

## REFERENCES

1. Parkin D.M., Pisani P. and Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int J Cancer*: 54: 594-606, 1993.
2. Ferlay J., Black R.J., Pisani P., Valdivieso M.T. and Parkin D.M. EUCAN 90: Cancer in the European Union. IARC Cancer Base n. 1. Internat. Agency for Research on Cancer, Lyon, 1996.
3. Muñoz N., Bosch F.X. The causal link between HPV and cervical cancer and its implications for prevention of cervical cancer. *Bull Pan. Am. Health Organ.* 30: 362-77, 1996.
4. Hakama M., Miller A.B., Day N.E., Eds. Screening for Cancer of the Uterine Cervix (IARC Scientific Publication n. 76), Lyon, 185-195; 1986.
5. Nieminen P., Kallio M., Hakama M. The effect of mass screening on incidence and mortality of squamous and adenocarcinoma of cervix utery. *Obst.Gyn.* 85: 1017-1021, 1995.
6. Advisory Committee on Cancer Prevention. Position Paper. Recommendations on cancer screening in the European Union. *Eur. J. Cancer* 36:1473-1478, 2000.
7. Chamberlain J. Reasons that some screening programmes fail to control cervical cancer. In: Hakama M., Miller A.B., Day N.E., Eds. Screening for Cancer of the Uterine Cervix (IARC Scientific Publication n. 76). Lyon, International Agency for Research on Cancer, pp. 161-168, 1986.
8. Moller Jensen O., Estève J., Moller H. and Renard H.: Cancer in the European Community and its Member States. *Eur. J. Cancer*, vol. 26: 1167-1256, 1990.
9. Morosini P.L., Lauriola P., Magliola E., (Italian Collaborators): European Community Atlas of Avoidable Death. W.W. Holland Ed. Oxford University Press, pp. 349, 1991.
10. Papanicolaou G.N., Traut H.F. The diagnostic value of vaginal smears in carcinoma of the uterus. *Am. J. Abst. Gynecol.* 42: 193-206, 1941.
11. Herrero R. Epidemiology of Cervical Cancer. *Journal of the National Cancer Institute Monographs.* 21, 1996.
12. Branca M., Bonelli L., Rossi E., Gustavino C., Ferreri M., Riti M. G., Giovagnoli M.R., Midulla C., Carraro C., Vecchione A. Il Pap Test: modalità di esecuzione del prelievo cervico vaginale. Manuale elaborato nell'ambito del progetto CNR/ACRO, Istituto Superiore di Sanità, Roma, July, 1994.
13. Rutala W.A. APIC guidelines for selection and use of disinfectants. *Am. J. Infection Control.* 18: 99-117, 1990.
14. Centers for Disease Control. Guidelines for Handwashing and Hospital Environmental Control. *Am J Infect Control* 14: 110-129, 1986.
15. FDA Department of Health and Human Services: Medical devices; patient examination and surgeon's gloves; adulteration; proposed rule. *Federal Register* 54: n. 223, 1989.
16. Posner T., Vessey M. Prevention of Cervical Cancer: The Patient's View., King Edward's Hospital Fund for London; 1988.
17. Austoker J. Cancer Prevention in Primary Care. Screening for cervical cancer. *BMJ* 309, 241-247, 1994.
18. Orr JW. et al. The efficacy and safety of the cytobrush during pregnancy. *Gynaecol. Oncol.* 1992 Mar; 44(3):260-2.
19. Buntinx F., Boon M.E., Beck S., Knottnerus J.A., et al. Comparison of cytobrush sampling spatula and combined cytobrush-spatula sampling of the uterine cervix. *Acta Cytol.* 35: 64-68, 1991.
20. Cecchini S., Bonardi L. e Ciatto S. Comparing methods of cervical smear sampling. *Acta Cytol.* 35: 659-60, 1991.
21. Coleman D., Day N., Douglas G., Farney E., Lynge E., Philip J., Segnan N. European Guidelines for Quality Assurance in Cervical Cancer Screening. *Europ. J. Cancer* 29/A, Suppl. 4, S1-S31; 1993.
22. Koss L. Letters to the editors replies to questions on quality assurance measures in cytopathology editorial: Quality assurance measures in cytopathology. *Acta Cytol.* 32: 923-939, 1988.
23. Husain O.A.N., Butler E.B., Evans D.M.B., McGregor J.E., Yule R. Quality control in cervical cytology. *J. Clin. Pathol.* 27: 935-944, 1974.
24. Melamed M.R. Quality control in the cytology laboratory. *Acta Cytol.* 20, 203-206, 1976.
25. Klinkhamer P.J.J.M., Vooijs G.P., Haan A.F.J. Intraobserver and interobserver Variability in the diagnosis of epithelial abnormalities in cervical smears. *Acta Cytol.* 32: 794-800, 1988.
26. Koss L.G. The Papanicolaou Test for Cervical Cancer Detection: A Triumph and a Tragedy. *Jama*, 261: 737-743, 1989.
27. Palli D., Confortini M., Biggeri A., Russo A., Cariaggi P. A quality control system involving peer review of abnormal cervical smears. *Cytopathology* 4: 17-25, 1993.
28. Gifford C., Coleman D.V. Quality Assurance in Cervical Cancer Screening: results of a proficiency testing scheme for cytology laboratories in the North West Thames Region. *Cytopathology* 5: 197-206,

# THE PAP TEST PROCEDURE



*The screening test for preinvasive and early invasive cervical cancer - the PAP test - is a simple, sensitive, non invasive and low cost method of preventing this disease providing the test is correctly performed. In this booklet we provide guidelines for sampling, processing and analysis of the sample so that smears are of optimal quality and women can be reliably informed of the results of their test. The booklets also recommends screening protocols for women who are most at risk of cervical cancer and some advice on management.*

*The handbook "THE PAP TEST PROCEDURE" has been prepared at the request of the Leonardo da Vinci programme which is a programme of education and training sponsored by the EU. The aim of the handbook is to make cytopathologists and smear takers aware of the scope and limitations of the PAP test and to help them understand the scientific basis of the test.*

*The booklet is not intended to be a comprehensive review of screening but is designed to heighten awareness of the need for quality standards and to give an overview of what is widely considered to be the best practice.*

*The guidelines are intended for personnel involved in taking cervical smears and drafts of the guidelines were sent with a questionnaire to training schools and key laboratories in Europe for comment before they were included in the text. We would like to thank all who responded to the questionnaire and wherever possible, comments where included in the text. We have had many favorable comments on the book and plan to publish it in several different languages.*

Cervical cancer is the second most common cancer in women: cancer Registry data indicate that about 500,000 new cases occur each year worldwide. The incidence varies from country to country, the highest incidence being recorded in the developing countries.<sup>1</sup>

In the European Union, about 13,000 women die each year from cervical cancer.<sup>2</sup> Although cervical cancer accounts for only 4% of all cancer deaths in women, it is one of the most common malignancies in women under 50. The major cause of cancer of the cervix uteri is likely to be a sexually transmitted agent, possibly certain types of human papillomavirus.<sup>3</sup> Secondary prevention by mass screening of precancerous lesions of the cervix leads to a reduced incidence and mortality of cancer of the cervix,<sup>4,6</sup> the best results being obtained when all women in defined age groups are invited personally to participate in community-based screening programmes.<sup>7</sup> The highest rates of cervical cancer are seen in Denmark, Portugal and the United Kingdom; rates about half of those of Denmark prevail in the other Member States.<sup>8</sup>

In view of the fact that death due to cervical cancer is avoidable, it is very discouraging to note that there is still an excessive number of deaths due to cervical cancer in women under 55 in the United Kingdom and Denmark, compared to other EU countries.<sup>9</sup>

Invasive cervical cancer can be prevented by a simple, effective, harmless and inexpensive test: the cervical smear or Pap test developed by George Papanicolaou.<sup>10</sup> The test allows rapid detection of precancerous lesions of the cervix, for which conservative treatment exists which blocks any further development.

