Liquid-based cytology for risk-adapted cervical screening

Reinhard Bollmann
Institute of Pathology Bonn-Duisdorf, Bonn, Germany

Summary
In Germany, in the early 70’s, two projects dealing with the development of computer-assisted cervical screening were carried out. It was soon realized, however, that “machine-readable” cytological preparations had to fulfill certain criteria that were difficult to meet using conventional Pap smears: i.e. isolated cells in one optical plane (monolayer), high contrast between nuclei, cytoplasm and background and a clear background. The first issue was solved by the introduction of single cell preparation (monodispersion) and the latter by cell separation and cleansing. This was the advent of liquid based cytology (LBC). Currently, there are three LBC-systems used in Germany: the ThinPrep®, the SurePath® and the PapSpin® method. There are only very few differences between the cytomorphology of LBC and conventional Pap smears. The main difference is the clear background provided by LBC, which makes it easier to identify and interpret abnormal cells. So far, evidence suggests that liquid-based cytopathological methods offer the following advantages (Tab. 1, 2) compared to traditional smear techniques: a) a reduction in the proportion of inadequate specimens, b) an improvement in sensitivity, and c) a possible reduction in specimen interpretation times.

In the USA, 98% of the Pap tests are nowadays liquid-based. This reflects the normous popularity of the method amongst cytopathologists, as demonstratively expressed by the director of cytopathology of the University of Vermont, director of cytopathology of the University of Vermont, amongst cytologists, as demonstratively expressed by the

Table 1: Advantages and disadvantages of liquid-based cytology

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparations are standardized.</td>
<td>• Greater demands on equipment and logistics.</td>
</tr>
<tr>
<td>Cells are treated gently to avoid damage.</td>
<td>• Cytological interpretation differs from conventional methods and users have got to be trained.</td>
</tr>
<tr>
<td>Almost all smear test cells can be examined.</td>
<td>• The number of cells used in a BD SurePathTM Pap Test is not standardized; the staining process follows automatically.</td>
</tr>
<tr>
<td>Possibility to carry out other tests on the rest of the liquid (cytometry, immunocytochemistry, molecular pathology, cell block method).</td>
<td></td>
</tr>
<tr>
<td>Cells are distributed randomly on the slide.</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Results of the IAC Cervix Cancer Screening Meeting from the 20th to the 27th of April 2004, on the realities of liquid-based cytology

1. The sample transferred from the sampling device to the slide is more representative in LBC than in conventional pap smears
2. Reduced number of unsuitable smears
3. Several tests can be carried out on just one specimen
4. Significant increase in the detection of HSIL (“high-grade squamous intraepithelial lesions”)

Introduction
The science of liquid-based cytology is still in its infancy. I bet that George Papanicolaou would be its greatest fan (Leiman 2007).!

In 1943, Papanicolaou and Traut published the famous monography on vaginal cytology as a screening method for cervical cancer. The methods of Aurel Babes in Rumania (Tasca et al. 2002) were also documented during the same period. The technique of extracting cell samples from the cervix, streaking the cells onto a slide and their subsequent fixing, staining, and microscopic evaluation, has not altered greatly since then. The method has proven itself and has led to a decrease in the number of cervical carcinoma diagnosed in Germany. However, the major challenge that remains are the false-negative Pap tests, i.e. when the malignancy or its precursors remain undetected. Published studies dealing with the percentage of false-negative results have become a political issue in Germany: some claim a high false-negative rate (Schneider et al. 2000; Petry et al. 2003) while others can prove a low false-negative rate (Marquardt et al. 2000). The truth probably lies somewhere in between. Based on meta-analyses, an international false-negative rate of about 50% is assumed (Nanda et al. 2000). This low sensitivity can be compensated by regular participation in screening. The possible causes of false-negative cytology are listed in table 3.

Table 3: Possible causes of false-negative Pap tests

<table>
<thead>
<tr>
<th>Cause</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No atypical cells in the preparation (incorrect sampling)</td>
<td>60-75%</td>
</tr>
<tr>
<td>Atypical cells are present but cannot be identified due to poor fixation and/or overlapping</td>
<td></td>
</tr>
<tr>
<td>Atypical cells are present, but are being overlooked by the screener (“screening error”)</td>
<td></td>
</tr>
<tr>
<td>Atypical cells are present and are identified but not correctly interpreted, thus they are falsely judged</td>
<td></td>
</tr>
</tbody>
</table>
It is difficult to standardize the preparatory steps, such as sampling the cells and transferring them onto the slides: over 80% of the specimens taken are discarded together with the sampling device (Hutchinson et al. 1994). The prepared smears demonstrate artistic variations (Fig. 1). As described later on in this article, thin-film cytology provides a solution to the problem of standardizing cell transfer to slides.

