A TECHNICAL GUIDELINE:
COLLECTION OF ADEQUATE PAP SMEARS OF THE
UTERINE CERVIX

Translation of the final report of the Working Group “Sampling”

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CERVICAL CANCER SCREENING IN THE FLEMISH COMMUNITY

A TECHNICAL GUIDELINE:
COLLECTION OF ADEQUATE PAP SMEARS OF THE UTERINE CERVIX

Translation of the final report of the Working Group “Sampling”

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Contents

1. Reports from the Working Group “Sampling” ................................................................. 4

2. Technical guidelines for the collection of a Papanicolaou smear ........................................ 5

3. Accompanying illustrations .............................................................................................. 14

4. Standard request form ..................................................................................................... 14

5. Thin-layer cytology ......................................................................................................... 15

6. References ...................................................................................................................... 16

7. Annexes .......................................................................................................................... 19
   1. Accompanying illustrations ......................................................................................... 20
   2. Quality judgement of a Papanicolaou smear according to The Bethesda System ........ 34
   3. Standard request form ............................................................................................... 36
   4. Summary: Collection of an adequate Papanicolaou smear of the uterine cervix .......... 37
   5. Comparison of the quality of cervical smears obtained with the spatula &
      Cytobrush® versus the Cervex-Brush® ......................................................................... 42
   6. Members of the Sampling Working Group ................................................................. 51
   7. List of used abbreviations ............................................................................................ 53
1. Reports from the Working Group “Sampling”

The conclusions of the working group will be published in the form of the following outputs:


b) Scientific paper (possibly with illustrations): "Technical guideline for the collection of a Papanicolaou smear”, to be submitted for publication in a Belgian medical journal.

c) Booklet: "Uniform guidelines for cervical cancer screening in Flanders: (1) collection of Pap smears, (2) cytological interpretation, (3) follow up of abnormal results”. Clear, readable, illustrated booklet summarising the conclusions of the three working groups (consistency in cervix cytology methods, follow-up, sampling).
To be distributed to all general practitioners and gynaecologists in Flanders (~8000 copies).
The “Working Group Sampling” guidelines are summarised in Appendix 4.

d) Technical record card:
Three boxed laminated cards on specimen collection, interpretation and follow-up.

e) Accompanying colour illustrations: overhead transparencies, slides, and files in a standard graphic format for electronic transmission.

f) Presentation of the final report as a downloadable Acrobat file on the Scientific Institute for Public Health's Website.
2. Technical guidelines for the collection of a Papanicolaou smear

Introduction

In late 1996, the Cervical Cancer Screening Steering Group, a subcommittee of the Flemish Advisory Board for Cancer Prevention (VACK, “Vlaamse Advies Commissie voor Kankerpreventie”), requested to set up a working party “the Sampling Working Group” which was to formulate guidelines for the collection of a Papanicolaou smear. Since the VACK was closed down in 1997, the Steering Group no longer operates. Therefore, the Sampling Group has been idle for some time now. A new Steering Group is scheduled to be resurrected in September 1999. The guideline below can be assessed and validated at this forum.

The diagnostic value of cervical smears for detecting cytological precursors of uterine cervical cancer is determined by the quality of the sampling technique used. Several experts even claim that inadequate sampling, more so than cytological interpretation errors, is responsible for false-negative results [Richart, 1965; Frost, 1969; McGooghan, 1997].

The anatomy and histology of the uterine cervix and more specifically the transition zone between the squamous and columnar epithelia is described briefly, as understanding of this is essential for the collection of a representative smear.

In addition, the criteria for quality judgement of a smear are discussed. These are specified using The Bethesda System for reporting cervical smears [Lundberg, 1989; Laff, 1992; Bethesda, 1993; Kurman, 1994], modified by the Working Group on Uniformisation of Cervical Cytology [WUCC, 1996].

A summary is provided of the factors that affect the interpretation of a smear. Physicians who take smears should take account of these as far as possible.

Finally, a technical guideline is provided on all the steps of the sampling process:
- preparing the patient and equipment;
- visualising the cervix;
- scraping cellular material from the uterine cervix with appropriate sampling devices;
- transferring them on a slide;
- fixing the specimen;
- completing the standard request form;
- transporting the sample to a cytology laboratory.

The choice of sampling device is discussed in detail. Data in the literature show that the combination of a spatula and an endocervical brush produce significantly better results than a spatula alone. The Cervex-Brush® allows a representative smear to be obtained with one single device. In a meta-analysis we have investigated whether this latter method produces results comparable to those from the combination of spatula/endocervical brush.

There is also a short explanation of thin-layer cytology, a new sampling method in which the cellular material is immersed in a container with transport fluid from which uniform thin smears can be prepared in the laboratory.

This guideline on the collection of a Papanicolaou smear completes a series of earlier published instructions on uniform cytology reporting and follow-up of screen-positive lesions, which were formulated by the Working Group on Uniformity in Cervix Cytology [WUCC, 1996] and the Follow-up Working Group [Arbyn, 1996].

The illustrations in this guideline are made by Herman Vanvinckenroye
Anatomy and histology of the uterine cervix

See Figures 1a-d, 2a-d and 3 in Annex 1.

The exocervix or portio protrudes into the vagina and is generally covered with multilayer squamous epithelium. Via the external os, the exocervix internally becomes the narrow endocervical canal. This canal branches into the endocervical crypts and is lined with monolayer columnar mucus-producing epithelium. The transition between the two epithelia is called the squamocolumnar junction (SCJ). In prepuberty, this SCJ is usually situated inside the endocervical canal, close to the ostium externum. After puberty, as a consequence of a change in shape of the uterine cervix, with use of the contraceptive pill and still under the influence of the first pregnancy, the endocervical columnar epithelium everts. This cervical ectopy or ectropion is perfectly physiological during the reproductive period. It is visible as a red zone (erroneously termed cervical erosion) contrasting with the pinker squamous portio. The extent of the ectropion varies from person to person [Ferenczy, 1994]. Gradually, this exocervical columnar epithelium is replaced by metaplastic squamous epithelium. Two junctions can be distinguished: the distal transition between squamous and metaplastic epithelium (the primary SCJ) and the more proximal junction between metaplastic squamous and columnar epithelium (the secondary or physiological squamocolumnar junction [Boon, 1993; Ferenczy, 1994]. This area between the original and the current junction is called the transformation zone (TZ) or transition zone. The shape of the TZ is often irregular and at first comprises multifocal islands, which gradually go on to coalesce. Sometimes, there are still residual gland openings visible. Later, the metaplastic squamous epithelium may mature into fully mature multilayer squamous epithelium. The functional SCJ migrates inwards with increasing age. In post-menopausal women, this junction is almost always inside the endocervical canal.

Virtually all squamous cell neoplasias of the cervix arise initially around the physiological SCJ and the preferential location for dysplasia corresponds with the topographical distribution of the TZ [Burghardt, 1970; Richart, 1973]. Therefore, locating the transformation zone and sampling its entirety is essential to obtaining a representative smear.

Therefore, according to several authors, , an adequate smear should contain metaplastic and/or columnar endocervical cells as well as squamous cells [Elia, 1983; Vooijs, 1985; Boon, 1993; Kurman, 1994].

Evaluating the quality of a smear

The Bethesda System for reporting smears provides three categories for evaluating the quality of the smear: “satisfactory for evaluation”, “satisfactory for evaluation but limited by ….,” and “unsatisfactory for evaluation …. “

The assessment is based on the following criteria:
- presence of sufficient number of cells;
- presence of metaplastic and/or columnar cells as well as squamous cells;
- fixation of the cell material;
- absence of excessive numbers of erythrocytes or inflammatory cells;
- absence of extensive cytolysis of the squamous cells;
- even, thin spread of the cellular material, absence of aggregations of cells;

A smear suitable for cytological evaluation should also be labelled with identifying details. These refer to a request form that contains adequate clinical information. The specimen must, of course, not have been irreparably broken.

With these guidelines, the Working Group Sampling, hopes to help minimizing the percentage of unsatisfactory smears. (see Annex 2)

Factors that adversely affect smear quality

Table 1 on the next page lists a number of factors that adversely affect the quality of a smear. These are then discussed and recommendations given to limit the detrimental elements.

Table 1 Factors that adversely affect smear quality

<table>
<thead>
<tr>
<th>Factors that adversely affect smear quality</th>
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<tbody>
<tr>
<td>Menstruation, blood loss or breakthrough bleeding</td>
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<td>Vaginal inflammation/infection</td>
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<tr>
<td>Severe genital atrophy (menopause)</td>
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<td>Pregnancy (and lactation)</td>
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<td>Foregoing digital examination</td>
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<tr>
<td>Disinfectant cream or fluid, lubricating jelly</td>
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<td>Vaginal medication (less than 48 hours before)</td>
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<td>Vaginal douche (less than 24 hours before)</td>
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<td>Foregoing colposcopy with acetic acid (less than 24 hours before)</td>
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<td>Previous smear (less than 3 months before)</td>
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<td>Cervical surgery (less than 3 months before)</td>
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<td>Radiotherapy</td>
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Effect of menstrual cycle on smear quality

The best time for a smear to be taken is between the seventh and fifteenth day of the menstrual cycle [Vooijs, 1987]. At this time, there is no longer menstrual blood or cell debris and there is virtually no cytolysis of squamous cells. However, this does not mean that a doctor should propose a woman to come back if she presents at a different time in her cycle. The presence of a small amount of blood does not necessarily have any adverse effect on the quality of the smear, but smears should not be taken during menstruation itself or when there is significant blood loss or breakthrough bleeding, as the presence of excess blood obscures a clear view of the epithelial cells causing reduction of the sensitivity of the screening test.

