Introduction
Carcinoma of the cervix is a slow growing cancer, which is preceded by precancerous lesions named cervical intra-epithelial neoplasia (CIN). The slow progression of precancerous lesions allows detecting the lesion at this stage. The cervical smear allows detecting cytological abnormalities at the microscope. The cells are taken by a spatula or a brush, smeared on glasses and stained by the method of Papanicolaou. The screening by the conventional cervical smear has reduced the incidence and the mortality of cervical cancer (1). The interval between two smears is variable depending if the screening is organized or opportunistic. In the later case, a percentage of women do not have cervical smears, in particular women who are in precarious situation, immigrant women or postmenopausal women without treatment (2). Women who develop cervical cancer have not had a cervical screening or got it in a too spaced way in 60% of cases. In 10% of cases, they had a smear but not an appropriate follow-up. At least, in 30% of cases, they had a regular cervical smear. In this case, cancer is due to a false negative linked to the sampling or to the interpretation.

Terminology of Cervical Smears
Specimen Adequacy
Sampling quality was one of the most innovative proposals made in the Bethesda terminology in 1988. Three categories were proposed: satisfactory, satisfactory but limited, and unsatisfactory. The second category was used for smears not containing endocervical or metaplastic cells, which are the proof of sampling the transformation zone, or partial inflammatory smears. This category was eliminated since clinicians felt obliged to redo the smears. It is now suggested that along with the evaluation of smears, note should be made regarding the presence of less than 10 endocervical cells, inflammation clouding evaluation of less than 75% of the smear, and that the clinician must make the decision whether or not to redo a new smear. If the inflammation, blood and cellular debris clouding are more than 75% of the smear, it should be considered unsatisfactory. Between 8000 and 12000 squamous cells must be present in a conventional smear, and 5000 cells for a liquid smear. Smears with paucicellularity, without appropriate patient identification, or those, which arrive broken, must also be considered unsatisfactory.

Interpretation/Results
Negative for Intraepithelial Lesion or Malignancy
Table I summarizes the Bethesda 2001 System (3). The category “absence of intraepithelial squamous lesion or suspected malignant cell” regroups the categories “normal and benign alterations” (Figure 1). Microorganisms replace the term infection. Alterations due to inflammation, irradiation, or the presence of an intrauterine contraceptive device (IUD) are classified within normal smears.

Epithelial Squamous Cell Abnormalities
Atypical Squamous Cells (ASC)
Following numerous discussions, based on the practicality of maintaining or not an invalid category, it has been decided to keep this category, which is associated with approximately 10% of high grade CIN or CIN2+ from biopsies. On the other hand, the subdivisions of this category have been modified (Table I). The general term for this category is no longer “atypical cells of undetermined significance” (ASCUS). It is
Table 1. The 2001 Bethesda System.

SPECIMEN ADEQUACY
- Satisfactory for evaluation
- Unsatisfactory for evaluation because of (specify reason)

DESCRIPTIVE DIAGNOSES

NON NEOPLASTIC
- Negative for intraepithelial lesion or malignancy (include in the absence of neoplastic abnormalities, with or without entities listed below)

MICROORGANISMS:
- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida
- Shift in vaginal flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with Actinomyces
- Cellular changes associated with Herpes simplex virus

- Reactive cellular changes associated with
  - Inflammation (includes typical repair)
  - Radiation
  - Intrauterine contraceptive device (IUD)
  - Benign-appearing glandular cells status-post hysterectomy

OTHER
- Endometrial cells (in a woman ≥ 40)

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL
- Atypical squamous cells
  - Undetermined significance (ASC-US)
  - Not exclude HSIL (ASC-H)
- Low grade squamous intraepithelial lesion (LSIL) encompassing:
  - HPV/mild dysplasia/CIN 1.
- High grade squamous intraepithelial lesion (HSIL) encompassing:
  - Moderate and severe dysplasia, CIS/CIN 2 and CIN 3
  - Squamous cell carcinoma

GLANDULAR CELL
- Atypical
  - Endocervical cells
  - Endometrial cells
  - Glandular cells
  - Atypical glandular/endocervical cells, favor neoplastic
  - Endocervical adenocarcinoma in situ
  - Adenocarcinoma (endocervical, endometrial, extraterine, NOS).

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The Bethesda 2001 system has the same 2 categories as proposed in 1998, which are “low grade intraepithelial squamous lesion” (LSIL) (Figure 4) and “high grade intraepithelial squamous lesion” (HSIL) (Figure 5) (Table I). Precancerous and invasive cancer is associated with a high-risk human papillomavirus (HPV) infection in 95% of the cases. HPV is a highly infectious virus; most of infections are latent and regress spontaneously without intervention. Neoplasia is a very rare event and a complication of HPV infection. This infection may be productive. It is characterized by the presence of koilocytosis and corresponds to LSIL according to the Bethesda terminology. LSIL (1% of the smears) regress spontaneously in at least half of cases, especially in young patients. They evolve slowly to a HSIL only in 20% of cases.
Abnormalities of basal cells define HSIL. HSIL (0.5% of the
smears) are associated with persistent viral infection and with
high grade CIN detected from biopsy and can progress to an
invasive lesion.