#### **IN ORDER TO BE EFFECTIVE, ALL PHASES OF THE PAP TEST MUST BE CARRIED OUT CORRECTLY.**

The aim of this booklet is to help cytologists and smear takers to achieve this goal.

#### **AETIOLOGY AND RISK FACTORS**

Our current concept of invasive cancer of the cervix is that the invasive stage of the disease is preceded by a precancerous stage known as cervical intraepithelial neoplasia (CIN) and more recently squamous intraepithelial lesion (SIL).

The Pap test permits detection of these precancerous lesions which can readily be removed thereby preventing progression to invasive cancer.

Cancer of the uterine cervix is considered to be a disease with a multifactorial aetiology; both viral and chemical factors are probably responsible for its development.<sup>11</sup>

The following correlated factors may, directly or indirectly, promote the development of cancer:

- sexual intercourse at an early age;
- multiple sex partners;
- first pregnancy at an early age and a high number of births;
- viral infections (particularly high-risk types of HPV Human Papilloma Virus);
- history of sexually transmitted disease;
- low socio-economic status;
- immunodepression (HIV infection, chemo and/or radiotherapy, transplants, dialysis);
- cigarette smoking (20-40 cigarettes a day);
- use of contraceptives that do not act as barriers.

**However, considering the effectiveness of the Pap test, one can say that the most relevant risk factors are represented by:**

- **never having had a Pap test;**
- **not having had a Pap test for a long time (more than 5 years).**

## WHAT IS SCREENING ?

A MASS SCREENING PROGRAMME is a series of diagnostic and therapeutic services offered actively and free of charge to an apparently healthy population and promoted by a National Health Service with the aim of early diagnosis of the disease, before symptoms appear.

Screening for cervical cancer should ideally take place in the framework of a well-organized mass screening programme.

Mass screening programmes for cervical cancer should be carried out by mailing letters annually over a three year period, offering a smear test to women in the risk age group, according to the UICC/IARC recommendations, the 25-64 age group,<sup>4</sup> living in the chosen areas. A systematic monitoring of all phases of the screening programme is absolutely necessary to improve the technical procedures of the test (sampling and preparation of the cytological smear, reading, etc.) and also for evaluating and improving women's participation in the programme.

## FREQUENCY OF PAP SMEAR SCREENING

Cost-effective analysis suggest the Pap test should be routinely performed every 3 years; it is more important to screen all women than to screen the same women at more frequent intervals.

Some countries have decided to screen every 4 or 5 years in order to concentrate their effort to reach all women, especially the women at risk.<sup>6</sup>

The age range varies from 20 to 69, but most screening programmes recruit women between the ages of 25 to 64.

## REASONS FOR FAILURE TO DETECT CERVICAL NEOPLASIA

- Incorrect sampling of cervical epithelium
- Failure of transfer of significant biological material from sampling devices to the slide
- Incorrect slide processing, administrative and clerical errors
- Incorrect interpretation of the smear at light microscope (reporting error)
- Non desquamating cervical lesions (not amenable to quality improvement)

## ERRORS OF REPORTING

The main objective of all quality programmes for cytopathological laboratories is to reduce the risk of errors of reporting. The main cause of errors of reporting are due to misinterpretation of the smear by the cytologist. Errors of screening may result in *false negative report* or *false positive report* being issued.

A *false negative* report may be issued if abnormal cells are present in the smear but are not detected by the cytologist. False negative reports have a serious impact on patient management. They may give the patient and her doctor a false sense of security by leading them to believe the patient is free of malignant disease. Thus a CIN lesion may remain untreated and develop into invasive cancer which carries a poor prognosis for the survival of the patient.

In contrast *false positive* report may be issued when the report mentions a precancerous or cancerous lesion but no cancerous lesions are present on the cervix. False positives are less critical, because colposcopic and/or histological confirmation are usually required. However they create unnecessary discomfort and distress and lead to inappropriate further examinations and in some cases to inappropriate treatment.

Other causes of error of reporting are:

- incorrect recording of personal data;

