous metaplasia, the Cyto-Spatula sample was the most accurate. It is concluded that the Szalay Cyto-Spatula method is superior to the other cervical sampling methods because it provides well-preserved cells from both the endocervix and the ectocervix in one smear. The Cytobrush should be used in conjunction with spatula sampling (combination method) for effective sampling of the cervix. The Cytobrush alone is effective mainly for endocervical sampling while the Ayre spatula alone is effective mainly for ectocervical sampling; the cotton swab is ineffective for both endocervical and ectocervical sampling.

The Papanicolaou smear, which has been used for cervical cytologic screening since the 1940s,<sup>25</sup> is widely regarded as a reliable and inexpensive method for screening for cancer of the uterine cervix,<sup>16</sup>. The reliability of the method depends on proper sampling of the transformation zone (squamocolumnar junction)<sup>8,10-12,15,20,28,34,35,37,45</sup> because most epithelial abnormalities originate in this area,<sup>26,43</sup>. Since the upper margin of the transformation zone is formed by columnar endocervical epithelium, one can only be certain that the entire transformation zone has been adequately sampled if endocervical cells are present in the smear.<sup>10,14,44,45</sup> While some consider the presence of squamous metaplastic cells alone, without columnar endocervical cells, to be a marker of adequate endocervical sampling,<sup>14</sup> many do not.<sup>5,21,45</sup>

A number of studies have shown that sampling errors are an important source of false-negative diagnoses of Papanicolaou smears,<sup>2,4,7,13,14,17,23,24,29</sup> Nevertheless, the sampling method remains a matter of dispute. A variety of sample collection techniques have been introduced,<sup>21,32,34,38-41,44</sup> in an attempt to improve the yield of endocervical cells and increase the percentage of adequate smears. Such improvements could reduce the need for repeat smears, which double work and costs and cause unnecessary anxieties to the patient.

This paper reports a comparative study of the use of the cotton swab, the traditional wooden Ayre spatula, the Cytobrush designed by Dr. Stormby<sup>39</sup> (distributed by Medscand AB, Malmö, Sweden) and the plastic pointed Cyto-Spatula designed by Dr. Szalay<sup>40</sup> (distributed by **CSM Graf GmbH, Steinach, Switzerland**, [www.csmgraf.ch](http://www.csmgraf.ch), [www.zervixzytologie.com](http://www.zervixzytologie.com)). The cellular composition of the smears prepared from samples obtained by these devices, the efficacy of each one of these devices and, consequently, the adequacy of the Cytologia diagnosis were investigated.

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### Materials and Methods

Cervical samples were collected from 109 women of premenopausal age who presented to our hospital for a routine Papanicolaou smear. Questionnaires were given to each prospective participant, requesting information on prior douching or coitus, last menstrual period, method of contraception, marital status, number of pregnancies, previous cytologic examinations and general gynecologic history. The study group was limited to nonpregnant premenopausal women without prior douching or coitus who had normal menstrual periods and no history of menstrual bleeding, conization or hysterectomy. The cervix was visualized by the sample taker at the time of sampling in all cases. Under these circumstances, endocervical cells should be present in properly made cervical smears.

The majority of the examined women had come for routine screening, without any symptoms; eight women complained of vague symptoms, such as itching and pain connected with coitus. Nine of the examined women were using oral or other contraceptives. The gynecologic history included cervical cauterization within the last year for two cases and cervical cryosurgery within the last year for five cases.

All samplings were performed by one cytopathologist (I.A.) to achieve uniformity of smearing. After the first screening, which was done by the two cytopathologists who participated in this study, all smears were reviewed and evaluated by the senior author as a quality-control procedure.

The sampling devices were used in the following sequence in this study. The cotton swab was used first since it produces less scraping and trauma and at the same time collects most of the cervical mucus, which contains the largest number of accumulated exfoliated cells. The traditional wooden Ayre spatula was used next, followed by the Cytobrush and the Cyto-Spatula in that order. Each sample obtained was smeared on a separate slide (marked beforehand for the sampling device used) and immediately fixed. Of the three available sizes of the Cyto-Spatula, the No. 2 was used more often in this study, the No. 3 less often and the No. 1 only rarely.