Screening errors (atypical cells are overlooked) can be avoided by computer-assisted screening.

**The history of liquid-based cytology**

In the 1970s, the German state financed two research projects on computer-assisted cervical screening: LEYTAS (Leyden Texture Analysis System) and FAZYTAN (automated early detection cytological analysis). During the projects, it became clear that if they were to be used with machines, the cytological preparations would have to fulfill certain criteria (Tab. 4). In Germany, this realization led to the development of single cell techniques and to cell separation and cleansing (see Tab. 5) as the main steps in liquid cytology (synonyms: thin-film cytology, monolayer cytology, liquid-based cytology). Ideally, the aim of the single cell technique (monodispersion) is to produce isolated cells in one optical layer (monolayer) and thus avoid the formation of cell clumps.

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Technical solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Isolated cell mounting</td>
<td>• Single cell separation (monodispersion)</td>
</tr>
<tr>
<td>• Mounting in one optical plane (monolayer)</td>
<td>• Cell separation and cleansing</td>
</tr>
<tr>
<td>• High contrast between the nucleus, cytoplasm and background</td>
<td></td>
</tr>
<tr>
<td>• Clean background of the preparation</td>
<td></td>
</tr>
</tbody>
</table>

While the term “monodisperse cell separation” focuses on isolated mounting of the individual cells, the expression “monolayer preparation” emphasizes the fact that the cells lie in one plane. However, the liquid-based cytology technique does not yet meet these criteria: liquid-based cytology preparations contain more three-dimensional cell structures than those of conventional cytology, requiring the screener to constantly refocus (Fig. 2). It is therefore consistent to replace the term monolayer cytology with thin-film or liquid-based cytology.

**Table 4: Cytological preparations for use with machines**

![Figure 2 a-c: Three-dimensional dysplastic cell structures in a Thin-Prep® preparation (Pap IVb) have to be refocused, therefore the term “monolayer” is not appropriate](image)
Liquid-based cytology systems

Three liquid cytology systems are presently available in Germany: ThinPrep® (Hologic), BD SurePath™ PapTest® (Becton Dickinson) and PapSpin® (ThermoShandon), and other similar methods (see below).

The main differences between the systems are shown in tables 5 and 6.

Table 5: Methodological requirements of standardized liquid-based cytology

<table>
<thead>
<tr>
<th>Single cell isolation (monodispersion)</th>
<th>Cell separation and cleansing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mechanical</strong></td>
<td></td>
</tr>
<tr>
<td>· Vortexation (PapSpin®)</td>
<td></td>
</tr>
<tr>
<td>· Stirring (ThinPrep®)</td>
<td></td>
</tr>
<tr>
<td>· Filtration (SurePath®)</td>
<td></td>
</tr>
<tr>
<td>· Cannulation (SurePath®)</td>
<td></td>
</tr>
<tr>
<td>· Homogenization and ultrasound</td>
<td></td>
</tr>
<tr>
<td>· Density gradient centrifugation</td>
<td>Density gradient centrifugation</td>
</tr>
<tr>
<td>· Membrane filter (ThinPrep®)</td>
<td>Membrane filter (ThinPrep®)</td>
</tr>
</tbody>
</table>

**Chemical**
- Enzymes
- EDTA
- Dithiotreitol (PapSpin®)
- Detergents

Table 6: Differences between different liquid-based cytology methods

<table>
<thead>
<tr>
<th></th>
<th>ThinPrep® (Hologic)</th>
<th>SurePath® (Becton Dickinson)</th>
<th>PapSpin® (Shandon)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single cell isolation</strong></td>
<td>Rotation</td>
<td>Capillaries</td>
<td>Vortexation</td>
</tr>
<tr>
<td><strong>Cell separation and cleansing</strong></td>
<td>Membrane filter</td>
<td>Density gradient centrifugation</td>
<td>No</td>
</tr>
<tr>
<td><strong>FDA-licensed</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

*In the following short SurePath®

The procedure for cell sampling is always the same: the instrument used for sampling is either thoroughly rinsed in the liquid (ThinPrep®), or the head of the instrument is removed at the predetermined breaking point and placed in the liquid (SurePath®). The manufacturers of the equipment provide training material on how to take the smear.