- clerical and administrative errors
- to mistake one slide for another

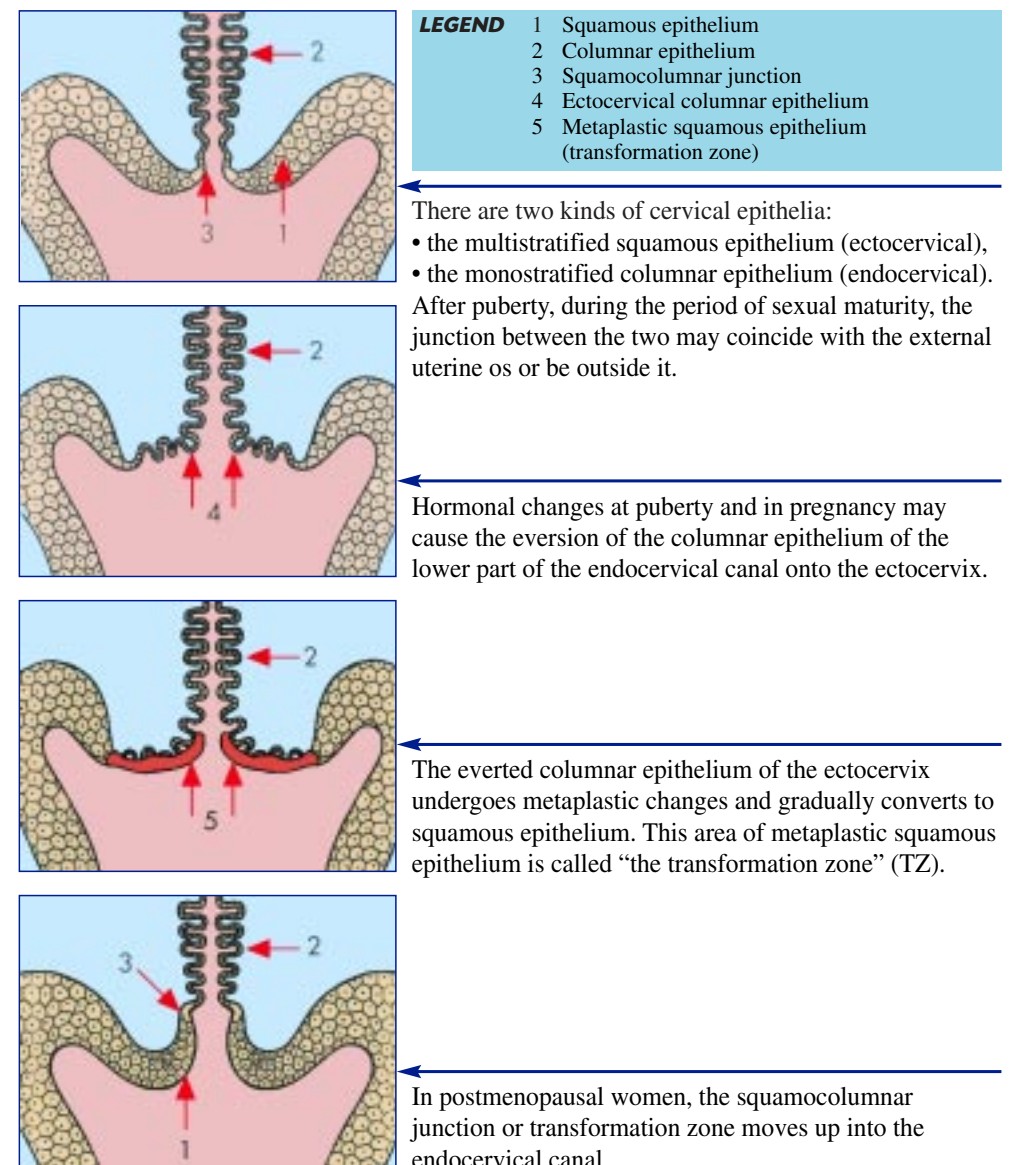
Another source of error is the misinterpretation of inadequate smears. A statement of smear adequacy is provided on page 12, 13 and 16.

## CERVICAL SAMPLING TECHNIQUE - GENERAL INFORMATION

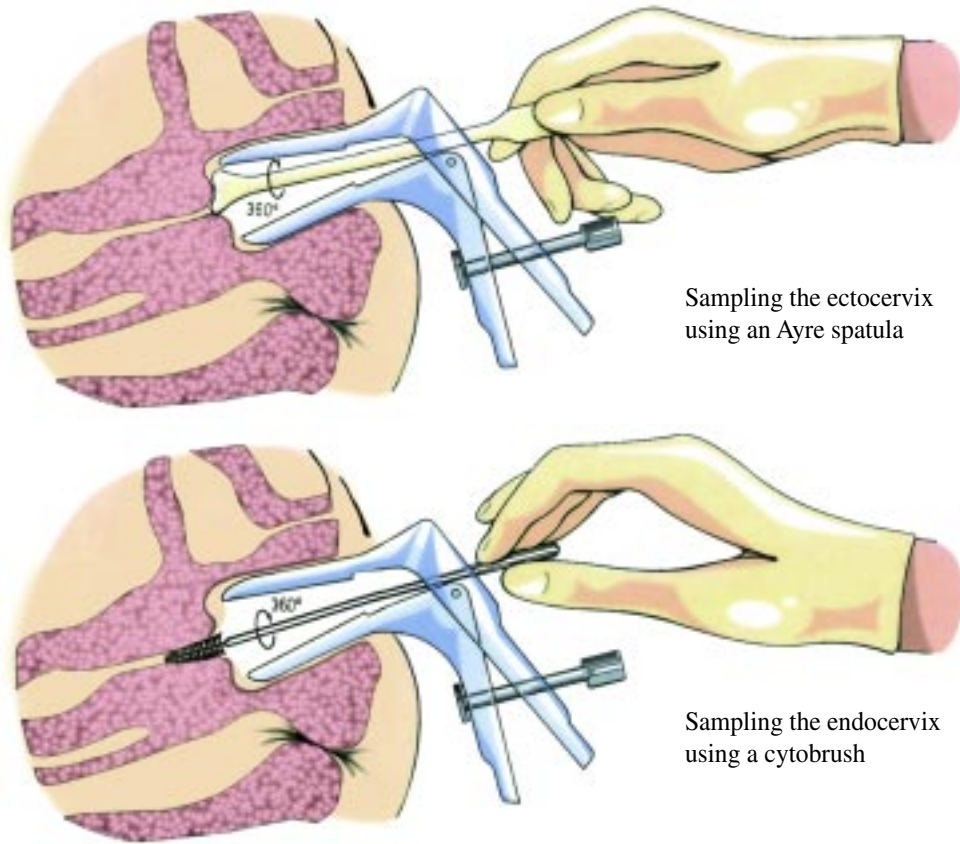
To obtain accuracy in Pap testing it is necessary first of all to obtain a representative cellular sample for examination. The sample should contain cells from the ectocervix, endocervix and the transformation zone (TZ). To ensure this it is important that the cervix is visualized and that both ectocervix and endocervical canal are sampled thoroughly, because CIN lesions occur currently in the (transformation zone). It is particularly important to obtain cells from this region for examination.

It is not always possible to identify the TZ because its position changes during the course of a woman's lifetime, according to her age and phase of her reproductive life. It is also necessary to sample accurately both the ecto and the endocervical areas (fig. 1). Whenever the endocervix cannot be reached easily with the Ayre spatula, a cyto-brush or cone-shaped endocervical brush must be employed: due to its flexibility the

FIG. 1 - THE PHYSIOLOGY OF THE CERVIX



**FIG. 2 - ILLUSTRATION OF THE TECHNIQUE USED FOR CYTOLOGICAL CERVICAL SAMPLING**



brush can penetrate into the endocervical canal and so it can collect a sample of endocervical cells.

It is therefore advisable to take two samples taking each one separately using different instruments: an Ayre spatula for the ectocervical sample and a cytobrush for the endocervical sample<sup>12</sup> (fig. 2).

It is possible to detect lesions which may lead to cervical cancer only from samples of good quality, taken from all risk areas, thus allowing therapy to begin which leads to complete recovery in almost 98% of cases.

The equipment necessary for optimal cervical sample taking is shown in fig. 3. Specula of different size (small, medium, large) may be used, preferably made of sterile disposable plastic.