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*Thompson [1989] considers the finding of squamous metaplasia as an important indicator that a sample has been taken from the target zone. Moreover, the presence of endocervical columnar cells indicates that cellular material beyond the transformation zone has been included in the specimen. However, the presence of squamous metaplastic and/or columnar cells as well as squamous cells is no proof that the entire transformation zone has been sampled [Kurman, 1994; Solomon, 1995]. Recent longitudinal studies do not support the thesis that the absence of endocervical cells is associated with a higher risk of false-negative results [Kivlahan, 1986; Mitchell, 1991]. Mitchell [1992] was able to confirm this correlation only for the presence of metaplastic, but not for columnar endocervical cells. Boon [1993] holds with the contention that the presence of immature metaplastic cells and/or columnar cells is associated with the detection of high grade intraepithelial lesions. If low grade lesions are also included in the definition of a positive smear, this association disappears. Boon [1993] also states that the progressive character of squamous lesions increases the closer they are to the metasquamo-columnar junction and therefore also pleads for a double endocervical and exocervical sample. The exclusive presence of columnar cells (without pavement cells) indicates a non-representative smear.
Inflammation/infection
Purulent infections/inflammations that are clinically obvious, should be treated appropriately before a smear is taken. The presence of large numbers of inflammatory cells inhibits a clear microscopic view on the epithelial cells. Inflammation or infection can sometimes give rise to atypia, which usually disappears after treatment [WHO, 1988].

Atrophy
A highly atrophic cervix is best treated with hormones before a smear is taken.

Pregnancy and postpartum
During pregnancy and up to 3 months postpartum, screening smears should not be taken [NHG, 1996]. The cytology of the cervix is affected by pregnancy and lactation and difficult to interpret [Ziekenfondsraad, 1993]. Several authors report an increased risk of false-negative results or under-interpretation of epithelial lesions [Hellberg, 1987; Ritter, 1995].

With pregnant women who have not had a previous smear, and for whom the chance of future participation in screening is low (for example in the case of low socio-economic status), it may be opportune to take a smear as a precaution at the prenatal or postnatal consultation.

Mechanical manipulation or chemical irritation of the cervix epithelium
A smear should not be taken if vaginal medication has been administered less than 48 hours before, or less than 24 hours following a vaginal douche.

Diagnostic epithelial cells can be removed or damaged by scraping with a sampling device, by vaginal digital examination or as a result of application of acetic acid prior to a colposcopic examination [Griffiths, 1989]. It can take several weeks for such lesions to heal. Any vaginal digital examination, or colposcopic examination should therefore be done after the smear has been taken.

A cytological check, indicated in case of ASCUS, L-SIL or unsatisfactory quality judgement should only be done after a minimum interval of three months, since lesions can be temporarily absent, or more difficult to detect after a smear, thus increasing the likelihood of false-negative results [FU working group, 1995]. There is little correspondence between the assessment of two smears taken from the same woman at an interval of less than 3 months [Meisels, 1990].

In addition, after surgical intervention (biopsy, conisation, partial excision, laser or cryo therapy) one should wait three months before taking another smear, as reparative changes can give rise to false-positive results.

Radiotherapy can in itself give rise to fairly extensive cell abnormalities.

Contra-indications for screening smears

Hysterectomy
Screening smears should no longer be taken after total hysterectomy. Following total hysterectomy, because of cervical neoplasia, the need for follow-up smears (or other investigations) is determined on an individual basis.

The Follow-up Working Group recommends that screening smears should not be taken from pregnant or lactating women until 6 months after delivery or breast-feeding [Follow-up Working Group, 1995; Arbyn, 1996]. The Sampling Working Group revised this recommendation and reduced the interval to three months post delivery.

A supplementary smear during colposcopic examination has very little diagnostic value and is therefore not recommended [Beckwith, 1995].

Following total hysterectomy no further invitations should be sent.
Macroscopically suspect lesions
If there are macroscopic suspect abnormalities of the cervical surface (irregular elevated lesion with ulceration, covered with blood or necrotic material) no smear is taken, but the patient is referred to a gynaecologist for biopsy under colposcopic examination [NCCLS, 1994; NHG, 1996; NHSCSP, 1998].

Information to the woman
The woman should be given adequate information on how the smear will be taken [Buntinx, 1989], why it is being taken and the possible consequences of any abnormalities [NHG, 1996]. The advice is given that one correct smear every three years - in the absence of gynaecological complaints and if previous results were negative - is almost always sufficient to exclude the appearance of uterine cervical cancer or to enable prompt detection and treatment.

It is explained that a smear sometimes has to be repeated because of technical problems relating to the quality of the smear beyond any suspicion of (pre-)cancerous lesions. [NHG, 1996]. It is recommended that the woman be systematically questioned on whether a copy of the results may be sent to another doctor (her general practitioner, or her attending gynaecologist)\footnote{We cite here four clauses from the consensus reached within the Flemish Steering Group on Cervical Cancer Screening, Discussion concerning Communication between General Practitioners and Specialists, - Brussels 9 February 1996: Clause 2: Gynaecologists support the contention that the general practitioner is the manager of the central medical file of his patient. They also agree that if the screening is done by a gynaecologist, a copy of the protocol should be sent to the general practitioner. Feedback on diagnosis or treatment of a referred patient is part of good medical practice. Clause 3: The general practitioner in his turn will notify the gynaecologist on the further (gynaecological) course of any patient who has been monitored and/or treated in the past. Clause 6: A section "send copy to Dr. ....... " is to be inserted into the request form for cytological investigation that accompanies the smear. Clause 7: This section will only be filled in if the woman gives her consent. The physician must ask this question systematically.} [Flemish Steering Group on Cervical Cancer Screening, 1996].

A formal arrangement is made concerning when and how the patient will be notified of the result. In that way, it can be agreed that no message within two weeks means that the smear is normal. The woman may, if she so wishes, make a telephone call herself to find out the result.

If a repeat smear, other additional examination or referral to a gynaecologist is required, the doctor himself will do whatever is necessary to contact the patient [NHG, 1996; Follow-up Working Group, 1995].

Preparation of the smear
The examination couch should be equipped for gynaecological examination. A suitable light-source must be available. The doctor taking the smear should be familiar with gynaecological examinations and trained in taking cervical smears.

All the equipment should be laid out ready and within hand-reach so that the interval between taking the smear and fixing it is as short as possible (at most a few seconds) (see Figure 4). Any digital vaginal examination should be postponed until after the smear has been taken.
Equipment required:
- specula (various sizes, virgo-specula)
- gloves
- microscope slides
- pencil
- forceps with swabs for removing any excess mucus
- atraumatic forceps
- sampling devices: spatula + endocervical brush (Cytobrush\textsuperscript{R}) or Cervex-Brush\textsuperscript{R}
- fixative: aerosol spray or alcohol solution.

Slides, sampling devices and aerosol spray are supplied by most cytological laboratories for physicians who take smears. It is recommended that the top from the aerosol spray be removed and to check that the spray is neither clogged nor empty.

The microscope slide is labelled on the frosted end with lead pencil with the name or reference number (see Figure 6).

The speculum is preferably inserted without specific lubricant and the portio cervicis is visualised as clearly as possibly. For the comfort of the patient, some people recommend that the speculum first be warmed for example in tepid water or in a warming drawer. Contact between the speculum and the cervix should be avoided as far as is possible. In the event of extreme retroversion or anteversion of the uterus, the cervix can be manipulated with closed atraumatic forceps towards the centre to enable a smear to be taken. The position and aspect of the transformation zone is checked visually (see Figure 7).

If there is excessive mucus, discharge or blood, it should be very carefully removed with a swab.

**Number of specimens**

One or two microscope slides can be used for smears of exocervical and endocervical mucus [Buntinx, 1989]. The use of one slide leads to less weight and volume for transport and archiving, and to less surface and time for reading. Some older experiments show that the sensitivity of the Pap test can be increased by a double smear spread over two slides [Sedlis, 1974; Shulman, 1975; Luthy, 1978; Beilby, 1982]. However, there is not enough evidence on whether this increase in sensitivity is due to the effect of a second reading or to the examination of more cell material.

Moreover, these studies report nothing about the value of a double exocervical/endocervical smear spread over one slide. The reports also show that in routine use, the two smears are not examined with the same care if two smears are taken from each patient without indication of a special reason for so doing [A. Hanselaer, personal communication].

The Working Group therefore pleads for the use of **one slide only**.

**Sampling devices**

Cervical screening always requires an endocervical and an exocervical specimen taken with the appropriate sampling devices. Only in that way is there a reasonable chance that the transformation zone is sampled satisfactorily [Boon, 1993; Buntinx, 1996]. The presence of an ectropion does not in any way change the requirement for dual sampling [Garite, 1978; Buntinx, 1995].