Epithelial Glandular Cell Abnormalities

Atypical Glandular Cells (AGC)
The term “atypical endocervical, endometrial or glandular
cells” replaces the term “atypical glandular cells of
undetermined significance” (AGUS) (Table 1). The nature of
glandular, endocervical or endometrial cells should be noted;
this will permit a more appropriate diagnostic approach,
cervical biopsy, and examination of the endocervix to identify
endocervical lesion, or endometrial biopsy to identify an
endometrial lesion.

Endocervical Adenocarcinoma in situ (AIS)
“In situ adenocarcinoma” is a new category, which corresponds
to specific morphological abnormalities (Figure 6); some
have already been using this term for several years. These
abnormalities, which are based on morphological changes,
permit to differentiate AIS from invasive adenocarcinomas
of endocervical origin. This category allows for a more
aggressive diagnostic approach such as a conization if the
initial diagnostic findings are negative or for the complete
ablation of the lesion.

Adenocarcinoma
The category “atypical glandular or endocervical cells
suggesting neoplasia” is badly defined on morphological
grounds. It should permit early identification of intraepithelial
glandular lesions eventhough it requires prospective studies
for confirmation. The category for invasive adenocarcinomas
remains identical.

Other
Cervical cytology is not a good diagnostic assay for
endometrial cancer. Morphologically benign endometrial
cells were not mentioned in the 1988 Bethesda system,
except referring to menopausal women. The category “other”
is now proposed to classify smears without morphological
abnormalities but which have apparently benign endometrial
cells, in women over 40 years. The presence of these cells
indicates an increased risk for endometrial cancer, and
therefore an endometrial biopsy is recommended. Benign
glandular cells found after hysterectomy should be noted as
“absence of malignant-like cells”.

The Conventional Smear
The smear should be performed remotely of sexual activities
(48 hours), outwards of menstrual periods, of local therapy or
infection, and if necessary after estrogenic treatment for the
menopausal woman. Vaginal touch should be avoided before taking a smear such as lubricant. The sampling should concern the transformation zone, which is located most of the time around the external os, specifically in premenopausal women. The Ayre spatula is recommended with associated endocervical brush if the squamo-columnar junction is located in the endocervix. The Cervex Brush or a modified Ayre spatula allows sampling the cervical os and the endocervix simultaneously. The sampling is smeared on the glass in uniform pattern. The fixation should be done immediately.

The Liquid Based Cytology

The liquid based cytology (LBC) corresponds to a sampling where cells are put in suspension in a conversation liquid. For the clinician, the sample is made the same manner as that of the conventional smear by using a plastic brush, which can take the squamo-columnar junction and the endocervix, or by combining the use of a spatula and an endocervical brush. The taken material is then immediately rinsed in the bottle, which contains a fixative allowing transport to the laboratory. The clinician does not have to deal with any spreading, which is done at the laboratory. Currently, two technical methods, which use automats, were validated by Food and Drug Administration (FDA) and are used frequently. One is proceeding by filtration and collecting cells vacuum-packed on a membrane with transferring cells on a glass (ThinPrep®, Cytyc®). The other is proceeding by centrifugation and sedimentation through a gradient of density (Surepath®, Tripath Imaging®). Spreading out in thin layer which results from these techniques eliminates a great part of the inflammatory cells, necroses and of red blood cells, outcome to “a cleaning” of spreading out. The LBC makes it possible to avoid the majority of the artefacts of superposition of the conventional smear but the dispersion of the cellular material removes also usual visual reference marks. The cytologists are used to reading smears fixed in a liquid for the urines, the serosa or the ovaries. It imposes an analysis element by element and a training at least 6 months to readjust the morphological criteria. The cells are not flattened on the support but deposited and the pictorial aspects are some modified. The nuclei are not hyper chromatic any more but take a vesicular aspect. The cytoplasms are important to differentiate the cellular origin.