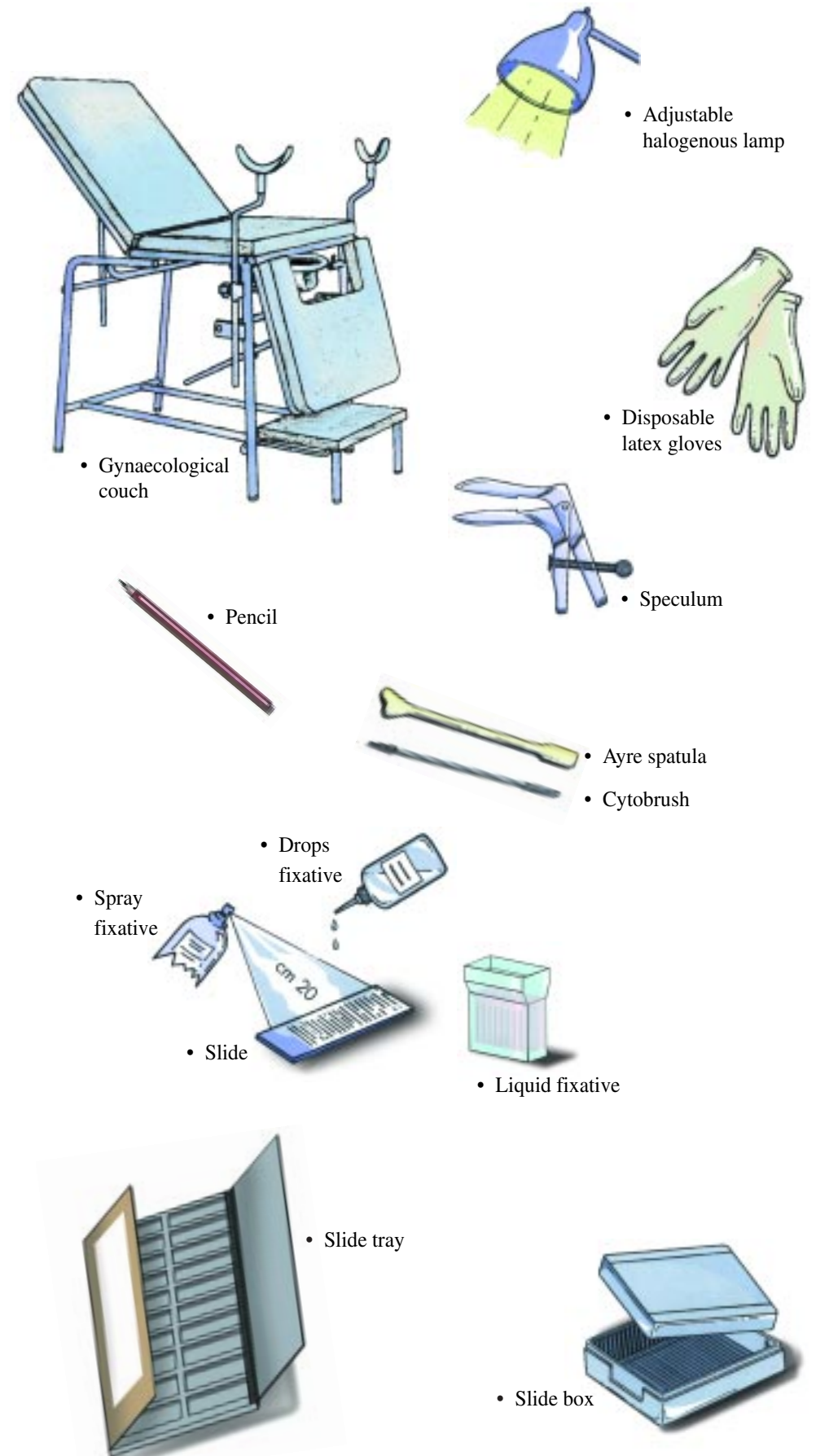
If metal specula are used, they should be thoroughly cleaned and washed before being sterilized. The presence of blood or other organic matter allows microorganisms to survive the sterilization or disinfection procedures. After washing, the specula must be sterilized in an autoclave at 121°C for 20 minutes or in a dry oven at 170°C for 1 hour.<sup>13,14</sup>

A good source of light is necessary to visualize the portio or cervix: the best way is to use an halogenous lamp with a mobile arm which can be moved into a suitable position. The smear taker should use high quality latex gloves, up to ASTM (American Society for Testing Material) standards.<sup>15</sup>

## THE ANATOMY AND PHYSIOLOGY OF THE CERVIX

The uterus is composed of the cervix and the body. The cervix can be subdivided into ectocervix and endocervix. The cervix opens into the vagina through the external uterine os. Clinical examination allows distinction of the ectocervix, which is contin-

**FIG. 3 - EQUIPMENT NEEDED AT THE SAMPLE TAKING CENTRE**



uous with the vagina, and of the endocervical canal which is lined by columnar mucus secreting cells and extends from the external cervical os to the internal cervical os which is continuous with the body of the uterus. The cervical canal has a diameter of a few millimetres and may contain glandular secretions in the form of a mucus plug in fertile women.

The ectocervix is mostly covered by non-keratinized stratified squamous epithelium without glands, while the endocervix is covered by columnar epithelium, which changes only slightly during the menstrual cycle. The junction between the squamous epithelium and the columnar epithelium corresponds to the **squamocolumnar junction**.

The position of the squamocolumnar junction changes during a woman's lifetime: before puberty the junction is within the endocervical canal. After the onset of menstruation or with the first pregnancy, the increase in volume of the uterine cervix causes an eversion of the columnar epithelium; the squamocolumnar junction is consequently located distally from the external uterine os. In the second and third decade of a woman's life, the position of the squamocolumnar junction changes again. In the menopausal woman, the cervix begins to atrophy and the squamocolumnar junction recedes into the endocervical canal. The **transformation zone** is the name given to the area of columnar epithelium which undergoes metaplastic changes to a squamous epithelium. The metaplastic changes occur in the everted columnar epithelium as it is become exposed to the vaginal environment (see figure 1). The metaplastic process is often patchy. The final outcome is usually the formation of a layer of mature squamous epithelial cells which constitute, together with the columnar epithelium, a new squamocolumnar junction adjacent to, or confluent with, the external uterine os (fig. 1). The transformation zone is an area where most cases of cervical cancer occur and precancerous and cancerous lesions of the cervix arise. The cervical sample must therefore always include, not only squamous and endocervical cells from the ecto and endocervix, but also cells from the transformation zone.

**The lesions which precede cancer of the cervix can be detected only if the sample is of good quality and if it includes representative elements of all risk areas.**

## PHASES OF CERVICAL SMEAR TAKING

### 1) PRELIMINARY INTERVIEW

Before cervical smear taking, the woman should be briefly interviewed. The staff of the centre should bear in mind that for many women Pap testing still creates embarrassment and worry; they should consequently behave with courtesy and respect for the woman's privacy, reassuring her and explaining the meaning of the Pap test and the procedures that will be carried out; this will also help to improve co-operation with future tests. It must also be borne in mind that most women associate the idea of an abnormal smear with invasive cancer.<sup>16</sup>

Women's anxiety about cervical screening can be reduced by providing adequate information before taking the smear. In particular, that cervical screening can detect lesions before the cancer develops and the likelihood of a negative result is about 93%.<sup>17</sup>

During the first interview, data must be gathered on general health and previous gynaecological problems, such as discharges or loss of blood not due to menstruation.