The composition of the liquids is patented and is therefore unknown. The liquids probably consist of buffered cell mediums with a relatively low alcohol content (methanol), as they must fulfill both the requirements of a cell transport medium and a cell fixative.

Aims of liquid cytology

- To recruit all exfoliated material for the cytological examination
- Have method-immanent evidence on the cellularity as a quality assurance measure
- Optimize visual morphological examination through an improved presentation of the material
- Fulfill the criteria for computer-based screening
- To temporarily document the material for further morphological and non-morphological examinations
- Enable biomedical assistants to work more safely and in a less tiring manner, partly due to the elimination of disruptive elements (e.g. inflammatory cells, mucus, fibrin)

Production of cytological preparations

ThinPrep® (Hologic)

Figure 3 shows the automated steps of the ThinPrep® method that take place in the T2000 Processor. Firstly, a rotating cylinder mechanically isolates the cells. Following this dispersion, the cells are filtered. The lower end of the cylinder is covered with a micropore filter and a vacuum is mounted at the upper end. The liquid containing the cells is thus sucked through the filter. The pores of the filter are large enough to allow neutrophil granulocytes and erythrocytes to pass, but too small to let epithelial cells pass. Gradually, epithelial cells obstruct the pores of the filter. The processor can detect when all the pores of the filter are obstructed and complete the filtration process. The filter is then thrown onto an electrically charged slide in order to transfer the cells onto the slide, which is stained in the following step.
Figure 3: Automated steps of the T2000 Processor in the ThinPrep® technique

a: 1. Cells in the liquid; 2. A rotating cylinder causes cell dispersion (mechanical single cell isolation); 3. A vacuum is then mounted on the cylinder and the cells in the liquid are sucked through the filter; 4. The cylinder with the membrane filter is then “thrown” onto an electrically charged slide and the cells are thus transferred to the slide.

b: Step 3 in detail

c: ThinPrep® Processor T2000
With these techniques, the cells are isolated by vortexation and are subsequently centrifuged. The cells are neither separated nor cleaned beforehand. However, these techniques are not yet authorized by the FDA (Bollmann and Jordan 2005).

**Pro and contra: licensed versus unlicensed methods**

Due to high costs, the methods that are not authorized by the FDA (PapSpin®, amongst others) do not include the main steps of standardized preparation, i.e. cell separation and cleansing (see Tab. 6), so that the preparation produced still contains inflammatory cells, blood, fibrin and detritus.
However, if the cells are also separated and cleansed, as in the ThinPrep® Pap test (Hologic) or the SurePath® Pap test (Becton Dickinson), the diagnostically relevant cells can be enriched. The methods that do not include previous cell separation and cleansing with simple centrifugation deliver preparations having coincidental, questionably relevant accumulation of cells, as the diagnostically important cells are not enriched.

Despite this, it appears that these less expensive methods provide an alternative to the established methods if the preparations are carefully processed and if the cell content of the liquid is determined turbimetrically. This has been suggested by a study carried out at the John Hopkins Hospital, Baltimore (Rosenthal et al. 2006). One can only hope that the manufacturers of the methods licensed by the FDA follow a different pricing policy in the future so that in Europe, the equipment becomes available at reasonable prices, as is the case in Belgium (ThinPrep®). We have to consider that the WHO only approves of less expensive methods for “mass screening”. Correspondingly, the German Cytological Society (DGZ) is politically right when rejecting liquid-based cytology due to its high costs. However, it is surprising that a scientific society enlists the price of a method as an argument against its use.

Staining
On completion of the automated ThinPrep® process, the so produced unstained preparation can be stained using a variety of automatic staining apparatus. The author’s own experience shows that it is advisable to slightly modify the staining protocol used for conventional smear preparations. In the SurePath® technique, staining is an integral part of the process, whereby the results of staining may differ from staining conventional preparations, especially with regard to the coloration of cytological cornifying processes. Keratinized cells are, however, an important marker both in detecting alterations caused by HPV as well as keratinized carcinomatous solitary cells. The fact that keratinized cells do not stain well led to a relatively poor rating for the SurePath® staining method in an external technical quality assurance program carried out in Great Britain (TEQA) (NHS CSP 2004).