All four smears in each case were microscopically evaluated for (1) the presence or absence of endocervical columnar cells and (2) the presence or absence of any noteworthy cytologic findings (i.e., squamous metaplastic cells, inflammatory changes and epithelial abnormalities). The relationship between such findings and the presence or absence of endocervical cells was then analysed. Statistical analysis of the results utilized the Z-test with an alpha of 0.05.

### Results

None of the 109 cases evaluated showed invasive carcinoma. The routinely examined patients without symptoms included 4 cases of cervical intraepithelial neoplasia (CIN), 1 case of severe "tissue repair" related to an unknown agent, 3 cases of severe inflammatory cellular changes due to fungi, parasites or bacteria (for which a repeat of the test after treatment was recommended) and 15 cases of routine inflammatory changes due to fungi, trichomonads or bacteria. The eight cases with symptoms included one with fungal inflammation and six with bacterial inflammation; the last case had cytologic evidence of an abnormal tissue repair, without a visible bacterial, parasitic or viral agent. Of the nine patients using oral or other contraceptives, one was found to have a bacterial inflammation and one, who had been using contraceptive therapy for one year for the treatment of an ovarian cyst, was found to have CIN II.

Table I shows the presence or absence of endocervical cells in the different smears prepared in the 109 cases. Endocervical cells were present in 50 cotton swab smears (45.9%), in all but one Cytobrush smear (99.1%), in all but one Cyto-Spatula smear (99.1%) and in only 18 Ayre spatula smears (16.5%). In addition, another 24 cotton swab smears and 26 Ayre spatula smears contained only strands of mucus with trapped polymorphonuclear leukocytes and degenerated naked nuclei, probably of endocervical origin, among the squamous cells. The Cytobrush smears contained only endocervical cells, with no squamous or squamous metaplastic cells, in 21 cases;

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those cases included ectocervical epithelial abnormalities that were missed. Endocervical cells were found in the cotton swab smears, Cytobrush smears and Cyto-Spatula smears of all seven cases with a past history of cryosurgery or cauterization, but in only one Ayre spatula smear (a cryosurgery case).

**Table I Cellular Composition of Smears Prepared with Different Sampling Devices**

Devices used	Endocervical columnar cells present		Squamous cells only*		Squamous cells and mucus only*		No squamous cells present	
	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
Cotton swab	50	45.9	35	32.1	24	22.0	--	--
Cytobrush	108	99.1	1	0.9	--	--	21	19.3
Ayre spatula	18	16.5	65	59.6	26	23.9	--	--
Cyto-Spatula	108	99.1	1	0.9	--	--	--	--

\* No endocervical columnar cells present.

The cytologic findings in the 109 cases are given in Table 11. As shown in Table 111, the percentages of epithelial abnormalities found in smears without endocervical cells were significantly lower ( $P < .001$ ) than the percentages of abnormalities found in smears with endocervical cells for three of the four sampling methods. The exception was the Ayre spatula smears, in which most of the epithelial abnormalities were diagnosed in smears that did not contain endocervical cells.

The 28 inflammatory lesions included 3 Trichomonas infections, 5 fungal infections, 11 bacterial infections 2 Haemophilus infections, 5 cases of tissue repair atypia without visible cause and 2 human papillomavirus (HPV) infections (Table IV). For all inflammatory lesions, the spatula samples were more accurate and diagnostic than the nonspatula ones (Tables II and IV). Inflammatory cellular lesions were diagnosed in 13 cotton swab smears and 9 Ayre spatula smears containing endocervical cells versus 5 cotton swab smears and 17 Ayre spatula smears without endocervical cells present (Table III).

The five cases of CIN in this study included four cases of CIN II and one case of CIN III All five were diagnosed in the Cyto-Spatula smears (Table II One case of CIN II was missed by the Ayre spatula, Cytobrush and cotton swab methods, and one more case of CIN II was missed by the cotton swab method only (Table II). Consequently, the Cyto-Spatula sampling was the most accurate method for diagnosing CIN lesions; only the Cyto-Spatula smears contained endocervical cells in all five CIN cases (Table III).