Endocervical sampling alone, for example just with a Cytobrush\textsuperscript{R}, is to be avoided just as much as is an exocervical smear alone, for example with an Ayre-spatula [Spurett, 1989; Buntinx, 1991].

Comparable detection percentages are found if either a spatula + endocervical brush, the sharp + blunt
The first two methods result in a larger number of smears with endocervical cells [Buntinx, 1994], which is considered to be an indicator of good quality [Elias, 1983; Vooijs, 1985; Boon, 1993]. The good performance of the combination spatula + endocervical brush depend little on the experience of the smear-taker [Boon, 1985; 1986a; Szarewski, 1990]. The likelihood of detection of glandular lesions is also greater if the endocervical brush is used [Boon, 1986]. Therefore, the original preference of the Sampling Working Group was for the combined use of the appropriate end of the combination spatula and the endocervical brush (Cytobrush®). Recent experience of a few pathologists in the Working Group, however, shows that the Cervex-Brush® scores very highly in terms of technical interpretability of the smear. A supplementary literature survey reveals that the detection rate for cytological lesions and the presence of metaplastic squamous cells and columnar cells does not differ significantly between the Cervex-Brush® and the combination of spatula + Cytobrush® [Arbyn, 99; Annex 5]. The cost of the sampling devices are comparable in the two methods (~10 BEF). Given these arguments, the Working Group has decided that both the Cervex-Brush® and the combination of the spatula with the Cytobrush® can now be recommended (see Figure 5).

The possibility of obtaining a representative smear with the Cervex-Brush®, i.e. with a single device offers a definite saving in time, so the specimen can be fixed more quickly. Moreover, the Cervex-Brush® is highly suitable for thin layer cytology (see Section 5).

The endocervical brush often results in a very slight bleeding, which usually causes minor if any problems [Buntinx, 1989; Boon, 1989]. In the case of pregnancy or a cervix that bleeds easily, it is best to use the Cervex-Brush®.

After surgical intervention on the cervix, in post-menopausal women, and in follow-up of glandular lesions, the Cytobrush® is often required for obtaining endocervical cells. The combination method with an endocervical brush is then preferred [Thompson, 1989; Szarewski, 1991].

**Sampling technique**

1. **Cervex-Brush® sampling** (see Figure 8)

Endocervical cells and exocervical cells are sampled simultaneously. The long bristles are applied to the endocervix to pick up endocervical cells, while the short bristles are put into contact with the exocervical region. The handle of the brush is rotated between thumb and forefinger a few times (ideally 5) in a clockwise direction, under gentle pressure, b [Ferris, 1992]. The sample is spread onto the slide with a painting action, using both sides of the brush (see Figure 9) and fixed immediately (see Figure 10).
2. Combination sampling with spatula and endocervical brush

- 2.a. Spatula sampling (see Figure 11)

In theory, the end of the spatula is used that is most suitable to the anatomy of the portio. In nullipara, the narrow end (Aylesbury spatula, with elongated tip) is usually used; in multipara the wider end is used (Ayre spatula, with shorter tip). With the tip at the ostium, the spatula is rotated 360° under gentle pressure. It will be necessary to change the hold at least once. The tip scrapes cells from the ostium while the less protruding part scrapes the surface of the portio. Care should be taken to scrape the (physiological) squamocolumnar junction over its whole circumference. If there is extensive ectropion, the outer edge of the portio is scraped separately.

After the sample has been taken, the spatula is put aside with the specimen face-up. The danger of drying out is less if the cell material plus mucus remain in contact with the sampling device. Only after the Cytobrush® has been used has the material is spread onto the slide.

- 2.b. Cytobrush® sampling (see Figure 12)

Then, the brush is inserted two thirds into the endocervical canal and gently rotated 90 to 180°. A full turn as with the spatula sampling is not necessary since the bristles come in contact with the entire surface of the endocervical canal [NCCLS, 1994]. Rotating over a larger angle only increases the likelihood of cell damage and endocervical bleeding.

The Cytobrush® is immediately rolled (not wiped) over the outer third of the slide. The sample from the spatula is then smeared over the central third as quickly as possible (see Figure 13). Fixation is done immediately. The interval between sampling and fixation should be as short as possible, because drying artefacts can appear after just a few seconds.

The rolling and spreading is done is a single movement (not in a zigzag) and without pressure, so that an even thin smear is obtained.

Because of blood loss associated with use of the Cytobrush®, the spatula sample should be taken before the one with brush.

If the double sample is spread over two slides, the spatula sample is spread and fixed before proceeding to the endocervical brush sampling.

**Fixation**

Immediate fixation kills bacteria, denatures enzymes and eliminates the likelihood of drying artefacts in the cells on later staining [Boon, 1993].

Epithelial cells stay moist for longer if they are left on the sampling device [Boon, 1993]. Once spread onto the slide, they dry out very quickly, which leads to alterations in the chromatin pattern of the cells if they are not fixed immediately (air drying effects) [Boon, 1993]. The speed of fixation is very important, particularly if the specimen contains little or no mucus. Endocervical and immature squamous cells, reserve cells, atrophic and dysplastic cells are extremely sensitive to drying. Mature squamous cells are somewhat more resistant.
Fixation is done with a special spray. Commercial hairsprays are not recommended given the variety in composition.

It is best to shake the aerosol can well and remove its top before starting to take the smear. The specimen is fixed by spraying the slide at right angles from 20 cm (see Figure 10). If the distance is less, the cells may be blasted away or frozen; if on a slant, the material is blown into ridges. Formation of droplets should be avoided: so do not spray too much [Buntinx, 1989].

Fixation can also be done by immersion for 5 to 30 minutes in 95% ethanol [NCCLS, 1994]. The top of the container should be removed beforehand so that no precious seconds are lost.

Inadequate fixation reduces the specificity of the examination [Buntinx, 1992a; Arbyn, 1997]. The sensitivity is also affected adversely.

**Transport to the cytology laboratory**

After fixation (spray fixation or by immersion), the specimen is allowed to dry fully in air. It is then put into a cardboard or plastic holder for transport to the laboratory. A wet specimen can stick at the edges if it is put into a plastic folder. The holder is labelled with identifying details matching those on the request form (see Figure 14).

Biopsies in formalin are not sent in the same package as smears because the vapour can affect the fixation of the smears.

**Feedback on the quality evaluation of the specimen**

Research has demonstrated that systematic feedback to the physician on the quality of the smears he/she has taken can significantly improve the quality [Boon, 1986; Buntinx, 1993]. Periodic notification of the average quality score of an individual doctor compared with the general or regional distribution of smears taken by all doctors is an important incentive element in quality assurance of the screening process [Buntinx, 1993; Wilson, 1999].

All cytology laboratories are also encouraged to provide regular feedback. In the future, a central register can take on the task of providing physicians with feedback.

**Training of physicians**

The experience and dedication of the person taking the smear also plays a crucial role in achieving a good smear [Lundberg, 1989b; Boon, 1993; Szarewski, 1993; Bar-Am, 1997]. According to some authors, the collector effect is even more important than the choice of equipment [Wolfendale, 1991]. This is insufficiently stretched in comparative literature. Theoretical and practical training in taking Pap smears needs to be a permanent feature of training programmes in the medical faculties and in post-graduate courses [NHSCSP, 1998].
3. Accompanying illustrations
These guidelines are illustrated with figures (see Annex 1), made by H. Vanvinckenroye, graphic artist at the Institute of Tropical Medicine in Antwerp.

These illustrations are available in colour in the form of slides and overhead transparencies.

4. Standard request form
Clinical details are important for accurate interpretation of a smear [Craig, 1985; Bethesda: Lundberg, 1989; Luff, 1992; Kurman, 1994; NVVP, 1996; WUCC, 1996].
The Working Group Sampling presents a standard request form with a minimum list of essential details that can contribute to the diagnostic value of the cytology report and the pertinence of any follow-up advice.

Annex 3 contains a standard request form for cervico-vaginal cytology.

This form can be used for screening purposes, and also for follow-up or clinical indications.

Correctly completed identification and dating of the request and specimen are obviously indispensable to the processing of the sample. The identification details of the patient can also be provided in the form of a sticker or badge.
The cytopathologist needs relevant clinical information in order to increase the reliability of the cytology result. This information is essential to the interpretation of certain cell pictures (e.g. presence of endometrial cells according to the cycle, menopausal status, hormone therapy). Based on the clinical data, specimens can also be selected for specific review (e.g. in the case of follow-up of low grade lesions). Advice relating to further management depends both on the cytological appearance and on clinical details. If this information is lacking, a specimen cannot be processed optimally. In this case, it is recommended that the cytopathologist reports as follows: “satisfactory for evaluation but limited by: lack of clinical data” [Luff, 1992; NVVP, 1996]. The Working Group Sampling recommends the use of specific request forms for cervix cytology. Requests for clinical biological investigation are usually unsuitable for this particular purpose and provide insufficient space for the clinical details required.

Date of birth and date of last menstrual period must always be provided; other details should be included if they apply to the patient. The use of the standard request form (Annex 3) allows all the pertinent details to be listed simply and efficiently.
The minimum clinical data needed (origin of sample, gynaecological status, interventions) can be provided by means of a check-box system. In this way, data relating to screening history and reason for smear can also be specified.