Staff should also check the woman has an empty bladder.

### 2) FILLING UP THE SMEAR REQUEST FORM

The form should be designed to allow easy reporting of data and computer entry. It should allow hard copy for laboratory record and copy for the sender and general practitioner.

The data sheet should include the following information:

- first name and surname, maiden name, address and post code;

- the patient's date of birth;
- identification number;
- date of this test;
- the sender's name and address;
- name, address and phone number of the woman's GP;
- clinic or centre where the smear has been taken;
- date of the last Pap test and its outcome (if known);
- first day of last menstrual period and duration of cycle;
- reason for smear (i.e. screening programme or clinically indicated);
- type of sample (i.e. ectocervical sample, endocervical sample, vaginal pool);
- reproductive status (reproductive age, pregnancy, post-partum, post-menopause);
- relevant clinical data: number of pregnancies; date of last delivery; contraceptive measures in use; hormone therapy; medical or surgical therapy (local destructive or excisional, hysterectomy) with particular reference to the uterine cervix, and reasons for such therapy; specific symptoms such as leucorrhoea, leucorrhoea, intermenstrual, post-coital or post-menopausal bleeding; description of cervix;
- name of the laboratory where the smear will be examined;
- laboratory number assigned to smear;
- name and/or code of the smear taker and signature.

The request form must be completed as far as possible at the sample taking centre before taking the smear. The request form must be planned to make the subsequent task of computer filing as easy as possible to allow for statistical analysis.

A clean slide must be prepared with the identifying data on the frosted edge, using a permanent marker: woman's name and surname, date of birth and identification.

## 3) SAMPLE TAKING FOR PAP TESTING: THE TECHNIQUE

### CONDITIONS FOR OPTIMAL SAMPLING

In women of reproductive age, sample taking should be carried out at least 5 days after the end of the menstrual cycle and at least 5 days before the expected date of onset of menstruation. Ideally, smear taking should be carried out at least 2 days after intercourse; in the previous 5 days a diaphragm, vaginal suppositories or vaginal creams should not have been used. The same applies to vaginal douches or ecographies with an intravaginal probe. **Endovaginal exploration should be performed, if necessary, after the sample taking.** It is generally accepted that an ecto-endocervical sample can be taken during pregnancy.<sup>18</sup>

### INSTRUMENTS FOR TAKING CERVICAL SAMPLES

- Ayre spatula or spatula of similar design;
- cytobrush.

Examination with the speculum allows the sample taker to observe the ectocervix and locate the external uterine os and the access to the cervical canal. Since it is not always possible to identify the squamocolumnar junction due to the change in location of the junction during reproductive life, it is essential to take two samples, using two different instruments: the Ayre spatula and the cytobrush. These instruments, if properly handled, allow collection of naturally exfoliating or scraped cells from the ectocervix, the transformation zone and the endocervix (fig. 2). After menopause, when the endocervix cannot be easily reached with an Ayre spatula, only endocervical cone-shaped brush (cytobrush) can penetrate the endocervical canal and a sample can be collected by brushing with a circular screw like movement. Some smear takers prefer to take a third sample from the posterior vaginal fornix using the rounded end of the Ayre spatula (VCE smear).

## PROCEDURES FOR SAMPLE TAKING, TRANSFERRING AND PREPARING THE SLIDES

Before sample taking, the woman should be informed that she will feel a slight vaginal pressure.

The unlubricated speculum, which can be dipped, if necessary, in warm running water, must be inserted in the vagina - visually checking the operation - along the axis of the vaginal opening, rotating it by 90° when it is halfway; the speculum must not be opened until it is completely inserted and should be delicately manoeuvred to show the portio. In case of difficulties in inserting the speculum, it is useful to invite the woman to cough, after breathing in deeply.

Should it be difficult to see the portio, the cervix can be exposed using a delicate manual manoeuvre (bearing in mind that the glove should not have either talcum or lubricants on it). Avoid cleansing the cervix with a tampon before sample taking: only if there is an excess of mucus or purulent exudate, a delicate cleansing with a piece of gauze soaked in saline solution can be used to wipe the cervix before direct sampling.

The two samples, the ectocervical sample collected with the Ayre spatula and endocervical with the cytobrush, should be carried out separately exerting a delicate,

but firm pressure, to collect the cells. The first sample to take must be the ectocervical, to avoid the possibility of contamination by blood from the endocervical canal.

The elongated end of the Ayre spatula is inserted in the external cervical os, scraping the ectocervical mucosa, so as to remove the flaking cells and should be rotated clockwise through 360° (fig. 4.1). The rotation should be repeated a second time, if the sample taker is not sure of having sampled material from some area of the cervix. Then, keeping the spatula with the sample in the other hand for a moment, the endocervical sample is quickly taken by delicately and completely inserting the cytobrush in the endocervix and rotating it by 360° (fig. 4.2).

This manoeuvre permits both specimens (ecto and endocervical) to be smeared on two preselected areas of the same slide (or on separate slides) in rapid succession (fig. 4.3) and fixed immediately (fig. 4.6).

The cytobrush has been shown to be the best technique for taking samples of the endocervical canal cells.<sup>19,20</sup>

In order to transfer the total or the majority of the collected cells on the slide from the spatula and the cytobrush, it is important to carry out the operation carefully,

in order that all cells smeared be representative of the total population sampled.

The ectocervical sample must be smeared in one direction lengthwise along 2/3 of the slide using **both sides** of the spatula (fig. 4.4). The endocervical sample should be smeared from top to bottom in an anti-clockwise direction on the remaining third of the slide (fig. 4.5). It is recommended that only slight pressure be used to smear the material in order to preserve the cells' integrity, trying at the same time to obtain an even consistency overall, neither too thin nor too thick.

