

# Contribution of the cytobrush to determining cellular composition of cervical smears

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## Abstract

**A study was made of the contribution of the cytobrush to determining the composition of the cervical smear. From a population of women screened in The Netherlands in 1987, 733 women were selected whose smears for the second time in a row lacked endocervical cells. Note was made of method of contraception, age of the woman, and day of the menstrual cycle. A control group was formed of women whose smears contained endocervical cells. Highly significant differences were found in the results between the two groups.**

**Two new samples were collected, one made with the modified wooden Ayre spatula, the other with the cytobrush from the research group. The number of smears containing endocervical cells increased from 44% (by spatula alone) to 79% (spatula plus cytobrush). The cytobrush alone produced a high percentage of unsatisfactory smears (17.5%) due to a low content of squamous epithelial cells. No differences could be observed in the rate of cellular atypia because of the small number of cases.**

The Papanicolaou (Pap) smear, obtained with a wooden extended tip Ayre spatula, is a reliable and inexpensive method for the detection of premalignant and malignant cervical lesions. In several countries this method has been successfully used for years for screening for cervical neoplasia.<sup>1</sup> Nevertheless, in a screening programme using the Pap smear, follow up studies showed that 2-31% of the cervical (pre)malignant lesions went undetected. For example, in the screening programme carried out in The Netherlands between 1976 and 1985 the false negative rate in cervical cytology was about 17.5%.<sup>2</sup>

This high percentage of non-diagnostic Pap smears is attributed to several factors, including laboratory error, physician sampling error, and patient factors. It is a serious problem in cervical screening programmes, and attempts should be made to minimise the rate of inadequate smears. Quality control in the laboratory and quality control of the smear is therefore of great importance. In our laboratory we carry out a strict quality control programme as recommended by the WHO,<sup>3</sup> with rescreening.<sup>4</sup> We also try to reduce the number of preparations screened by one cytotechnologist a day, as proposed by Vooijs *et al*,<sup>5</sup>

to avoid diagnostic errors due to fatigue.

The reduction of sampling errors is difficult to achieve in our population owing to a large number of different sample takers. Studies have shown that the composition of the cervical smear is an important indicator of its quality.<sup>6</sup> Most cervical neoplasia originates from the transformation zone—that is, the place where the endocervical columnar cells transform in squamous epithelial cells, also known as the squamo-columnar junction. The transformation zone can be located in both the endocervical canal and on the ectocervix. An adequate smear should contain endocervical cells, unless the absence can be explained satisfactorily—for example, hysterectomy.

In some cytological laboratories it is the policy to consider smears adequate if they contain squamous metaplastic cells or endocervical columnar cells.<sup>7</sup> In our laboratory we only accept endocervical columnar cells, otherwise a repeat smear is asked for after one year instead of after three years.

A significantly higher number of moderate and severe epithelial abnormalities were found in smears containing endocervical cells than in smears without these cells, so the presence of endocervical columnar cells has been an important variable in assessing the reliability of the cervical smear.<sup>5,6,8</sup>

Several investigators have stated the usefulness of other devices to improve the rate of smears containing endocervical cells. These methods include various spatulae (plastic), endocervical aspiration devices, wet or dry cotton swabs, and the newer bristle brushes.<sup>9-12</sup> Recently the combination of an ectocervical scrape with the Ayre spatula and the endocervical smear with the cytobrush was evaluated.<sup>7,13</sup> In this laboratory we do not favour the routine use of the combination cytobrush-spatula because of the double workload. When using the cytobrush the general practitioner has to make two smears instead of one; and it means double screening time in the laboratory. We therefore tried to select a group of women at a higher risk of not having endocervical cells in their smears. We hoped that selective use of the extra cytobrush smear could be advised.

A study was made to establish the relation between the presence or absence of endocervical cells in the smear and clinical data. Furthermore, we tried to evaluate the role of the cytobrush to obtain endocervical cells in women who had had at least two successive smears without endocervical cells. We also evaluated the detection of cervical epithelial

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Accepted for publication  
16 November 1989

dysplasia using the cytobrush compared with the spatula.

### Methods

The data for this study were obtained from all cervical smears sent to our laboratory by general practitioners. In our laboratory about 45000–50000 cervical samples are processed each year, comprising 10000 smears made on behalf of the screening programme and 35000–40000 smears made for diagnostic purposes.

In January 1987 all known contributors were informed about the forthcoming project. The project itself lasted 30 weeks from May until December 1987. All women whose smears did not contain endocervical columnar cells for the second (successive) time were selected for this study.

The general practitioner of each woman was sent a letter with an explanation of the procedure plus equipment and was requested to take a new sample. A conventional smear with the traditional spatula had to be made, then an endocervical smear with the cytobrush. Both slides had to be fixed immediately. The slides were to be coded according to their method of preparation. Extra attention in providing clinical data was asked for to ensure a proper evaluation of the results. In our laboratory the slides were processed and recorded in the usual way. The smears were screened routinely by one cytotechnologist. In cases of cytologically positive smears—that is, cells indicating moderate dysplasia, severe dysplasia, or carcinomas—the slides were also examined by the chief cytotechnologist and the cytopathologist.