So as not to overload the request form, space is provided for free text for reporting relevant data that may influence the cell picture but are less frequently encountered (e.g. radiation, chemotherapy, topical treatment of condylomas).

Finally, correct identification and signature of the requesting doctor are required. To aid communication between the first and second echelon, the requesting doctor is recommended to indicate whether a colleague (general practitioner or attending gynaecologist) is to receive a copy of the report.
5. Thin-layer cytology

Thin-layer cytology is a new technique for transferring the cellular material to the microscope slide. The Cervex-Brush\(^a\) is usually the device recommended for taking the sample. However the combined use of spatula and endocervical brush is also an option.

The smear is not transferred in the usual way onto a slide. The sampling device\(^1\) carrying the material is immersed in a container with a special liquid transport medium. The container is then sent to a specially equipped laboratory. At present, there are several commercial systems available. Only Thinprep\(^a\) (Cytec) and Cytorich\(^a\) (Autocyte) have so far been approved in the United States by the Food and Drug Administration. With the first system, the liquid is sucked through a membrane and the cellular material sticks to the filter, which is then stamped onto a slide in the form of a monolayer. With the more automated Autocyte\(^a\) machine, the material is sedimented through a density gradient [Howel, 1998].

A primary advantage of these methods is that almost all the sampled cells are rinsed into the liquid while with the conventional smear a selective portion of the cellular material may remain stuck to the sampling device [Rubio, 1977; Hutchinson, 1993]. Transfer by means of a liquid increases the likelihood of representative smears. In addition, fixation of the cell material is optimal. The altered background requires a degree of adaptation on the part of the cytologist, however. Red blood cells and mucus are for the most part absent and leukocytes are more evenly distributed. Epithelial fragments, which are difficult to interpret on a classical smear, are for the most part disaggregated during the preparation, while diagnostic clusters of columnar or metaplastic cells are usually preserved. The microscopic visualisation of a calibrated thin line of beautifully distributed cells is more comfortable for cytological interpretation, which should improve the evaluability and diagnostic quality of the investigation [Linder, 1997; Austin, 1998].

Multiple smears can be made or additional investigations done (e.g. HPV-DNA detection) with the residual material [Ferenczy, 1997; Sherman, 1997].

A significant disadvantage is the high cost - both the capital investment and the operating costs. The cost-benefit ratio needs further investigation before this technique can be recommended for general use.

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\(^1\) The head of the Cervex-Brush\(^a\) can easily be removed from the handle. The brush is then immersed in the transport fluid. The container (brush plus fluid) is then sent off to the laboratory. The top of the Cytobrush and spatula should be broken off. The Cytobrush needs a pair of wire-clippers. Recently, endocervical brushes have also become available with a notch, which facilitates snapping.
6. References


7. Annexes

1. Accompanying illustrations
2. Quality judgement of the Papanicolaou smear according to The Bethesda System.
3. Standard request form
5. Literature review: Comparison of the quality of cervical smears obtained with spatula/Cytobrush® versus Cervex-Brush®
6. Members of the Working Group Sampling
7. List of used abbreviations
Annex 1

Accompanying illustrations.

Fig 1 a - d       Fig 2 a – d
Figure 1. Schematic presentation of the ontogenesis of the transformation zone (after Ferency, 1994).

1a: The cervix is completely covered with squamous epithelium. The endocervical canal and the underlying crypts are covered by mucin-secreting columnar epithelium. The original squamo-columnar junction (SCJ) is situated at the external ostium.

1b: Cervical ectropion: columnar epithelium has migrated more distally; the squamo-columnar junction is situated at the exocervix.

1c: The exocervical columnar epithelium is replaced by metaplastic squamous epithelium. This is termed the transformation zone (TZ). This TZ constitutes the predilection place for the development of squamous intra-epithelial neoplastic lesions detectable by the Papanicolaou smear test.

1d: The transformation zone is fully matured and covered with squamous epithelium. There is only one physiological squamo-columnar junction left. This physiological SCJ migrates inwards and is situated in the endocervical canal in post-menopausal women.

Figure 2. Different aspects of the cervical portio.

2a: View of the immature cervix: the squamo-columnar junction at the ostium

2b: Cervical ectropion

2c: View of the complete transformation zone (both junctions are visible)

2d: The squamo-columnar junction is situated inside the endocervical canal, only squamous epithelium is visible from outside.

The visualisation of the portio and the localisation of the transformation zone are essential for the collection of an adequate smear.
Figure 3.
A: Squamous cells
B: Metaplastic cells
C: Columnar cells

An exo- and endocervical smear give the best guarantee to find cytological abnormalities. Such a smear contains squamous and metaplastic and/or columnar cells.
Figure 4.: Required material for sampling.

Microscope slide, pencil, gloves, sampling devices, fixative, slide holder and request form.
Figure 5. Sampling devices.

5a: Combined spatula with an Ayre-pole (under) and an Aylesbury pole (on top)
5b: Cytobrush®
5c: Cervex-Brush®
Figure 6. Labelling the slide.

Identification data are written by pencil on the frosted end of the slide.
Figure 7. Visualising the cervix.

After insertion of the vaginal speculum the cervix is visualised and the position of the transformation zone is checked.
The rubber bristles of the Cervex-Brush® are flexible and adapt themselves to most of the cervixes. This instrument permits to take an exo- and endocervical smear in one manoeuvre. The central bristles of the Cervex-Brush® are inserted with gentle pressure in the endocervical canal until the lateral bristles bend against the exocervix. The broom is then turned five times 360° by rolling the handle clockwise between thumb and index finger.
Figure 9. Transferring the sample to the slide using Cervex-Brush®

The sample is spread onto the slide with a painting action, using both sides of the brush.
Figure 10. Fix immediately.

The sample is fixed as soon as possible with a fixation spray. The spray is directed at right angles at about 20 cm distance.
Figure 11. Specimen collection using spatula.

An exocervical smear should be taken using the end of the spatula which corresponds best with the anatomy of the cervix (the Aylesbury pole in nullipara and the Ayre pole in multipara). The point of the spatula is inserted in the ostium. The whole circumference of the exocervix is sampled under gentle pressure with the broad end of the spatula.
Figure 12. Collection of an endocervical smear with the Cytobrush™

The brush is inserted for two thirds in the endocervical canal and gently rotated 90° to 180°.
Figure 13. Transferring the sample to the slide using Cytobrush® and spatula.

The Cytobrush® is rolled over the outer third of the slide.  
The material on the spatula is spread in a thin layer in one movement over the middle third of the slide.  
Fixation is done immediately in the same way as prescribed in Figure 10.
Figure 14. Standard request form

The fixed specimen is left to air dry and subsequently packed in the holder. The request form is completely filled in.
Annex 2

Quality judgement of a Papanicolaou smear according to The Bethesda System

The *Bethesda System* for reporting cervical smears, developed by the *National Cancer Institute*, awards a significant role to quality evaluation of the specimen [Kurman, 1994; Nielsen, 1993]. The Flemish standard protocol of the *Working Group on Uniformisation of Cervical Cytology (WUCC)* is based on this American model [Cuvelier, 1998]. Three categories are distinguished:

- satisfactory (synonyms: adequate, optimal)
- satisfactory for evaluation but limited by … (qualify with reason) (synonym: suboptimal)
- unsatisfactory (qualify with reason), (synonym: inadequate or poor)

Four kinds of criteria are used for the quality evaluation of the smear: identity details of the woman and specimen, availability of adequate and pertinent clinical data, cellular composition and technical interpretability.

The quality of the smear to a great extent determines the accuracy of the cytological interpretation.

Below is the definition of the three categories of quality evaluation:

**Satisfactory**
- Satisfactory labelling of the specimen matching the identification details on the request form.
- Relevant clinical information.
- Adequate number of well-preserved and clearly visible squamous cells: visible over more than 10% of the specimen surface.
- Presence of an endocervical/transformation zone (EC/TZ) component: at least two clusters of at least 5 endocervical or metaplastic squamous cells.

The requirement for the presence of a transformation zone/endocervical component does not change with age and applies equally for pre and post menopausal women. If there is extensive atrophy, however, metaplastic and endocervical cells can scarcely be distinguished from parabasal cells and absence of a TZ/EC-component is not reported.

The presence of metaplastic squamous cells and/or columnar cells as well as squamous cells or mucus indicate that the transformation zone has been sampled. However, they do not prove that the entire TZ was included in the sampling. On the basis of the cellular composition alone, the cytologist cannot guarantee that the entire circumference of the transformation zone has been sampled. It is ultimately up to the doctor who takes the smear to integrate the information on the composition of the specimen with the clinical data (examination, history) and thus assess the adequacy of a smear from an individual woman [Kurman, 1994; NHSCSP, 1995].

**Satisfactory for evaluation but limited by …**
- Between 50% and 75% of the specimen cannot be read because of interfering elements (blood, inflammation, aggregation of cells, defective fixation, drying artefacts, cytolysis, contamination etc.).
- Absence of a component from the transformation zone: two clusters of at least 5 endocervical or metaplastic cells (applies to all women, irrespective of age).
- Absence of pertinent clinical information.