Screening methods for liquid-based cytology
The screening fields of the slide are round-shaped and much smaller than the fields in conventional preparations (Fig. 5). Screening methods therefore differ from those used for conventional smear preparations: abnormal cells can be found in any position and are not grouped together in so-called streets, as observed in conventional preparations. The fields must therefore overlap in this type of screening. Additionally, screening of LBC preparations is altogether more intensive compared to conventional preparations, as atypical cells can be “hidden” in the gaps between the normal cells. Additionally, it is oftentimes necessary to change from the 10mm objective (screen enlargement) to the 40 mm lens to be able to precisely evaluate the solitary cells. Overall, the liquid-based cytology screening process requires greater concentration of the technician.

Adequate cell number for liquid-based cytology preparations
In the Bethesda Classification, 5000 cells are considered as adequate. McQueen and Duval (2006) were able to show that at least 87 atypical cells must be present in a preparation in order to detect them with certainty. However, according to mathematical calculations this can only be achieved with a total of 10,000 cells. In Wales, a limit of 15,000 cells has been established (Cervical Screening Wales 2006). A study presently being carried out by the National Health Service (NHS) aims at solving the cell number issue for Great Britain. The cell number can only be correctly judged with a great amount of experience, thus having completed intensive training and use of comparative images (Solomon and Nayar 2004).

Cell morphology of liquid-based cytological preparations in comparison to conventional preparations
Liquid-based cytology preparations are very similar to those of the regular smear method, although their morphology can sometimes differ fundamentally (Tab. 7). The most important difference – in comparison to regular methods – is the clear background of liquid cytology preparations, which enables an easy “visual access” to abnormal cells, thus facilitating their interpretation (Fig. 6).
Apart from the differing localization of atypical cells ("missing streets"), liquid-based cytology preparations also differ from conventional preparations in that there is less blood, fibrin, and necrotic debris on the slides due to their special processing. For many years, such artefacts have served as evidence for malignant processes in conventional cytology. Although this so-called tumor diathesis is also always found in preparations in liquid-based cytology, it is much more discreet and hangs like wallpaper on the surface of the cells and cell structures (Tab. 9). The squamous epithelial carcinoma should rather be recognized due to its characteristic morphology and not due to such debris (Tab. 10, Fig. 7a-d). Glandular neoplasias also present a characteristic picture in thin-film cytology (Tab. 11, Fig. 8a-d) and are easily recognized.

Table 7: Morphology of liquid-based cytology preparations in comparison to conventional preparations

- More similarities than differences
- Lower number of pathological cells/slide
- Easier "visual access" to abnormal cells due to the clearer background
- Shrunk cells (fixation in alcohol)
- Cells are more circular in solution
- Cytoplasm and nuclear structures are more easily recognized (wet fixation, lack of air-drying artefacts)
- Thus lower nuclear hyperchromasia

Table 8: Morphology of high-grade lesions (HSIL) in liquid-based cytology

- Increased number of isolated dysplastic cells
- Smaller cells with a decreased nucleus are more frequently found
- Cells with a greater nuclear-cytoplasmic relation are frequently found

Table 9: Discreet tumor diathesis in carcinomas using liquid-based cytology

- Diathesis consisting of blood, fine granules of fibrin and necrotic debris is almost always found, but is discreet
- Diathesis "hangs" like wallpaper on the surface of the cells and cell structures ("clinging diathesis")

Table 10: Morphology of the squamous epithelial carcinoma shown using liquid-based cytology

<table>
<thead>
<tr>
<th>Cornified type</th>
<th>Noncornified type</th>
</tr>
</thead>
<tbody>
<tr>
<td>- There is no difference in keratinized cells in comparison to conventional preparations</td>
<td>- Tumor cell structures are more three-dimensionally layered</td>
</tr>
<tr>
<td></td>
<td>- Cytoplasm is more strongly contracted and denser</td>
</tr>
<tr>
<td></td>
<td>- The nucleus appears smaller</td>
</tr>
<tr>
<td></td>
<td>- Chromatin is distributed more evenly (cells appear &quot;less malignant&quot;)</td>
</tr>
<tr>
<td></td>
<td>- Nucleoli are more prominent</td>
</tr>
<tr>
<td></td>
<td>- At a lower magnification, tumor cell structures may be mistaken for benign squamous epithelial metaplasia</td>
</tr>
<tr>
<td></td>
<td>- Malignant nuclear characteristics are preserved (pleomorphism in size and shape plus the irregular contours of the nucleus)</td>
</tr>
</tbody>
</table>
Cytological details are well preserved in liquid-based cytology, for instance enabling the clear depiction of the nuclear chromatin. This has led to a renaissance of the "non-classical HPV signs", which had already been described many decades ago (Schneider et al. 1987).