## FIXING THE SMEAR

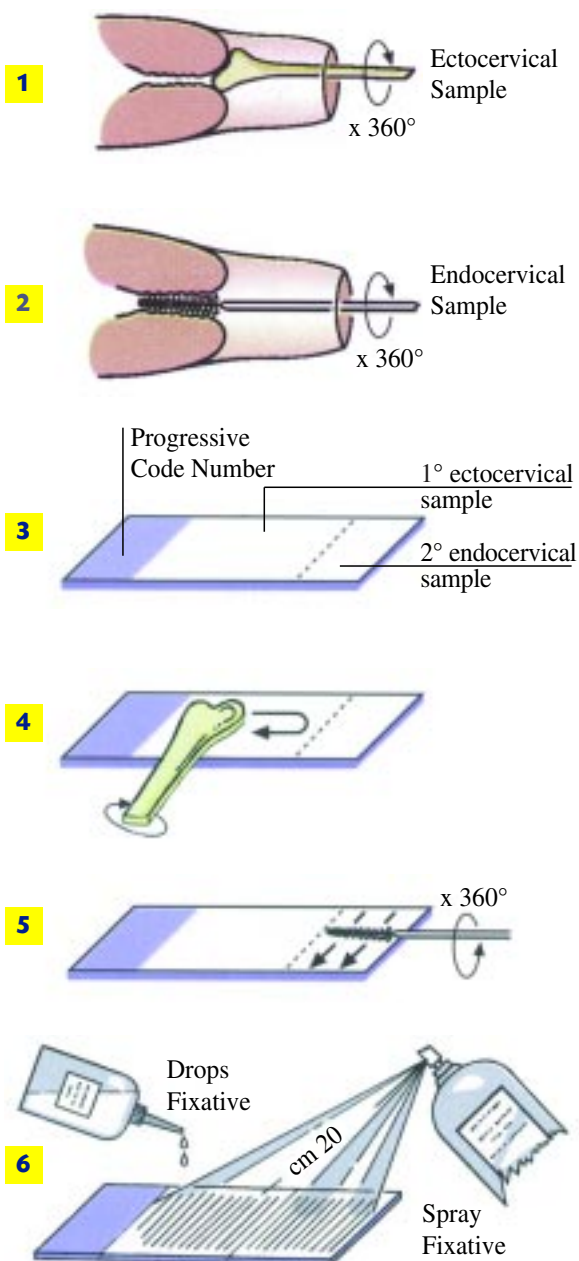
The slide must be fixed immediately to avoid cellular changes in the nucleus or cytoplasm due to drying of the sample.

*The Cytological Fixative (95° alcohol and carbowax):*

- a spray, of the ecological kind without gas propellant, to be used at a distance of at least 20 cm., so as to spray the entire slide (fig. 4.6);
- in drops, using 4-6 drops per slide, then left to dry (fig. 4.6);
- jar of alcohol.

After being fixed, the slides must be handled with care, avoiding contact with paper (prescription, notes, etc.) and put into a slide tray or into a proper transport box. In the cytopathological lab the slides, after having being stained by the Papanicolaou technique, must be rapidly mounted with 24x50 minimum mm slide coverslips or plastic film which guarantees complete coverage of the smear.

FIG. 4 - CERVICAL SAMPLES



## TRAINING OF SMEAR TAKERS

The doctors, midwives and nurses with appropriate qualification involved in taking cervical samples must be given at least 3 days theoretical and practical training in the technique.

It has been recommended that nurses should have adequate theoretical knowledge and practical experience before they have given responsibility without supervision of taking smears (20 hours of theory and 50 smears from women in different age groups).

Training must be carried out by a doctor in charge of a sample taking centre.

Staff in charge of sample taking must be familiar with the anatomy and physiology of the female genital tract and must be trained to:

- a) insert a speculum, identify the cervix with the naked eye and evaluate it for the presence or absence of disease, i.e. note ulcerated areas, discharge, bleeding, etc.;
- b) take samples from the ectocervix, the transformation zone and the endocervix;
- c) prepare a smear and fix correctly the sample taken.

The staff in charge of sample taking must also know the basic principles of population screening strategy and of effective communication with women and also be capable of interpreting correctly the cervical smear report.

It is the duty of the director of the sample taking centre to check the percentage of inadequate smears; inadequate smears should be kept to a minimum and should they exceed 5% the reason should be investigated. Smear takers may need further training and one to one meeting with the director of the smear taking centre should be arranged.<sup>21</sup>

## TRAINING OF ADMINISTRATIVE STAFF IN SMEAR TAKING CENTRE

In smaller centres administrative duties can be carried out by midwives who can interview, fill in the request forms and take the samples. In centres which carry out a large number of smear tests (in excess of 15,000 per year) administrative duties should

be undertaken by office personnel. In both cases, such staff must be trained to:

- use a computer and run an office;
- use the registration, filing and data retrieval systems;
- handle the slides following laboratory safety procedures;
- be familiar with the relevant medical terminology.

Furthermore, they must be aware of the importance of accuracy and confidentiality in the transfer of patients' details.

“On site” training must also be provided for administrative personnel.

## PROCESSING CERVICAL SMEARS

### STAINING

The smear should be stained by the Papanicolaou method using reputable, high quality stains which are within the stated expiry dates. In order to maintain a consistent coloration of cervical smears, staining machines are recommended. The stains should be replaced at regular intervals or when there is any noticeable deterioration in the coloration.

### COVERSLIPPING

The cellular material on the slide should be covered by a glass coverslip or plastic film. The coverslip must be optically flat, clean and free from glass dust and no more than 0.17 mm in thickness to permit the use of high power objectives which have short working distances.

The coverslip must completely cover the preparation so that the whole slide can be screened, otherwise possible tumour cells may be missed. A 24 x 50 minimum mm coverslip is recommended. The mountant should be dry before the slide is screened.

### LABELLING

The slide should be labelled with the slide laboratory number, woman's surname, name of the center and date.

The slide should then be matched with the request form.

### ASSESSMENT OF SMEAR TAKING AND SLIDE PROCESSING

The initial evaluation and classification of the prepared slide should include evaluation of the adequacy of the sample. The following features should be checked:

- the staining quality (all cytological details are well preserved and can consequently be interpreted at the light microscope level);
- the presence of material which might interfere with the reading (excess of inflammatory cells or erythrocytes, excessive cytolysis or extraneous material);
- cell content (presence of both squamous and endocervical cells).

### ADEQUATE SMEAR

In cervical cytology, adequacy is the set of criteria which the smear must meet to be considered suitable for diagnosis.

Many factors can make a smear difficult to analyse:

- air-drying and poor fixation;
- extensive cytolysis;
- large number of leukocytes, red blood cells or other contaminants.

The assessment of adequacy is a subjective exercise, but the following criteria are widely used:

- well-preserved and well-visualised squamous cells should cover more than 10% of the slide surface;
- at least 50% of epithelial cells smeared should be evaluable;
- transformation zone component: minimum 2 clusters of well-preserved endocervical and/or squamous metaplastic cells, each cluster composed of a minimum of

at least 5 cells.

A smear containing abnormal cells should never be categorised as inadequate (The Bethesda System 1991).

It may be emphasized also that all slides should have appropriate labelling and identifying information and be accompanied by relevant clinical information (at least age and last menstrual period).

In post-menopausal women (excluding those on hormone replacement therapy) cells from transformation zone often are not always easily to sample and are often not recognisable from squamous parabasal cells: this type of specimen can be considered adequate even if there is not clear evidence of transformation zone sampling.

Some laboratories prefer to use the phrase “*satisfactory for evaluation but limited by...*” in the case of:

- absence of endocervica/transformation zone component;
- partially obscuring blood, inflammation, thick areas, poor fixation, air-drying artifact, contaminant, etc. which precludes interpretation of approximately 50-75% of epithelial cells;
- lack of pertinent clinical patient information (age, date of last menstrual period).

### INADEQUATE SMEAR

A inadequate smear is a smear not suitable for diagnosis.