**Unsatisfactory**
- Too few squamous cells: well-preserved and recognisable squamous cells covering less than 10% of the area of the smear.
≥ 75% of the specimen unreadable because of blood, inflammation elements, areas that are too thick, drying artefacts because of insufficient fixation, cytolysis or contaminants [Kurman, 1994].
- Identity data lacking on slide or missing on the accompanying request form.
- Broken, irreparable slide.

Smears in which abnormalities of the epithelial cells are discovered are not labelled “unsatisfactory”.

With the specification of “satisfactory for evaluation but limited by...” and “unsatisfactory” the reason is given (see Table 2).

Table 2: quality evaluation of Papanicolaou smears from the uterine cervix and summary of the possible reasons for less than satisfactory and poor quality [WUCC, 1996].

<table>
<thead>
<tr>
<th>Quality evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Satisfactory 0 Satisfactory but limited by … 0 Unsatisfactory</td>
</tr>
<tr>
<td>Reasons:</td>
</tr>
<tr>
<td>exclusively endocervical cells</td>
</tr>
<tr>
<td>no endocervical cells</td>
</tr>
<tr>
<td>poor or unsatisfactory fixation</td>
</tr>
<tr>
<td>too little cellular material</td>
</tr>
<tr>
<td>too much blood, pus</td>
</tr>
<tr>
<td>extensive cytolysis</td>
</tr>
<tr>
<td>poor smear technique</td>
</tr>
<tr>
<td>other: ............................................</td>
</tr>
</tbody>
</table>

Thompson [1989] considers the finding of squamous metaplasia to be a strong indication that a sample has been taken from the target zone. The presence of endocervical columnar cells proves that cellular material from beyond the transformation zone has been included in the specimen. The presence of squamous metaplastic and/or columnar cells as well as pavement cells, however, does not prove that the entire transformation zone was sampled. [Kurman, 1994; Solomon, 1995]. Many observations support the thesis that the presence of endocervical or metaplastic squamous cells increase the likelihood of finding cytological lesions. And, vice versa, the absence of such cells leads to more false-negative results.

A few recent longitudinal studies do not support this contention [Kivlahan, 1986; Mitchell, 1991]. Mitchell [1992] was able to confirm this correlation only for the presence of metaplastic, but not for columnar endocervical cells. Boon [1993] stands by the contention that the presence of immature metaplastic cells and/or columnar cells is associated with the detection of high-grade intra-epithelial lesions. If low grade lesions are also included in the definition of a positive smear, this connection disappears.

Boon [1993] also reports that the progressive nature of squamous cell lesions increases the closer they are to the metasquamo-columnar junction and therefore also pleads for double endocervical/exocervical sampling. Histological examination of conisation material and colposcopic observations confirm this latest thesis [Burghardt, 1998].

The exclusive presence of columnar cells (with no squamous cells) indicates a non-representative smear.
AANVRAAGFORMULIER VOOR CERVICO-VAGINALE CYTOLOGIE

Identificatie patiënt
Naam en voornaam: ............................................................................................................................................................................................
Adres: ........................................................................................................................................................................................................................................
Geboortedatum: ..................................................................................................................................................................................................................
Mutualiteitsgegevens: ...........................................................................................................................................................................................................

Datum afname staal: ______________________ Oorsprong staal

<table>
<thead>
<tr>
<th>€ cervix</th>
</tr>
</thead>
<tbody>
<tr>
<td>€ vagina</td>
</tr>
</tbody>
</table>

Gynaecologische status
Datum laatste menses: ______________________

- £ Anticonceptie
  - € pil: ......................................................
  - € spiraal
  - € andere: ..................................................
- £ Zwanger ... weken
  - € Postpartum
  - € Menopauzaal, beginjaar: .......
  - € Substitutie  € nee
  - € ja

Reden tot onderzoek
- £ Georganiseerde screening
- £ Opportunistische screening
- £ Klacht of klinisch letsel: ..............................................................
- £ Follow-up onderzoek
  - reden: ..........................................................

Gynaecologische ingrepen
- £ Hysterectomie
  - € cervix aanwezig
  - € cervix afwezig
- £ Conisatie, lusexcisie of analoge ingrepen
- £ Andere:

Afname-instrument
- £ Gecombineerde spatel
- £ Spatel en Cytobrush®
- £ Cervex-Brush®
- £ Andere: (preciseer) ..................................................

Gevolgde therapie
- £ Chemotherapie
- £ Pelvische radiotherapie
- £ Anti-oestrogenen

Andere relevante inlichtingen uit de anamnese of het klinisch onderzoek
........................................................................................................................................................................................................
........................................................................................................................................................................................................
........................................................................................................................................................................................................

Handtekening, datum en stempel van de aanvragende arts

Kopij verslag aan Dr.  ..........................................................................................................................
Annex 4

Summary: Collection of an adequate Papanicolaou smear of the uterine cervix

Importance and objective of the guideline on sampling
The correct sampling of a cervical smear with appropriate equipment and in the approved way contributes to a significant extent to the diagnostic value of the screening test. A smear taken unsatisfactorily is a significant cause of false negative and false positive results.

Below is a concise summary of “A technical guideline: collection of adequate Pap smears of the uterine cervix” compiled by the technical Sampling Working Group. The aim is to propagate the consensus amongst all general practitioners, gynaecologists, pathologists and other healthcare workers involved in the detection of cervical cancer in Flanders.

Indicators of the quality of a smear
The cytological precursors of cervical cancers arise mainly in the transformation zone (TZ) between the multilayer squamous epithelium and the endocervical columnar epithelium. Therefore, it is important that cell material be sampled primarily from this zone. An optimum smear contains metaplastic and columnar endocervical cells as well as squamous cells. The presence of significant quantities of blood cells or inflammatory cells can reduce the quality of the smear. The cellular material must be spread over the slide in a homogeneous thin layer. The specimen must be fixed as quickly as possible. Quality evaluation of the smear (satisfactory, satisfactory but limited and unsatisfactory) is an essential component of the cytology report.

Contra-indications for screening smears
Total hysterectomy, cervical amputation, and the presence of a suspect, macroscopically visible lesion in the area of the cervix are contra-indications for screening. In the latter case, the woman must be referred for colposcopic examination and/or biopsy.

Factors adversely affecting the quality of a smear
The quality of a smear is adversely affected by:
- menstruation, blood loss, breakthrough bleeding
- vaginal inflammation/infection
- severe genital atrophy (menopause)
- pregnancy and lactation
- physical manipulation or chemical irritation such as: preceding digital vaginal examination, disinfectant cream or liquid, lubricating jelly, vaginal medication (less than 48 hours before), vaginal douche (less than 24 hours before), prior colposcopy with acetic acid (less than 24 hours before), previous smear (less than 3 months before), cervical surgery (less than 3 months before)
- radiotherapy

It is essential to recognise these factors and reduce their effect to a minimum. Relevant clinical information must be recorded on the request form.

Information to the woman
The woman is informed of the aim of the smear and its procedure. She is informed that sometimes the examination has to be repeated within 3 to 6 months, if the smear was not of satisfactory quality. The doctor makes a clear arrangement how the woman will be notified of the laboratory result.
Preparation of the smear

- The equipment required for the smear is laid out ready in advance: specula (various sizes), gloves, microscope slides, pencil, tampon tongs plus swabs, atraumatic forceps, sampling devices: spatula + endocervical brush (Cytobrush® or Cervex-Brush®), fixative spray or alcohol solution, request form.
  - The doctor should take special care to keep the interval between taking the smear and fixing it as short as possible. So: remove top from aerosol can, check that the can is not blocked or empty.
- The slide is labelled on the frosted end in lead pencil with the name or reference number.
- The speculum is inserted preferably without any lubricating jelly and the cervix is visualised as clearly as possible. If there is extreme retro or anteversion of the uterus, the cervix can be manipulated into a good position with a pair of closed atraumatic forceps. The position and aspect of the transformation zone is checked visually. Contact with the portio should be avoided as far as is possible. Excessive mucus, discharge or blood can be removed carefully with a swab (not wiped away).

Number of specimens

A single specimen is usually sufficient for a smear. The exocervical and endocervical material can be spread over a single slide.

Sampling devices

Cervical screening always requires an endocervical and an exocervical sample, taken with the appropriate instruments.

Two methods are recommended as the best choice:
- Cervex-Brush® or endocervical brush (Fig. 1c)
- Combination of a spatula (Figure 1a) for the exocervical sample and the Cytobrush® (Figure 1b) for the endocervical sample.
  - The wooden spatula (Figure 1a) with an Ayre end and Aylesbury end (with elongated tip) is best.
The Cervex-Brush is best if the woman is pregnant or has a cervix that bleeds easily. The combination method is best if the squamocolumnar junction is high (often post menopausal), after cervical surgery or if there is extensive ectropion of the columnar epithelium.

**Technique for taking the smear**

1. **Cervex-Brush**

Endocervical cells and exocervical cells are sampled simultaneously - the long bristles pick up endocervical cells while the short bristles collect exocervical cells.
- The long bristles are positioned endocervically.
- With gentle pressure the brush is turned five times 360° by rolling the handle clockwise between thumb and forefinger.
- The sample is spread onto the slide with a painting action, using both sides of the brush.