Squamous metaplasia was present in a total of 54 cases. Squamous metaplastic cells were present in 30 cotton swab smears (55.6%), 41 Ayre spatula smears (75.9%), 42 Cytobrush smears (77.8%) and 53 Cyto-Spatula smears (98.1%) (Table II). The Cyto-Spatula sampling was thus also the most accurate method for detecting squamous metaplasia; it again produced the highest percentage of smears containing endocervical cells (Table III).

The diagnostic differences between the spatula methods (Ayre spatula and Cyto-Spatula smears) were not statistically significant; however, the diagnostic differences between the nonspatula methods (cotton swab and Cytobrush smears) and the spatula ones were statistically significant. The Z-test gave values of 3.68 for cotton swab samples, 3.84 for Cytobrush samples, 1.76 for Ayre spatula samples and 1.76 for Cyto-Spatula samples. The sensitivities were 63.6% for the cotton swab, 90.9% for both the Ayre spatula and the Cyto-Spatula and 60.6% for the Cytobrush when all the lesions were recorded; the specificity was 100% for all of the devices used.

### Discussion

Most investigators agree that the presence of endocervical columnar cells is important for a cervical smear to be considered adequate<sup>3,5,8,10,14,15,32,34,35,37,38,41,44,45</sup> only a few studies observed no difference in the rates of atypia between women with and women without endocervical cells on

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serial Papanicolaou smears.<sup>21,22,46</sup> In our study, one of two cases of severe "tissue repair" lesions and one of five cases of CIN were diagnosed in cotton swab smears without endocervical cells while four of five cases of CIN were diagnosed in Ayre spatula smears without endocervical cells. The presence of endocervical cells is only an index that a transformation zone containing abnormalities has been adequately sampled. In our cases, the area of the slide containing the endocervical material did not contain material from squamous or metaplastic abnormalities of the transformation zone; the latter was found elsewhere in the slide.

**Table II** *Distribution of Cytologic Findings in Smears Prepared with Different Sampling Devices*

Devices used	Inflammation		CIN		Squamous metaplasia		Any of the three findings	
	No. of cases with affected cells present	%	No. of cases with affected cells present	%	No. of cases with affected cells present	%	No. of cases	%
Cotton swab	18	64.3	3	60.0	30	55.6	51	58.6
Cytobrush	17	60.7	4	80.0	42	77.8	63	72.4
Ayre spatula	26	92.9	4	80.0	41	75.9	71	81.6
Cyto-Spatula	25	89.3	5	100.0	53	98.1	83	95.4
Total*	28		5		54		87	

\*As diagnosed in any sample.

**Table III**

Relationship Between Cytologic Findings and the Presence of Endocervical Cells in Smears Prepared with Different Sampling Devices

Device used	Smears with endocervical cells								Smears without endocervical cells							
	Inflammation		CIN		Squamous metaplasia		Total		Inflammation		CIN		Squamous metaplasia		Total	
	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
Cotton swab	13	46.4	2	40.0	23	42.6	38	43.7	5	17.9	1	20.0	7	12.9	13	14.9
Cyto-brush	17	60.7	4	80.0	42	77.8	63	72.4	--	--	--	--	--	--	--	--
Ayre spatula	9	32.1	--	--	12	22.2	21	24.1	17	60.7	4	80.0	29	53.7	50	57.5
Cyto-spatula	25	89.3	5	100.0	53	98.1	83	95.4	--	--	--	--	--	--	--	--
Total*	28		5		54		87		28		5		54		87	

\*As diagnosed in any sample.

The location of the squamocolumnar junction (transformation zone) varies with the patient's age<sup>6</sup> shifting upward with increasing age.<sup>31</sup> This is considered to be one of the factors influencing the presence of endocervical cells in cervical smears. The incidence of "inadequate" smears lacking endocervical cells is reported to be 36% to 48% in postmenopausal women, but only 8% to 22% in premenopausal women<sup>14,42</sup>; the incidence in premenopausal women is influenced by prior cervical surgery, increasing up to 33% after conization.<sup>42</sup> In our series, no such "inadequate" smears were obtained from women with a history of prior cauterization or cryosurgery. In addition, several features specific to the patient, such as evidence of inflammation, menstrual bleeding, prior douching, recent coitus and pregnancy, reportedly affect the yield of endocervical cells.<sup>6,12,18,38</sup> Finally, the sampling devices used<sup>32,34</sup> and the skill of the sample takers<sup>44</sup> also influence the quality of the smears and, indirectly, the number of epithelial abnormalities detected.