For the purpose of this study the smears were recorded regarding the presence or absence of endocervical cells. The smears were subdivided into three groups: (1) smears unsatisfactory for diagnosis (Pap 0); (2) smears containing endocervical cells (EC+); (3) smears lacking endocervical cells (EC–).

A note was made of all clinical data provided by the GP of the women lacking endocervical cells in two successive smears. Age, methods of contraception and day of the menstrual cycle were given special attention. Data were compared with the clinical data of women whose cervical smears had been sent to this laboratory in 1987 ( $n = 48212$ ). Of this group, the women who had endocervical cells in one smear were selected (72.8%) to form a control group.

The  $\chi^2$  test for contingency tables was used to test differences in prevalence.

### Results

During the 30 week period of research, 1854

women were selected who, for the second time in a row, had a smear lacking endocervical cells. A request for a repeat smear combined with a cytobrush smear was complied with in 872 cases. In 139 cases the returned slides were not properly marked, so only 733 cases were available for our investigation (response rate of 40%). The clinical data of this group of 1854 women were compared with those of a control group. The control group consisted of the women who had their smears sent to our laboratory in 1987 (48212 smears) and who had endocervical cells present in their smears ( $n = 35097$ ).

Table 1 shows the clinical data of the groups, expressed in percentages. In the research group more women were using oral contraceptives than in the total screened population in 1987, and fewer women were using intrauterine devices. Furthermore, there was a slight difference in age distribution. In the group of women whose smears lacked endocervical cells in two successive smears, twice as many women were postmenopausal (older than 56 years). Statistical analysis of these data showed these results to be significant ( $p < 0.0001$ ).

The day of the menstrual cycle was also of great influence on the presence or absence of endocervical cells. In the group of women who lacked endocervical cells in two successive smears more samples were taken in the second half of the menstrual cycle. Another risk factor for having no endocervical cells is when the portio was not visible at the moment of obtaining the cervical sample. No such case was present.

Table 2 shows the relation between the composition of cytobrush smears and that of the spatula smears. If a conventional spatula alone was used (after two successive samples without endocervical columnar cells) an adequate sample was obtained on the third occasion in 44% of the cases. If used in combination with the cytobrush, this rate increased to 79%. Table 2 also shows the high rate of unsatisfactory smears when only a cytobrush was used—17.5%.

When the cytobrush was used in combination with the spatula the rate of unsatisfactory smears declined from 4.4% with the spatula method alone to 1.6% in the combined method.

Finally, we looked at the contribution of the cytobrush to the detection of premalignant stages of cervical neoplasia in the smears. Table 3 shows the atypia found in the squamous epithelial cells in the 733 smears obtained in this study. With the spatula method, a moderate atypia was diagnosed in four cases and with the cytobrush method in five cases.

Table 1 Clinical data of research group compared with those of control group

	Method of contraception		Age (years)			Day of menstrual cycle			
	Hormonal	IUD	<35	35–55	>56	0–3	4–13	14–28	28
Control group (with endocervical cells) $n = 35097$	30.0%	6.0%	38.4%	56.9%	4.5%	1.5%	39.4%	49.7%	9.4%
Research group (lacking endocervical cells for the second time in a row) $n = 1854$	45.2% $p = 0.0001$	3.6% $p = 0.0001$	37.7% NS	52.6% $p = 0.0001$	10.3% $p = 0.0001$	1.6% NS	31.5% $p = 0.0001$	55.7% $p = 0.0001$	11.2% $p = 0.001$

Table 2 Cellular composition of cytobrush smear compared with spatula smear

Cytobrush smear	Spatula smear			Total (%)
	Not satisfactory	Endocervical cells		
		Present	Absent	
Not satisfactory	12	67	49	128 (17.5)
Endocervical cells present	16	234	241	491 (67.0)
Endocervical cells absent	4	21	89	114 (15.6)
Total No (%)	32 (4.4)	322 (43.9)	379 (51.7)	737 (100.0)

Only two cases overlapped with the spatula method; in one case a moderate atypia was found that would have been overlooked had only a cytobrush smear been made. This was the same for the cytobrush method. No cases of severe atypia or CIS (CIN 3) were found in these 733 cases.

Table 4 shows the atypia found in the endocervical columnar cells. In 579 cases columnar endocervical cells were present, and in 356 cases there were not enough endocervical cells to diagnose or exclude an endocervical cell atypia. So in only 223 cases (38.5%) could the value of the cytobrush to detect abnormal endocervical cells be evaluated. The only case of moderate atypia in the columnar cells was found with the spatula method, not with the cytobrush method.