- The specimen is fixed immediately by being sprayed at right angles from a distance of 20 cm. If closer, the cells are blown away or frozen, if on a slant, the material is blown into aggregates. Droplet formation should be avoided: so do not use too much fixative. A very fast fixation, within a few seconds, is essential to prevent drying artefacts.
2. Combination of spatula and Cytobrush®

- Take the spatula. The end of the spatula should be used that is most appropriate to the anatomy of the portio. For nullipara, this is usually the Aylesbury end, for multipara the broader Ayre end.
- The spatula is then rotated 360° under slight pressure with the point at the level of the ostium. The hand-hold will need to be changed at least once.
- The tip scrapes the ostium while the less protruding part scrapes the surface of the portio. Special care should be taken to scrape the squamocolumnar junction as fully as possible. If there is extensive ectropion, the outer part of the portio should be scraped separately.
- After the sample has been taken, the spatula is put aside with the specimen face-up. The danger of drying out is less if the cell material and mucus remain in contact with the sampling device. Only after the Cytobrush® has been used the material is spread onto the slide.

![Diagram of spatula usage](image1)

- Take the Cytobrush®. Insert it for two thirds into the endocervical canal and rotate gently 90 to 180°.
- The Cytobrush® is immediately rolled (not wiped) over the outer third of the microscope slide.
- The spatula is then streaked as quickly as possible onto the central third.
- The rolling and wiping should be done in one movement (not in a zigzag) and without pressure, so that an even thin smear is obtained.

![Diagram of Cytobrush usage](image2)

- If a double sample is taken, spread over two slides (rarely necessary), a primary fixation is done immediately after the spatula sample has been taken, before proceeding to taking the endocervical sample.
Transport to the cytology laboratory
After fixation, the specimen is allowed to dry completely. Then it is put into a cardboard or plastic holder for transport to the laboratory. A wet specimen can stick at the edges if it is put into a plastic folder. The holder is labelled with identification details matching those on the request form.

Standard request form for cytology laboratory
Clinical details are important for accurate interpretation of a smear. The standard form (see Annex 1) is filled in as fully as possible. The identification details required for recording the cytological data must not be missing. It is indicated whether the result of the examination should be sent to a different doctor.

Feedback on the quality evaluation of the specimen
The cytological report on the smear, evaluated according to the guidelines of the Working Group on Uniformity in Cervix Cytology (WUCC) always includes an assessment of the quality of the smear. Every doctor periodically receives a summary of the quality evaluations of the smears he/she has taken compared with general distributions. This feedback, provided by the laboratory or a central register, should help to improve the average quality of smears.
Annex 5

Comparison of the quality of cervical smears obtained with the spatula & Cytobrush* versus the Cervex-Brush*

In order to study the differences in smear quality obtained with the two methods a review of the cytological literature was undertaken. The following outcome measures were studied: ratios of detection rates of intraepithelial lesions and the presence of endocervical cylindrical/metaplastic squamous cells in Papanicoloau smears obtained with the considered sampling methods. Crude relative risks for individual studies and weighted relative risks for the pooled studies were calculated applying the same procedure as in Buntinx’ meta-analysis [1996].

1. Combination spatula with extended tip & Cytobrush* versus Cervex-Brush*

1. A. Detection rate of cervical lesions (Table 1)

1A1. Low SIL:
- No separate study showing any significant association.
- Crude pooled analysis: significantly more lesions found with the combination method: RR=1.24 (CI=1.09-1.41).
- Weighted pooled analysis: the contrary is observed: Mantel-Haenszel RR =0.94 (CI=0.83-1.07). Nevertheless: the adjusted association is not significant.

1A2. High SIL:
- No separate study showing any significant association.
- Crude pooled analysis: no significant relation RR=0.80 (CI=0.58 -1.09).
- Weighted pooled analysis: no significant relation: Mantel-Haenszel RR =1.01 (CI=0.73-1.38).

1. B. Presence of endocervical cells/transformation zone component (Table 2)

1B1. Presence of endocervical cells (EC):
- 2 separate studies (Hutchinson 92 & Svarevski 93) showing a significant association: more EC found with the combination method.
- Crude pooled analysis: significantly more EC found with the combination method: RR=1.08 (CI=1.07-1.08).
- Weighted pooled analysis: same result: RR =1.07 (CI=1.06-1.08).

1B2. Presence of metaplastic squamous cells (MSC):
- 1 separate study (Järvi 97) showing significantly more MSC with the Cervex Brush*.
- Crude pooled analysis: significant relation RR=0.90 (CI=0.85-0.95).
- Weighted pooled analysis: same result: Mantel-Haenszel RR =0.90 (CI=0.84-0.94).

1B3. Presence of metaplastic squamous/endocervical cells (MSC/EC):
- No separate study showing any significant relation.
- Crude pooled analysis: not significant relation RR=1.01 (CI=0.98-1.03).
- Weighted pooled analysis: same result: Mantel-Haenszel RR =1.01 (CI=0.98-1.03).
2. Combination spatula & Cytobrush\textsuperscript{a} versus Cervex-Brush\textsuperscript{a}

2. A. Detection rate of cervical lesions (Table 4)

2A1. ASCUS and low SIL:
- 1 study showing significantly more l-SIL and ASCUS with combination (Williamson 98).
- Crude pooled analysis: significantly more l-SIL lesions found with the combination method: RR=1.13 (CI=1.03-1.24). Especially due to one study.
- Weighted pooled analysis: same result.

2A2. High SIL:
- No separate study showing any significant association.
- Crude pooled analysis: more h-SIL found with the combination, but not significant relation RR=1.18 (CI=0.93-1.51).
- Weighted pooled analysis: idem.

2. B. Presence of endocervical cells/transformation zone component (Table 5)

2B1. Presence of endocervical cells (EC):
- 1 separate study (Neinstein 92) showing a significant association: more EC found with the combination method.
- Crude pooled analysis: significantly more EC found with the combination method: RR=1.10 (CI=1.04-1.16).
- Weighted pooled analysis: same result: RR =1.06 (CI=1.00-1.06).

2B2. Presence of metaplastic squamous cells (MSC):
- No studies.

2B3. Presence of metaplastic squamous/endocervical cells (MSC/EC):
- 2 studies (Szarevski 90 & 91) showing more MSC/EC with the combination.
- Crude pooled analysis: significantly more MSC/EC with the combination RR=1.04 (CI=1.03-1.06).
- Weighted pooled analysis: idem.

2. C. Quality judgement of the smear (Table 6)

- Contradictory results: 2 studies (Fokke 93, Williamson 98) show more inadequate smears with the combination (1st not significant); 2 other studies (Szarevski 90 & 91) show more inadequate smears with the Cervex-Brush\textsuperscript{a}.
- Pooled analysis: the cervex smears have significantly less inadequate (adj RR=0.74; CI:0.68-0.81).

2. D. Sensitivity, specificity (compared with histology) (Table 7)

Risberg found higher specificity and comparable sensitivity for the cervex, resulting in higher positive predictive value and positive likelihood ratio.
Conclusion:

More low SIL or ASCUS and smears with EC found with the combination method. Differences are not spectacular. More smears found with metaplastic cells with the Cervex-Brush®. The Cervex-Brush® provides also less inadequate smears and is a little bit more specific.

I propose that the Cervex-Brush® should be considered as almost equivalent to the combination methods.

Comfort: Cervex-Brush® offers more comfort, sampling is somehow quicker, less risk for fixation problems.

Price of the Cervex-Brush® comparable to the combination (9-10 BEF).

One first choice cannot be recommended. It is to the lab or clinician to choose.

Marc Arbyn
Table 1: detection of cervical lesions in smears obtained with Cytobrush & spatula with extented tip (1) versus Cervex (2)

=> RR<1 means cervex is more effective
=> RR>1 means combination is more effective

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Ref</th>
<th>Outcome</th>
<th>+ve</th>
<th>-ve</th>
<th>% +ve</th>
<th>+ve</th>
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<th>CI up</th>
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<td>Acta Cytol</td>
<td>Mild</td>
<td>29</td>
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<td>14</td>
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<td>4</td>
<td>2.623</td>
<td>0.2%</td>
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<td>195</td>
<td>8.6%</td>
<td>21</td>
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<td>Mild</td>
<td>26</td>
<td>146</td>
<td>15.0%</td>
<td>35</td>
<td>132</td>
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<td>Acta Cytol</td>
<td>Mild</td>
<td>312</td>
<td>3.029</td>
<td>9.3%</td>
<td>380</td>
<td>3.448</td>
<td>9.9%</td>
<td>0.94</td>
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<tr>
<td>Szarewski, '93</td>
<td>Acta Cytol</td>
<td>Severe</td>
<td>64</td>
<td>3.277</td>
<td>1.9%</td>
<td>75</td>
<td>3.753</td>
<td>2.0%</td>
<td>0.98</td>
<td>0.7</td>
<td>1.36</td>
</tr>
<tr>
<td>McCord, '92</td>
<td>Am J Obst Gyn</td>
<td>Mild</td>
<td>29</td>
<td>472</td>
<td>5.8%</td>
<td>31</td>
<td>474</td>
<td>6.1%</td>
<td>0.94</td>
<td>0.58</td>
<td>1.54</td>
</tr>
<tr>
<td>Järvi, '97</td>
<td>Cytopathol</td>
<td>Mild</td>
<td>2</td>
<td>1.062</td>
<td>0.2%</td>
<td>4</td>
<td>955</td>
<td>0.4%</td>
<td>0.45</td>
<td>0.08</td>
<td>2.45</td>
</tr>
</tbody>
</table>