Our series included only those cases with theoretically optimal conditions for having a cervical smear taken. To avoid problems due to atrophic changes in postmenopausal women or hormonal abnormalities in premenopausal women, the women selected for this study were all of premenopausal age with normal menstrual periods. No patients who were pregnant, who had a history of menstrual bleeding, conization or hysterectomy or who reported prior douching or coitus were accepted for the study. The sample taker who performed all samplings was a well-trained physician. Since adequate visualization of the cervix is required for a successful collection of

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columnar endocervical cells during cervical sampling, only cases in which the cervix was seen were evaluated.

**Table IV** Incidence of inflammatory Lesions in Smears Prepared with Different Sampling Devices

Inflammatory lesions	Total no. of cases	Cotton swab		Cytobrush		Ayre spatula		Cyto-Spatula	
		Total no. of cases	%	Total no. of cases	%	Total no. of cases	%	Total no. of cases	%
Trichomonas	3	2	66.7	1	33.3	3	100.0	3	100.0
Fungi	5	3	60.0	3	60.0	5	100.0	5	100.0
Bacteria	11	10	90.9	7	63.6	11	100.0	11	100.0
Haemophilus	2	--		--		2	100.0	2	100.0
Tissue repair or endocervical cell atypia	5	2	40.0	5	100.0	3	60	2	40.0
HPV	2	1	50.0	1	50.0	2	100.0	2	100.0
Total	28	18	64.3	17	60.7	26	92.9	25	89.3

In one case in this study (0.91%), endocervical cells were absent from all four smears. In one report, endocervical cells were absent from the smears in 5.7% of the cases in which the cervix was seen<sup>44</sup> probably because the device could not be introduced deeply enough into the endocervical canal. In addition, cervical smears consistently do not contain endocervical columnar cells in 0.7% of cyclically menstruating women.<sup>10,45</sup> Attempts to improve the yield of endocervical cells by varying collection techniques have not eliminated the occurrence of smears without endocervical cells. Women with Papanicolaou smears without endocervical cells have a significantly higher chance of also having one or more subsequent smears without endocervical cells.<sup>21</sup> If a smear does not contain endocervical cells, the possibility of detecting epithelial abnormalities is significantly lower.<sup>44</sup> Yet, the sampling method should remain simple and easy to perform, especially because cervical smears are frequently not taken by gynaecologists, but by general practitioners or nonphysician sample takers.

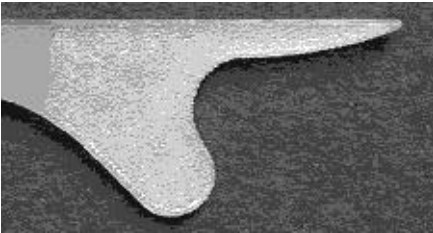
The importance of using the Cytobrush for endocervical sampling has already been emphasized, with most investigators agreeing that the cotton swab, whether moistened or not, should be abandoned.<sup>1,5,30,32,33,39,41</sup> Peng et al<sup>27</sup> however, found no significant differences in the detection rates for HPV positivity in samples collected with cotton swabs and Cytobrushes, and a few investigators who used cotton swabs in their series did not mention any problems of sample inadequacy.<sup>9,21,36</sup> Our findings are in agreement with previously reported comparisons of the cotton swab to the Cytobrush for endocervical sampling. While the endocervical cells found in the cotton swab and Ayre spatula smears generally showed prominent degenerative changes, endocervical material obtained by the Cytobrush was better preserved and of greater quantity than that collected with the conventional cotton swab. The reasons for the greater quantitative effectiveness of the Cytobrush relate to the characteristics of the device itself<sup>41</sup> In addition, the Cytobrush is able to sample immature lesions located higher in the endocervical canal (developing from metaplastic endocervical epithelium or atypical reserve cells) and to detect abnormalities of the columnar epithelium of the endocervix, including endocervical adenocarcinoma in situ.<sup>5</sup> The latter is important since there has been a proportional increase in adenocarcinoma of the cervix.<sup>19</sup>