### Discussion

The importance of endocervical cells in cervical samples has been established in several studies and is nowadays widely accepted. To obtain these cells an adequate smear taking technique is important. The skill and experience of the sample taker greatly influences the outcome. When smears were taken by specially trained people (as in the screening programme in the Netherlands in 1976–85) endocervical cells were found in 93% of the samples.<sup>8</sup> When smears were taken by general practitioners (38000 smears in our laboratory last year) 25% of the smears lacked endocervical cells. As the Dutch government has proclaimed that GPs should take over the

cervical screening programme which started in 1988, a great number of inadequate smears may well result. The consequence is that more women will be advised to have a repeat smear after one year instead of after three years. The financial and practical burden of recalling those women with inadequate smears is considerable.

Several devices have been developed to increase the chance of obtaining endocervical cells in the sample. These devices, nevertheless, have several disadvantages. Most of them are more expensive—for example, the price of one cytobrush is 10 times as high as the price of a wooden Ayre spatula. The double slide technique, as used with the cytobrush, will double the workload of cytotechnologists and so be more expensive. We don't favour the method used in several laboratories of making double smears on one slide, because we believe it will give poor morphology due to suboptimal fixation. Another disadvantage of several new devices is the risk of decreasing the amount of squamous epithelial cells in favour of endocervical cells. Therefore, the high content of endocervical cells in those cases will no longer be a valuable indicator of a reliable sample.

In our study we tried to define a group of women who were more likely to have cervical smears without endocervical cells. GPs could then use the combined cytobrush-spatula method in these cases. As the results show (table 1), there is an indication that women who are postmenopausal and those using hormonal contraceptives are more "at risk" of not having endocervical cells in their smears. These findings confirm the results of other studies. It is known that the transformation zone shifts into the endocervical canal in postmenopausal women, so it can be difficult to obtain endocervical cells. Nevertheless, most women in these groups do have endocervical cells in their smears, so we do not advise the routine making of a double smear in these groups. We decided to advise making an extra cytobrush smear only when the woman has a history of inadequate smears.

Table 3 Squamous epithelial cell abnormalities in spatula and cytobrush smears

Cytobrush smear	Spatula smear					Total No (%)
	Not satisfactory	No atypia	Minimal atypia	Slight atypia	Moderate atypia	
Not satisfactory	12	93	20	3	0	128 (17.5)
No atypia	19	396	58	0	0	473 (64.5)
Minimal atypia	1	45	72	3	1	122 (16.6)
Slight atypia	0	0	2	2	1	5 (0.7)
Moderate atypia	0	0	1	2	2	5 (0.7)
Total No (%)	32 (4.4)	534 (72.9)	153 (20.8)	10 (1.4)	4 (0.5)	733 100.0

Table 4 Endocervical columnar epithelial cell abnormalities in spatula and cytobrush smears

Cytobrush smear	Spatula smear				Total No (%)
	Not satisfactory	No atypia	Minimal atypia	Moderate atypia	
Not satisfactory	166	47	2	1	216 (29.5)
No atypia	272	183	15	0	470 (64.1)
Minimal atypia	22	11	14	0	47 (6.4)
Total No (%)	480 (62.8)	241 (32.9)	31 (4.2)	1 (0.1)	733 100.0

Unfortunately, we didn't succeed in our primary goal of defining a group of women in whom a routine combination cytobrush—spatula smear is advisable. From the results of our data in table 1 we can also conclude that smears taken in the first part of the menstrual cycle are more likely to contain endocervical cells as was found in a previous study.<sup>14</sup> We therefore also advise our sample takers to obtain the smears in the first part of the menstrual cycle if there is any difficulty in obtaining endocervical cells.

The value of the cytobrush in detecting atypical cells was also evaluated. Unfortunately, only a few cases of atypical smears were found. We therefore believe that we cannot predict the value of detecting more atypical cells when a double smear is made. It is interesting to note that the value of the cytobrush to detect endocervical atypical cells is limited because in only 38.5% (224 out of 733) of the cytobrush smears was the quality of the endocervical cells sufficiently satisfactory to give a diagnosis.

Again the results show that use of the cytobrush alone is unsatisfactory. In 17.5% of the cytobrush smears a reliable diagnosis was not possible. A cytobrush smear, in our opinion, always has to be combined with an ectocervical scrape.

While completing this study, a new sampling device was developed in the Netherlands, known as the Cervexbrush. This device is a bristle brush which also samples the ectocervix and does not have the disadvantage of the cytobrush in producing a high percentage of unsatisfactory smears. It can be used without a spatula and it has been developed by a Dutch general practitioner. The preliminary results are promising.

In our laboratory it is thought to be too expensive to make cytobrush smears a routine.

We advise using the cytobrush if a suboptimal smear is repeatedly obtained. We hope that the newer Cervexbrush will show good results, and that we will be able to provide our sample takers with one optimal screening device.

We thank Charles Kouwenhoven (CT) for his technical assistance, Dicky van der Baan and Julie Jones for reviewing the English text, and Joke Metselaar for typing the paper.

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