**Design**
- pop screening
- experienced sm takers
- randomised screening
- high risk population FU smears
- randomised
- fam plan, mostly young women
- FU smears
- outpat clinic, preg n-pregn women
- day gynecol,GP, technicians

**Setting**
- experienced sm takers
- 2 experienced clinicians
- FU smears
- experienced sm takers
- FU smears
- FU smears
- FU smears

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Ref</th>
<th>Outcome</th>
<th>+ve</th>
<th>-ve</th>
<th>% +ve</th>
<th>+ve</th>
<th>-ve</th>
<th>% +ve</th>
<th>RR</th>
<th>CI low</th>
<th>CI up</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL mild</td>
<td></td>
<td></td>
<td>416</td>
<td>9.017</td>
<td>4.4%</td>
<td>485</td>
<td>7.854</td>
<td>5.8%</td>
<td>0.76</td>
<td>0.67</td>
<td>0.86</td>
</tr>
<tr>
<td>TOTAL severe</td>
<td></td>
<td></td>
<td>73</td>
<td>7.410</td>
<td>1.0%</td>
<td>79</td>
<td>6.376</td>
<td>1.2%</td>
<td>0.80</td>
<td>0.58</td>
<td>1.09</td>
</tr>
</tbody>
</table>

**M-H RR**

| TOTAL mild   |     |         |    |       |       | 0.93 | 0.82 | 1.06 |
| TOTAL severe |     |         |    |       |       | 1.01 | 0.73 | 1.38 |
Table 2: detection of a TZ/EC component in smears obtained with Cytobrush & spatula with extended tip (1) versus Cervex (2)

=> RR<1 means cervex-sample contains more EC
=> RR>1 means combination-sample contains more EC

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Ref</th>
<th>Outcome</th>
<th>EC+</th>
<th>EC-</th>
<th>%+</th>
<th>RR</th>
<th>CI low</th>
<th>CI up</th>
</tr>
</thead>
<tbody>
<tr>
<td>McCord, '92</td>
<td>Am J Obst Gyn</td>
<td>EC</td>
<td>465</td>
<td>36</td>
<td>92,8%</td>
<td>454</td>
<td>51</td>
<td>89,9%</td>
</tr>
<tr>
<td>Hutchinson, '91</td>
<td>J Repr Med</td>
<td>EC</td>
<td>247</td>
<td>12</td>
<td>95,3%</td>
<td>251</td>
<td>57</td>
<td>81,6%</td>
</tr>
<tr>
<td>Hutchinson, '91</td>
<td>J Repr Med</td>
<td>MSC</td>
<td>129</td>
<td>130</td>
<td>50,0%</td>
<td>166</td>
<td>142</td>
<td>53,9%</td>
</tr>
<tr>
<td>Szarewski, '93</td>
<td>Acta Cytol</td>
<td>EC</td>
<td>3.097</td>
<td>244</td>
<td>92,7%</td>
<td>3.202</td>
<td>626</td>
<td>83,6%</td>
</tr>
<tr>
<td>Järvi, '97</td>
<td>Cytopathol</td>
<td>EC</td>
<td>714</td>
<td>30</td>
<td>96,0%</td>
<td>640</td>
<td>31</td>
<td>95,4%</td>
</tr>
<tr>
<td>Järvi, '97</td>
<td>Cytopathol</td>
<td>EC</td>
<td>270</td>
<td>48</td>
<td>84,9%</td>
<td>230</td>
<td>60</td>
<td>79,3%</td>
</tr>
<tr>
<td>Järvi, '97</td>
<td>Cytopathol</td>
<td>MSC</td>
<td>490</td>
<td>254</td>
<td>65,9%</td>
<td>518</td>
<td>153</td>
<td>77,2%</td>
</tr>
<tr>
<td>Järvi, '97</td>
<td>Cytopathol</td>
<td>MSC</td>
<td>192</td>
<td>126</td>
<td>60,4%</td>
<td>185</td>
<td>105</td>
<td>63,8%</td>
</tr>
<tr>
<td>Järvi, '97</td>
<td>Cytopathol</td>
<td>EC/MSC</td>
<td>717</td>
<td>27</td>
<td>96,4%</td>
<td>648</td>
<td>23</td>
<td>96,6%</td>
</tr>
<tr>
<td>Järvi, '97</td>
<td>Cytopathol</td>
<td>EC/MSC</td>
<td>274</td>
<td>44</td>
<td>86,2%</td>
<td>241</td>
<td>49</td>
<td>83,1%</td>
</tr>
<tr>
<td>Boon, '89</td>
<td>Acta Cytol</td>
<td>EC present</td>
<td>4.110</td>
<td>32</td>
<td>99,2%</td>
<td>2.512</td>
<td>402</td>
<td>95,7%</td>
</tr>
<tr>
<td>TOT EC present</td>
<td></td>
<td></td>
<td>8.903</td>
<td>402</td>
<td>95,7%</td>
<td>7.289</td>
<td>940</td>
<td>88,6%</td>
</tr>
<tr>
<td>TOT Metapl present</td>
<td></td>
<td></td>
<td>811</td>
<td>509</td>
<td>61,5%</td>
<td>869</td>
<td>400</td>
<td>68,5%</td>
</tr>
<tr>
<td>TOT EC/metapl present</td>
<td></td>
<td></td>
<td>991</td>
<td>71</td>
<td>93,3%</td>
<td>889</td>
<td>72</td>
<td>92,5%</td>
</tr>
</tbody>
</table>

Design: randomised
Setting: outpat clinic, pregn & n-pregn women

Design: randomised/period
Setting: fam plan, mostly young women experienced sm takers

Design: randomised/day
Setting: gynecol, GP, technicians <50 y

Design: randomised/day
Setting: gynecol, GP, technicians >=50 y
Table 3: distribution of quality judgments of smears obtained with Cytobrush & spatula with extended tip (1) versus Cervex (2)

=> RR<1 means cervex-sample less inadequate
=> RR>1 means combination-sample less inadequate

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Ref</th>
<th>Outcome</th>
<th>inad</th>
<th>n</th>
<th>inad %</th>
<th>inad</th>
<th>n</th>
<th>inad %</th>
<th>RR</th>
<th>Cl low</th>
<th>Cl up</th>
<th>Design</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Szarewski, '93</td>
<td>Acta Cytol</td>
<td>% inad</td>
<td>79</td>
<td>3.262</td>
<td>2.4%</td>
<td>119</td>
<td>3.709</td>
<td>3.1%</td>
<td>1.31</td>
<td>0.99</td>
<td>1.74</td>
<td>randomised/period</td>
<td>GP practice, mostly screening, sometimes clinic+</td>
</tr>
</tbody>
</table>
Table 4: detection of cervical lesions in smears obtained with Cytobrush & spatula (1) versus Cervex (2)

`=> RR<1 means cervex is more effective
`=> RR>1 means combination is more effective`

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Journal</th>
<th>Outcome</th>
<th>combination</th>
<th>cervex</th>
<th>RR</th>
<th>CI low</th>
<th>CI up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Fokke, '93   | Eu J Obst Gyn & Repr Biol | Mild dyspl. | 5 | 87 | 5,4% | 8 | 82 | 8,9% | 0,61 | 0,21 | 1,8 |
| Szarewski, '90 | Genitourin Med           | Mild dyspl. | 400 | 1.369 | 22,6% | 401 | 1.400 | 22,3% | 1,02 | 0,9 | 1,15 |
| Szarewski, '91 | Acta Cytol               | LSIL     | 23 | 358 | 6,0% | 30 | 391 | 7,1% | 0,85 | 0,5 | 1,43 |
| Szarewski, '91 | Acta Cytol               | HSIL     | 9  | 372 | 2,4% | 9  | 412 | 2,1% | 1,10 | 0,44 | 2,75 |
| Williamson, '97 | Cytopathol             | ASCUS    | 1,165 | 13.034 | 8,2% | 992 | 13.554 | 6,8% | 1,20 | 1,11 | 1,31 |
| Williamson, '97 | Cytopathol             | LSIL     | 409 | 13.790 | 2,9% | 321 | 14.225 | 2,2% | 1,31 | 1,13 | 1,51 |
| Williamson, '97 | Cytopathol             | HSIL     | 129 | 14.070 | 0,9% | 111 | 14.435 | 0,8% | 1,19 | 0,92 | 1,53 |
| TOTAL ASCUS  |                          |         |             |        |      | 1,165 | 13.034 | 8,2% | 1,20 | 1,11 | 1,31 |
| TOTAL LSIL   |                          |         |             |        |      | 837 | 15.604 | 5,1% | 1,13 | 1,03 | 1,24 |
| TOTAL hSIL   |                          |         |             |        |      | 138 | 14.442 | 0,9% | 1,18 | 0,93 | 1,51 |
Table 5: detection of a TZ/EC component in smears obtained with Cytobrush & spatula (1) versus Cervex (2)

=> RR<1 means cervex-sample contains more EC
=> RR>1 means combination-sample contains more EC