Table 11: Morphology of glandular neoplasias using liquid-based cytology

- Increased number of isolated tumor cells
- Glandular cell structures are thicker (three-dimensional), "side contours" are more difficult to recognize
- Smaller nuclei
- Chromatin is evenly distributed (cells appear "less malignant").
- Nucleoli are more prominent
- Malignant characteristics of the nucleus are preserved (pleomorphism in size and shape plus irregular contours of the nucleus)

Figure 7: Squamous epithelial carcinoma
a-b: Three-dimensional cell structures of a squamous epithelial carcinoma with severely atypical nuclei and fine granular deposits on the margins (wallpaper-like tumor diathesis)

Figure 8 a-d: Cell structures of varying size in an adenocarcinoma of the uterine cervix; glandular deposition and conspicuous atypical nuclei are easy to detect
The assessment of these criteria combined with the recommended type-specific HPV PCR analysis is an integral part of the Bonn Model of cancer prevention (Bollmann et al. 2007).

Apart from possible false-negative cytologies, another difficulty are the false-positive cytological findings, i.e. the low positive predictive value of cytology (ppv). The literature on the ppv of atypical cytologies shows results ranging from 11.4 % (Petry et al. 2003; Ronco et al. 2006) to 70.6 % (Schneider 2000), with respect to the histological diagnosis ≥ CIN II. In Germany, the follow-up costs to clarify findings and to treat nonspecific and abnormal findings found in cervix cytology amount to 55 million euro (Schneider et al. 2007). A method that increases the ppv could therefore signify enormous savings:

• An increase in the ppv of just 1 % in cancer prevention cytology would spare 1 200 women from unnecessary treatment and save health care costs (Baak et al. 2005).

• One of the main measures taken to improve the ppv is HPV triage of atypical cytological findings. In our material we achieve a ppv of 82 % (Bollmann et al. 2005). The cost-effectiveness of HPV triage was established in a decision-analytical model (Sheriff et al. 2007). A further improvement in ppv is possible if HPV triage is combined with static DNA cytometry.

High-Risk HPV infection may lead to disruptions in the cell cycle and to chromosomal aneuploidy.
Aneuploidy is today regarded as a progression marker and can be detected by fluorescent in situ hybridization (FISH) (Mehes et al. 2004) or by static DNA cytometry in routine diagnostics (Grote et al. 2004).

The results of DNA cytometric tests support the dual Bethesda classification: low-grade intraepithelial lesions (LSIL) are DNA-diploid, high-grade intraepithelial lesions (HSIL) are DNA-aneuploid (Bollmann et al. 2001).

Liquid-based cytology therefore provides an optimal platform for risk-adapted multimodal gynecological cancer prevention with a high ppv. By a simultaneous HPV genotyypization together with DNA image cytometry, an 88.2 % positive predictive value (ppv) for intermediate and higher-grade cervical dysplasias and carcinoma (≥ CIN II) can be achieved (Bollmann et al. 2007). In current literature, no method has reaches a higher ppv. This multimodal risk-adapted cervical screening avoids unnecessary surgery and saves costs. In addition, other molecular methods can be carried out with the liquid medium: determination of onco-genic HPV-E6/E7-mRNA (Varnai et al 2008), p16 ELISA test (Mao et al. 2007), or the simultaneous determination of a HPV infection and 3q26 and 8q24 aneusomia by FISH (Sokolova et al. 2007).

Cytological correlation (cell block technique)
Liquid-based cytology allows the production of histological preparations using the so-called cell block technique (Gupta et al. 2007) (Fig. 10). Liquid-based cytologies contain regular “mini-biopsies” (Yeoh u. Chan 1999), on which good immunohistochemical tests can be performed.