The Quality of the Smear

The performances were evaluated by several national agencies whose conclusions are convergent as for the improvement of the quality of the smear. The unsatisfactory smears or limited by the presence of inflammation and red blood cells are statistically less important with LBC than with the conventional method. The absence of cellular material due to a sampling of bad quality remains as frequent in LBC as in conventional smear. The presence of endocervical cells was evaluated of various manners. In the studies where the sampling was divided into a conventional spreading out and where the residual material was rinsed in the bottle (split-samples), the endocervical cells are fewer in LBC. In the studies where all the sampling was rinsed in the flask and results compared retrospectively to those in which smearing was made in a conventional way, the absence of endocervical cells is the same in the two methods. Scotland was the first European country to integrate LBC in an organized screening program (4). This decision was made on the results of a study of 70 000 smears concerning 3 centers. Cost-efficiency calculation was for the benefit of LBC because the rate of inadequate smears passed from 7% with the conventional smear to 1% with the smear in liquid medium. The definition of an inadequate smear in Scotland and England includes the smears deprived of endocervical cells. This definition explains the high percentage of inadequate cells. In the pilot study made in England, the rate of definite inadequate smear according to criteria’s of the National Health System Cervical Screening Programs (NHSCSP) is from 9.1% with the conventional smear to 1.6% with LBC (5).

The Diagnostic Performance

In the framework of the preparation of the new European Guidelines for Quality Assurance in Cervical Cancer Screening, a meta-analysis on test characteristics of LBC and conventional cytology (CP) was also prepared (6). Low-level and progressively higher-level inclusion criteria were considered on separated studies with concomitant testing and two-cohort studies. At the first level, studies that documented rates of cytological abnormality were accepted; at the second level, studies with colposcopic and histological verification of cytological positives were considered, and finally, at the third level, studies where all women were submitted to colposcopy and histology if colposcopic suspicion of lesions were selected. For all levels, ratios of test positivity (LBC/CP) have been computed, in addition for the second and third level ratios of positive predictive values, and for the third level only, relative sensitivity and specificity. Results were compiled according to cytological cut-off ASC-US, LSIL and HSIL and histological outcome threshold CIN categories. Also, the ratios of the proportion of unsatisfactory preparations and the duration of interpretation were analyzed. A series of test and study quality characteristics was established for multivariate analysis. 126 reports were retrieved from 109 studies matching selection criteria. Nevertheless, only 9 studies could be included in the third level meta-analysis. Results pooled from studies with concomitant testing showed nearly equal detection rates of HSIL and positive predictive value for CIN2+ in CP and LBC. However, in two-cohort studies, detection rates of HSIL were substantially and statistically significantly higher in LBC. The sensitivity and specificity of LBC at ASC-US+ and LSIL+ for CIN2+ pooled from studies of the third level never was significantly different from CP. In two-cohort studies, 34% (ratio: 0.66, CI: 0.42-1.02) and 83% (ratio: 0.17, CI: 0.10-0.32) less unsatisfactory smears were found in ThinPrep and AutoCyte/SurePath smears, respectively. Overall, interpretation of LBC required 30% less time to interpret than CP. It was concluded that no
evidence is available to claim higher accuracy of LBC to predict histologically confirmed CIN2+, but recognized that LBC improves the quality and speed of interpretation, and offers the possibility of additional molecular testing. Therefore both CP and LBC for screening in Europe are recommended. Preferences should be determined depending on local economical considerations.

The Automatisation

The smear in LBC was proposed in order to improve quality of cellular spreading out tracking and then make reading under the microscope easier but it was made to allow a reading under microscope more efficient too by cameras connected to computer software. The automated reading was created to increase cytology sensitivity by detecting small abnormal cells of squamous or glandular type, which are difficult to diagnostic in conventional reading. This should increase the specificity too by selecting only reproducible abnormalities. The automation of reading is made to increase productivity by excluding normal slides, by selecting abnormal pictures the pathologist must review.

The Cost

Choices of each country must be done on cost-efficiency studies, which are not transposable from one country to another. The over cost of LBC is more or less important according to the consumable, the equipment in material and the wages related to a manual or automated technique. The reimbursement of LBC in Europe varies from one country to another, but the majority of the systems of health reimburse LBC at the same price as the conventional smear. In England, the recommendations of NICE did not recommend a liquid medium of conservation rather than another (5). Certain countries authorize that the over cost is reimbursed by the private insurance, as in Germany, in Spain, in Italy or Portugal. This over cost is no more authorized in Switzerland since April 2003.

The Quality Assurance

The average ideal to control the quality of the cytological results would be to read again totality or a great part of the smears by using one or two observers or by correlating the results with a biopsy made under colposcopy (2). This remains of course a model, which is not applicable in routine because the women having a normal smear do not have, most of the time, histological follow-up. The misreading led to recommendations of quality assurance, which are single with cytology. The reading of 10% of the smears taken randomly is part of the recommendations of Clinical Laboratory Improvement Amendments (CLIA). The fast second reading of the whole of the smears and the targeted second reading of a risk population (patients with antecedents of abnormal smears, HIV positive, having a sexually transmitted disease) are considered more effective to detect false-negative cytological reading. The other methods generally recommended to detect the negative ones in a retrospective way are the rereading of previous negative smears when abnormalities appear on a smear or a biopsy under colposcopy.

References