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Journal</th>
<th>Outcome</th>
<th>combination EC+</th>
<th>EC-</th>
<th>%+</th>
<th>cervex EC+</th>
<th>EC-</th>
<th>%+</th>
<th>RR</th>
<th>CI low</th>
<th>CI up</th>
<th>Design</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neinstein, '92</td>
<td>J Ad Health</td>
<td>EC</td>
<td>77</td>
<td>7</td>
<td>91,7%</td>
<td>59</td>
<td>22</td>
<td>72,8%</td>
<td>1,26</td>
<td>1,09</td>
<td>1,46</td>
<td>randomised</td>
<td>Adolescent clinic</td>
</tr>
<tr>
<td>Risberg, '97</td>
<td>J Reprod Med</td>
<td>EC</td>
<td>77</td>
<td>19</td>
<td>80,2%</td>
<td>84</td>
<td>33</td>
<td>71,8%</td>
<td>1,12</td>
<td>0,96</td>
<td>1,3</td>
<td>randomised</td>
<td>Referred cases for biopsy</td>
</tr>
<tr>
<td>Cannon, '93</td>
<td>Obstet Gynecol</td>
<td>EC</td>
<td>115</td>
<td>17</td>
<td>87,1%</td>
<td>144</td>
<td>33</td>
<td>81,4%</td>
<td>1,07</td>
<td>0,97</td>
<td>1,18</td>
<td>randomised</td>
<td>clinic gyneco/obstet (&gt;1/3 pregnant women)</td>
</tr>
<tr>
<td>Cannon, '93</td>
<td>Obstet Gynecol</td>
<td>EC &gt;=mod</td>
<td>86</td>
<td>46</td>
<td>65,2%</td>
<td>107</td>
<td>70</td>
<td>60,5%</td>
<td>1,08</td>
<td>0,91</td>
<td>1,28</td>
<td>randomised</td>
<td>pregnant women (part of women in previous records)</td>
</tr>
<tr>
<td>Cannon, '93</td>
<td>Obstet Gynecol</td>
<td>EC</td>
<td>43</td>
<td>3</td>
<td>93,5%</td>
<td>58</td>
<td>9</td>
<td>86,6%</td>
<td>1,08</td>
<td>0,96</td>
<td>1,22</td>
<td>randomised</td>
<td>clinic gyneco/obstet (&gt;1/3 pregnant women)</td>
</tr>
<tr>
<td>Kavak, '95</td>
<td>Au NZ J Obst Gyn</td>
<td>EC</td>
<td>80</td>
<td>31</td>
<td>72,1%</td>
<td>88</td>
<td>22</td>
<td>80,0%</td>
<td>0,90</td>
<td>0,76</td>
<td>1,01</td>
<td>randomised</td>
<td>pregnant women (part of women in previous records)</td>
</tr>
<tr>
<td>Kavak, '95</td>
<td>Au NZ J Obst Gyn</td>
<td>EC &gt;=mod</td>
<td>57</td>
<td>54</td>
<td>51,4%</td>
<td>73</td>
<td>37</td>
<td>66,4%</td>
<td>0,77</td>
<td>0,62</td>
<td>0,97</td>
<td>randomised</td>
<td>clinic gyneco/obstet (&gt;1/3 pregnant women)</td>
</tr>
<tr>
<td>Szarewski, '90</td>
<td>Genitourin Med</td>
<td>EC/MSC</td>
<td>1.672</td>
<td>97</td>
<td>94,5%</td>
<td>1.639</td>
<td>162</td>
<td>91,0%</td>
<td>1,04</td>
<td>1,02</td>
<td>1,06</td>
<td>alternated/ period</td>
<td>mostly young wom genitourin clinic</td>
</tr>
<tr>
<td>Szarewski, '90</td>
<td>Acta Cytol</td>
<td>EC/MSC</td>
<td>360</td>
<td>21</td>
<td>94,5%</td>
<td>374</td>
<td>47</td>
<td>88,8%</td>
<td>1,06</td>
<td>1,02</td>
<td>1,11</td>
<td>alternated/ period</td>
<td>FU after laser trea exp takers</td>
</tr>
<tr>
<td>Fokke, '93</td>
<td>Eu J Obst Gyn &amp; Repr Biol</td>
<td>EC</td>
<td>82</td>
<td>10</td>
<td>89,1%</td>
<td>77</td>
<td>13</td>
<td>85,6%</td>
<td>1,04</td>
<td>0,93</td>
<td>1,16</td>
<td>randomised</td>
<td>Sexual health service clinic</td>
</tr>
<tr>
<td>Sparrow, '97</td>
<td>NZ Med J</td>
<td>EC</td>
<td>95</td>
<td>2</td>
<td>97,9%</td>
<td>76</td>
<td>5</td>
<td>93,8%</td>
<td>1,04</td>
<td>0,98</td>
<td>1,11</td>
<td>randomised</td>
<td>Sexual health service clinic</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>crude RR</th>
<th>CI low</th>
<th>CI low</th>
<th>M-H RR</th>
<th>CI low</th>
<th>CI low</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOT EC present</td>
<td>569</td>
<td>96</td>
<td>85,6%</td>
<td>586</td>
<td>169</td>
<td>77,6%</td>
<td>1,10</td>
<td>1,04</td>
</tr>
<tr>
<td>TOT EC&gt;=moderate</td>
<td>180</td>
<td>109</td>
<td>62,3%</td>
<td>230</td>
<td>124</td>
<td>65,0%</td>
<td>0,96</td>
<td>0,85</td>
</tr>
<tr>
<td>TOT EC/metapl present</td>
<td>2.032</td>
<td>118</td>
<td>94,5%</td>
<td>2.013</td>
<td>209</td>
<td>90,6%</td>
<td>1,04</td>
<td>1,03</td>
</tr>
</tbody>
</table>
Table 6: distribution of quality judgments of smears obtained with Cytobrush & spatula (1) versus Cervex (2)

=> RR<1 means cervex-sample less inadequate
=> RR>1 means combination-sample less inadequate

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Journal</th>
<th>Outcome</th>
<th>combination</th>
<th>cervex</th>
<th>RR</th>
<th>CI low</th>
<th>CI up</th>
<th>Design</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Williamsom, '98</td>
<td>Acta Cytol</td>
<td>% inad</td>
<td>1.154</td>
<td>13.045</td>
<td>8,1%</td>
<td>827</td>
<td>13.719</td>
<td>5,7%</td>
<td>0,70</td>
</tr>
<tr>
<td>Szarewski, '90</td>
<td>Genitourin Med</td>
<td>% inad</td>
<td>35</td>
<td>1.734</td>
<td>2,0%</td>
<td>62</td>
<td>1.739</td>
<td>3,4%</td>
<td>1,74</td>
</tr>
<tr>
<td>Szarewski, '91</td>
<td>Acta Cytol</td>
<td>% inad</td>
<td>1</td>
<td>380</td>
<td>0,3%</td>
<td>17</td>
<td>404</td>
<td>4,0%</td>
<td>15,38</td>
</tr>
<tr>
<td>Fokke, '93</td>
<td>Eu J Obst Gyn &amp; Repr Biol</td>
<td>% poor</td>
<td>6</td>
<td>86</td>
<td>6,5%</td>
<td>2</td>
<td>88</td>
<td>2,2%</td>
<td>0,34</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TOTAL POOR/INAD</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>combination</td>
<td>cervex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.196</td>
<td>15.245</td>
<td>7,3%</td>
<td>908</td>
<td>15.950</td>
<td>5,4%</td>
<td>1,35</td>
<td>0,21</td>
<td>1,80</td>
</tr>
</tbody>
</table>

Table 7: sensitivity, specificity, positive predictive value and positive likelihood ratio of smears obtained with Cytobrush & spatula (1) versus Cervex (2) for the detection of high grade SIL as compared with histology

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Journal</th>
<th>Outcome</th>
<th>Cyt+His+</th>
<th>Cyt+His-</th>
<th>Cyt+Hist-</th>
<th>Cyt+Hist-</th>
<th>TOT</th>
<th>SE</th>
<th>SP</th>
<th>PPV</th>
<th>PLR</th>
<th>Design</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risberg, '97</td>
<td>J Reprod Med</td>
<td>HSIL</td>
<td>46</td>
<td>28</td>
<td>2</td>
<td>41</td>
<td>117</td>
<td>62,2%</td>
<td>95,3%</td>
<td>95,8%</td>
<td>13,4</td>
<td>randomised</td>
<td>Referred cases for biopsy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>21</td>
<td>3</td>
<td>37</td>
<td>96</td>
<td>62,5%</td>
<td>92,5%</td>
<td>92,1%</td>
<td>0,3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p for H0: cervex & spat/cyt.brush smears equally valid 0,97 0,59 0,46
Annex 6.

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Annex 7

List of used abbreviations

AGUS atypical glandular cells of unspecified significance
ASCUS atypical squamous cells of unspecified significance
EC endocervical
HPV Human Papillomavirus
H-SIL High grade squamous intraepithelial lesion
L-SIL Low grade squamous intraepithelial lesion
MSC Metaplastic squamous cells
RR Relative risk
TZ transformation zone
SCJ squamo-columnaire junction
VACK Vlaamse Advies-Commissie voor Kankerpreventie
WIV Wetenschappelijk Instituut voor Volksgezondheid
WUCC Werkgroep voor Uniformisatie van Cervix-Cytologie