A specimen is “*unsatisfactory for evaluation...*” if any of the following criteria apply:

- lack of patient identification on specimen and/or requisition form;
- a technically unacceptable slide, defined as: one that is broken and cannot be repaired or as having cellular material that is inadequately preserved;
- scant squamous epithelial component (well-preserved and well-visualized squamous epithelial cells spread over <10% of the slide surface);
- obscuring blood, inflammation, thick areas, poor fixation, air-drying artifact, contaminants which precludes interpretation of approximately 75% or more of epithelial cells.

## 13 REASONS FOR INADEQUATE SAMPLING

- Carelessness in following procedures.
- Uncooperative patient because insufficiently reassured.
- Cervix inadequately exposed.
- Cervix not scraped firmly enough.
- Transformation zone and endocervix incompletely sampled.
- Material incompletely transferred to the slide.
- Sample poorly smeared (too thick or too thin or with excessive pressure causing distortion).
- Smear allowed to dry before fixation.
- Insufficient fixative.
- Incorrect staining.
- Smear consisting mainly of blood or inflammatory exudate.
- Contamination of the smear with lubricant, vaginal creams, spermicides or cream used for the ecographic probe.
- Menstrual smear containing large number of endometrial cells and red blood cells.

One should bear in mind that a badly taken and/or prepared smear can produce false negative results, creating a false sense of security in the patient.

The quality of sample taking must be systematically checked. The percentage of smears found to be inadequate after reading should not be more than 5%. The frequency of inadequate smears for each smear taker should be recorded.

**IF THE SMEAR IS INADEQUATE, IT IS AS IF NO SMEAR HAD BEEN TAKEN AND SO IT MUST BE REPEATED.**

### **MICROSCOPE READING**

The prepared slide should be read using a 10x objective and 10x or 12x eyepieces to obtain a general view of the quality of the smear; this same objective should be used to screen the specimen. Screening should be continued until all the fields within the coverslipped area have been examined. To see the general features of the cells better, a 20x or 25x objective is used and for the features of the nucleus a 40x objective.

In addition a 4x objective should be employed to mark the most significant fields.

### **CYTOLOGY WORKLOAD LEVELS**

In order to maintain and develop expertise it is essential that cytotechnologists see a wide range of cases. Laboratories should process at least 15,000 smears each year and a satisfactory staff workload ratio must be maintained. A cytotechnologist may be expected to screen up to 50 smears per day together with other duties.<sup>22</sup>

### **CHECKING THE QUALITY OF SCREENING**

To assure diagnostic reliability and accuracy the cytopathological lab must have in place a quality assurance programme.<sup>21-42</sup> This includes systematic evaluation of the adequacy of the smear, rapid rescreening of negative smears or full rescreening of a random sample of negative smears, systematic review of positive slides for cancerous and precancerous lesions, review of previous cytology in the case of a pathological specimen, review of cases with particular clinical history, cytohistological correlations, insertion of seeded slides in daily screening routine, participation in interlab comparison programmes with slide exchange. Other desirable organizational features are the participation of the laboratories to a programme of voluntary accreditation and systematic assessment of staff competence (e.g. proficiency and aptitude testing) and performance of quality improvement in auditing projects. For quality assurance procedures (see Tab. 1).

### **THE CYTOLOGICAL REPORT**

The cytological report should be accurate and concise, so that data can be easily understood and accepted by all medical staff involved (GPs, gynaecologists, clinicians) and local midwives.

It should also be descriptive and contain:

1. a description of the content of the smear;
2. a prediction of the underlying histology;
3. a statement as to whether the smear is satisfactory or unsatisfactory. If the latter is the case, a reason should be given;
4. advice on management of the patients, i.e. routine repeat (at 3, 4 or 5 years), early repeat or referral for colposcopy or gynaecological examination.

## **TAB. 1 - QUALITY ASSURANCE PROCEDURES**

### **1. INTERNAL QUALITY CONTROL PROCEDURES**

- Systematic assessment of smear adequacy
- Supervisory review of borderline and abnormal smears (they must be re-examined and reported by a pathologist or by an authorized person)
- Supervisory review of all cases with selected clinical characteristics
- Quality control of negative and inadequate smears should be carried out by one of the following methods:
  - Random rescreening
  - Rapid review
  - Automated systems
- Review of previous negative smears in cases of CIN2 or worse
- Peer review and discussion of all abnormal smears
- Biopsy/smears correlation
- Monitoring of workload for the cytologist and statistical distribution of diagnoses for the laboratory and individual cytologist

### **OTHER IMPORTANT PROCEDURES**

- Audit of procedure for identification and registration of slides
- Audit of slide processing
- Use of standard form with relevant clinical information
- Use of standard terminology and classification system
- Yearly laboratory workload (minimum 15,000)
- Satisfactory staff workload (approximately up to 50 slides per day)
- Establishment of staff training programmes and continuing professional education
- Storage of reports and slides (negative for a period of at least 5 years and “non negative” for 20 years)
- Monitoring turnaround time
- Seeding abnormal smears into the cytology workload
- Preparation of annual report

### **2. EXTERNAL QUALITY CONTROL MEASURES**

- Exchange of slides between different laboratories
- Aptitude test
- Proficiency test
- Accreditation/Certification procedures
- Comparison of laboratory performance with consensus standards, ideally national if they exist



## TAB. 2 - RECOMMENDED TERMINOLOGY

### STATEMENT ON SPECIMEN ADEQUACY

- Satisfactory for evaluation
- Satisfactory for evaluation but limited by (specify reason)
- Unsatisfactory for evaluation

#### Reasons for less than optimal / unsatisfactory specimen:

- Scant cellularity
- Poor fixation or preservation
- Presence of foreign material (eg. lubricant)
- Partially or completely obscuring inflammation
- Excessive cytolysis or autolysis
- No endocervical component in a premenopausal woman who has a cervix
- Not representative of the anatomic site
- Other (Specify)

### GENERAL CATEGORIZATION

- Within normal limits
- Benign cellular changes: see descriptive diagnosis
- Epithelial abnormality: see descriptive diagnosis

### DESCRIPTIVE DIAGNOSIS

#### Infection

- Trichomonas vaginalis
- Fungal organism morphologically consistent with Candida sp.
- Predominance of coccobacilli consistent with shift in vaginal flora
- Bacterial morphologically consistent with Actinomyces sp.

#### Reactive and reparative changes

- Inflammation (includes typical repair)
- Atrophy with inflammation (atrophic vaginitis)
- Radiation
- Intrauterine contraceptive device
- Cellular changes associated with Herpes simplex virus

### EPITHELIAL CELL ABNORMALITIES

#### Squamous cell

- Atypical squamous cells of undetermined significance (ASCUS)
- Low grade squamous intraepithelial lesion encompassing cellular change of HPV, mild dysplasia/CIN 1\*
- High grade squamous intraepithelial lesion encompassing moderate and severe dysplasia: CIN 2 and CIN 3/CIS
- Squamous cell carcinoma

#### Glandular cell

- Endometrial cells cytologically benign in postmenopausal woman
- Atypical glandular cells of undetermined significance (AGUS) (Qualify)
- Endocervical adenocarcinoma
- Endometrial adenocarcinoma
- Extrauterine adenocarcinoma
- Adenocarcinoma NOS

#### OTHER MALIGNANT NEOPLASM (Specify)

\* Previously termed koilocytosis, koilocytotic atypia or condylomatous atypia are included in low grade lesions

The European Federation of Cytology Societies/Committee of Quality Assurance Training and Education (EFCS/QUATE) has prepared a document on equivalent terminology for the reporting of cervical smears. It includes the classification recommended by the Bethesda System<sup>43</sup> (see Tab. 2).