Validity
A pilot study carried out in England by the Department of Health on the introduction of liquid-based cytology showed that the number of unsuitable preparations decreased from 9.1 % (conventional) to 1.6 % (liquid-based cytology) (Moss et al. 2002). In addition, screening had increased by 9 %. These results were sufficient reasons for including liquid-based cytology into England’s cancer prevention program, even if the results of the pilot study could not prove the superior sensitivity of liquid-based cytology when compared to the conventional smear technique. In the meantime, official reports (also from Scotland) have confirmed an increase in the detection rate and a higher ppv (department of health) (Williams 2006).

The FDA has established the following licensing guidelines for recognized methods:

For the ThinPrep® Test (Hologic):
• Licensed as an alternative to the conventional smear test (1996)
• Approved as a significantly more effective method in the detection of low- and high-grade lesions in comparison to conventional techniques (1996)
• Approved because the “sample quality is significantly better in comparison to conventional preparations” (1996)
• Approved as being significantly more effective (+59.7 %) in detecting high-grade lesions in comparison to conventional preparations (2003)
• Additional tests for HPV are approved using the PreserveCyt solution with the Hybrid-Capture method (2002)
• The PreserveCyt solution is approved for PCR screening for chlamydia (2003)
• Approved due to an increased rate of detection in abnormal glandular cells (2005)
• ThinPrep® Imaging-System: approved as a computer-assisted system for primary screening of cytological preparations. Focal Point identifies 25 % of the successfully screened preparations as being, with a high probability, normal, thus requiring “No Further Review”. Licensed for conventional and thin-film preparations (1998, respectively 2001).

For the SurePath® Test:
• Approved as an alternative to the conventional Pap test (1999)
• Approved as a significantly more effective method (+64.4 %) for detecting high-grade lesions in comparison to the conventional smear (2003)
• Focal Point System: approved for primary screening of cytological preparations. Focal Point identifies 25 % of the successfully screened preparations as being, with a high probability, normal, thus requiring “No Further Review”. Licensed for conventional and thin-film preparations (1998, respectively 2001).

At present, there is no comparable licensing for PapSpin® or other thin-film techniques.
A meta-analysis recently published in the Lancet concludes that liquid-based cytology is no better than conventional cytology. The number of unsuitable cytological preparations was neither lower, nor was the detection rate of high-grade lesions higher (Davey et al. 2006).

In an investigation carried out in Medline, the authors of the meta-analysis found 145 publications on the validity of liquid-based cytology. Of these publications, 116 (80 %) were discarded, as they did not fulfill the inclusion criteria postulated by the authors of the meta-analysis. 27 other studies, which were not found in Medline, were added to the remaining 29 studies. Of the remaining 56 studies, none were found to be “ideal”, 5 were judged to be of “high quality”, 32 “intermediate quality” and 19 were “poor quality”. The meta-analysis carried out by Davey and coworkers was based on the evaluation of 1.25 million slides. In conclusion, the meta-analysis does not support the proposition that liquid-based cytology produce better results and suggests that large randomized studies will have to be carried out.

When comparing the results of the study by Davey to those of the British study (Moss et al. 2002), we can draw the conclusion that the good English results are based on a better training program for screeners: liquid-based cytology requires experience and the method cannot be used without training. The screener must be aware of the differences between the various methods. In England, liquid-based cytology has been available to all women via the National Health Service since the end of 2008. In the USA, 98 % of the Pap tests are liquid-based (Institute of Management and Administration 2007), as in the USA a reflex HPV test is required in the case of ASCUS (“atypical squamous cells of undetermined significance”). This test is carried out with the preserved liquid. Therefore it is not necessary to recall the patient in order to collect new samples.

Incidentally, the College of American Pathologists (CAP) has discovered that in the cytological examinations prescribed by the state (“proficiency program”), cytologists and cytological assistants significantly more often detect lesions when using liquid-based cytology compared to conventional preparations (Renshaw et al. 2004).

It is regrettable that liquid-based cytology has led to a schism amongst cytologists with regard to the evaluation and use of the method and that the study by Davey has been greeted with such vehemence by the “partisans of the antiliquid league” (Leiman 2007) (Obwegeser and Schneider 2006).