The importance of using an extended-tip spatula (although not the plastic one used in our study) to improve the yield of columnar endocervical cells, as compared with the cotton swab or traditional Ayre spatula, has also been mentioned.<sup>3,5,8,17,38,40,44,46</sup> The use of such devices is the most efficient and reliable method for screening programs because the single scrape, single-sample method reduces the workload of sample takers and laboratory personnel.<sup>44</sup> The extended-tip plastic spatula used in our study collects abundant cells from both the endocervix and the ectocervix and transfers them to the slide without loss due to its rough surface (Figure 1). Due to its overall shape, the spatula comes in optimum contact with the endocervical surface epithelium and thus provides well-preserved endocervical cellular material; moreover, the different sizes and shapes in which the Cyto-Spatula is available (Figure 2) permits an optimal fit of the device to the form of the ectocervix and the size of the cervical canal of each patient. Its deep scraping removes

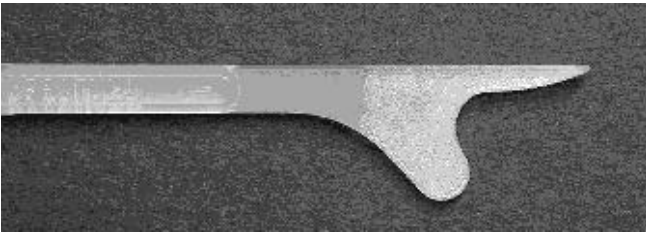
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keratinising superficial-cell layers that may cover underlying abnormal tissues. In less than 2% of the cases, minor contact bleeding of the cervix may occur as a result of the scraping of the surface epithelium with the Cyto-Spatula; this does not constitute a serious problem.

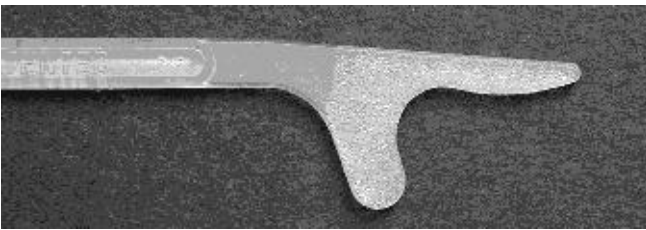
Although the Ayre spatula produced samples with a very high diagnostic accuracy, it only provides adequate ectocervical sampling. The Cytobrush was very effective in the detection of endocervical abnormalities and most of the abnormalities of the transformation zone; however, a few epithelial abnormalities were not detected in Cytobrush samples. Those cases were diagnosed by the extended-tip Szalay-type spatula, which detected endocervical changes almost as well as the Cytobrush and identified all abnormalities of the transformation zone. Since the ideal cervical smear should include both endocervical and ectocervical samples, a combined Cytobrush and spatula method should be used. If only one device is to be used, it should be the Szalay-type spatula, which is as effective and reliable as the combination method.



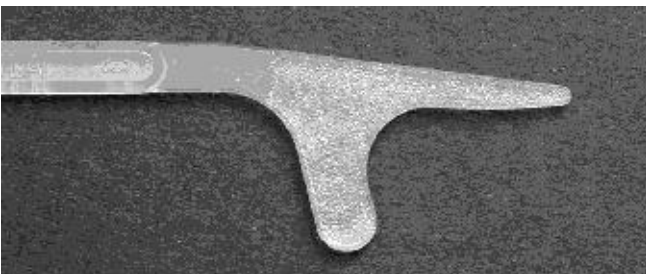
**Image 1** The pointed plastic Szalay Cyto-Spatula used in this study. The rough surface retains all of the collected material and does not allow any to be lost when transferred to the glass slide.



**Image 2** Szalay Cyto-Spatula No 1, The three different sizes and shapes in which the Szalay device is available facilitate its use with all forms of ectocervices and all sizes of cervical canals.



**Image 3** Szalay Cyto-Spatula No 2



**Image 4** Szalay Cyto-Spatula No 3

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