### FOLLOW-UP OF WOMEN WITH AN ABNORMAL CERVICAL SMEAR

Whenever the smear test results indicate the presence of a precancerous lesion, the patient must be recalled for further examination: referral for colposcopy, which permits direct observation of the cervix and closer inspection of the suspect area and biopsy, is essential.

In the event of an abnormal smear report, colposcopy and biopsy should be carried out within 3 weeks.

### STORAGE OF SLIDES

Both smears and reports should be kept for at least 5 years. In any case, for the purposes of quality assurance (e.g. re-examination of previous smears in the case of positive results, medico-legal matters, etc.) it is recommended that negative cytological specimens be kept for 10 years and positive ones for 20 years. In general, laboratories will be responsible for storing smears, whether taken for screening purposes or for clinical reasons. In general, laboratories will deal with smears performed for screening purposes and because of symptoms.

Although the reason why a smear was performed should be clearly identifiable, it is advisable that a single registration system includes all smears performed. This will reduce errors and allow data for evaluation and possibly for an integrated screening programme.<sup>21</sup>

### WOMAN'S CLINIC CARD

A clinic card is also useful to record the test dates: it should include some personal details and details of the results of every test, including the outcome. The card should be laid out to allow information on at least 8 smear tests to be recorded in a life time (between 25-64 years) (fig. 5).

The card can be given to women when they are first interviewed together with the recommendation that it should be completed when the cytological result is received. It should be stressed that they should bring their cards with them to each and every test.

FIG. 5 - WOMAN'S CLINIC CARD

DIARY OF CERVICAL SMEAR TESTS		
Name and Surname .....		
Signature .....		
<i>Please, complete the cytological report as soon as you receive it and bring the card with you to the next test.</i>		
Date	Result	Date of next Pap test
1	.....	.....
2	.....	.....
3	.....	.....
4	.....	.....
5	.....	.....
6	.....	.....
7	.....	.....
8	.....	.....

## NEW DEVELOPMENTS

### LIQUID-BASED CYTOLOGY

Liquid-based cytology is a new approach to the preparation of cervical scrapes.

This method permits the preparation of a thin smear which theoretically should be easily read by the cytologist. It involves sampling the cervix in the usual way with the sampling method in use: thereafter the device is rinsed off into a transport medium, so that all the cells removed are collected in a homogeneous suspension.

An aliquot of the cells is deposited on the slide ready for staining after processing.

The *advantages* of this technique are:

- all the material on the collection devices is available for the analyses;
- all the material is well fixed;
- the background of the smear is clearer: less blood, mucus, debris, inflammatory cells;
- possibility to apply other techniques i.e. HPV typing.

The *disadvantages* of this technique are:

- major changes in the routine collection, transport and disposal;
- necessity of retraining for cytotechnologists and cytopathologists;
- precious information from the background (tumoral dyathesis) may be missing;
- increase of consumable costs and preparatory time and workload.

Several systems are available commercially, e.g. Thin Prep® 2000 (Cytec), Auto-cyte Prep® (Neopath). Large scale studies<sup>46,47</sup> on Thin Prep® have shown significant improvement in sensitivity over conventional smears and Thin Prep® 2000 and Auto-cyte System® have been approved by Federal Drug Agency for clinical use as a method of preparation of cervical scrapes for primary screening. Other systems are still under evaluation.

For this new technique the cost-effectiveness needs to be validated.

### AUTOMATION

Cytologists have been aware for many years of the potential value of automation for the analysis of cervical smears.<sup>48</sup> Several devices have been developed commercially, of which only one so far has been approved by the FDA in the United States for primary screening. This device Autopap® 3000 (Neopath) can identify negative smears with a high degree of certainty.<sup>49,50</sup> It has been approved by FDA for primary screening under limited conditions namely up to 25% of smears submitted for analysis and classified as negative may be reported without further microscopic examination.

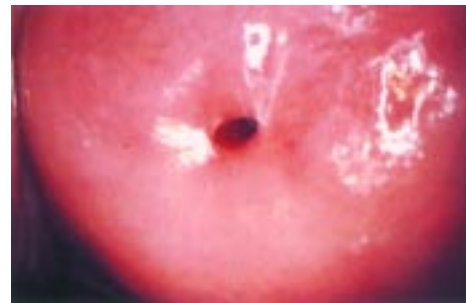
## WOMEN'S LEAFLET

A leaflet is helpful to inform women and improve their participation in the screening programme. It is also important to give it out to all users and potential users.

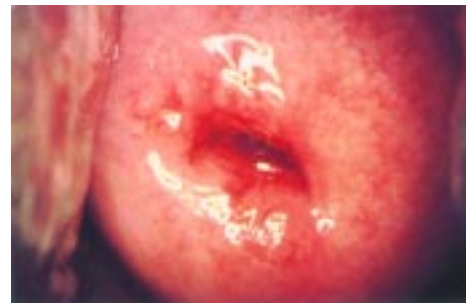
The leaflet should be written in plain, simple language and possibly have drawings and diagrams. The main contents<sup>44</sup> should be:

- Purpose and efficacy of the Pap test;
- Smear taking procedures emphasizing the lack of pain;
- Age range and frequency of Pap testing;
- Stage of the menstrual cycle most appropriate for smear taking;
- Warning signs of cancer and risk factors for cervical cancer;
- Address for asking further information;
- Right and duty to perform a Pap test.

## UTERINE CERVIX: NORMAL APPEARANCE



**Fig. 1** - Nulliparous Cervix.  
External cervical os is small and round.



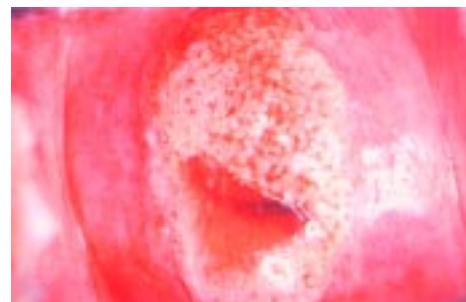
**Fig. 2** - Multiparous Cervix.  
External cervical os is wide and open



**Fig. 3** - Atrophic Cervix.  
The external cervical os in postmenopausal women is narrow.



**Fig. 4a** - Ectropion.  
The glandular epithelium is exposed on the ectocervix.  
Note mucus on the anterior lip.



**Fig. 4b**  
Same cervix after the application of acetic acid 3%  
showing eversion of endocervical epithelium onto the  
ectocervix.