The rigid stance of the German Society for Cytology is equally incomprehensible; the Society’s statement on liquid-based cytology, issued in 2003, has still not been revised, even if members of the board of this scientific society, such as the head of the board of directors, regularly make use of the method (Bayer-Pietsch and Flenker 2004). Above all, the DGZ considers the high costs of the tests and the financing of studies by the manufacturers as a reason for rejecting the method. To be consistent, the DGZ should also reject HPV vaccination, as it is financed by the industry and the vaccination is expensive. In contrast, the German HTA study (HTA: Health Technology Assessment; Siebert et al. 2003) came to the conclusion that the use of new screening technologies (thin-film preparation and computer-assisted evaluation methods) are acceptable per year of life saved, if screening takes place over a period of three or more years.

**Computer-assisted screening**

Liquid-based cytology was advanced by the development of computer-assisted cytology. Nowadays, there are technically developed systems that can be used in everyday diagnostics. Becton-Dickinson Imaging (a subsidiary of SurePath) has developed the Focal Point and Focal Point GS System, which is now distributed by Becton-Dickinson. Besides preparations produced by the SurePath technique, the system also scans conventional preparations. A good smear technique is a prerequisite, as the computer is not able to read suboptimal cytological smear preparations. In order to produce optimal preparations for computer-assisted systems, foam-based sampling devices can be used (e.g. Cervisoft®, PapCone®) (Bollmann et al. 2002). The system identifies abnormal cells using an adaptive morphometric algorithm and sorts them according to the probability of being abnormal. 25 % of the preparations are classified as “no review” and can be issued as a negative result in the USA. The procedure is licensed by the FDA. However, in Germany, supplementary information has got to be documented, such as the degree of proliferation and microbiology. The preparations must therefore be evaluated by cytological assistants. In Germany, the system is used together with the Focal Point GS. Pictures of atypical cells are taken and their coordinates are stored. With the aid of a motorized microscope table, the 15 most abnormal cells are presented at the microscope for evaluation before the whole of the slide is conventionally screened. Focal Point GS has not yet been licensed by the FDA.
The coordinates of 22 cells with abnormal densitometric values are stored and after scanning, are presented on the microscope. Both methods are significantly more sensitive than manual screening, or at least as sensitive (Davey et al. 2007; Wilbur et al. 1998) (Biscotti et al. 2005; Ronco et al. 2003).

Slight koilocytic dysplasias may be overlooked by TIS (Zhang et al. 2007). It is possible that these cells do not differ densitometrically from normal cells and are therefore not recognized by the system. In this regard, it should be mentioned that the percentage of invasive cervical carcinoma that are DNA-diploid is not low (Horn et al. 2002).

Increased productivity
An English pilot study showed a 9% increase in screening when using liquid-based cytology preparations (Moss et al. 2002). This percentage is even higher when combined with computer-assisted methods (Wilbur et al. 1998; Davey et al. 2007).

Technical problems associated with the processing of liquid-based cytology preparations
Liquids containing a lot of mucous or blood can block the pores of the cell filter in the ThinPrep® method (Bentz et al. 2002), producing preparations that are low in cell content and appear “moth-eaten” (Figure 11a, b). In such cases, a second preparation must be processed after mucolysis or erythrolysis. The possibility of cell carryover is an additional problem with the ThinPrep® technique, if the preparation is not carefully processed. The cylinder with the cell filter naturally carries cells on its outer walls that were immersed in the preparation liquid. The used cylinder is unscrewed after preparation of the sample and is replaced by another filter. It must be ensured that no cells are transferred from the used to the new cylinder by using disposable gloves. In the SurePath® method, cells may be lost (FDA).

Costs of liquid-based cytology and billing modes
Thin-film preparation costs 29.03 euro while conventional cytology only costs 17.28 euro (the latter cost compares with an award of only 165 points for the EBM code number 01733)! (EBM = the so-called “Unique Evaluation Table”, Einheitlicher Bewertungsmaßstab) (Siebert et al. 2003).

Following a ruling of the German Medical Association, liquid-based cytology is billed according to §6 GOÄ (Gebührenordnung für Ärzte – medical fee schedule) analogous with code number 4815. Fees can also be charged according to §10 GOÄ. In the new draft of the GOÄ, one code number, which was not previously included, has been added for sample extraction.

Patients covered by the statutory health insurance can only be charged under the IGeL system (privately paid health services). In any case, it seems appropriate to use code number 1 GOÄ for consultancy fees. Many gynecologists

Figure 11: Problems associated with processing in the ThinPrep® technique
a: Cell-free, moth-eaten fields on the stained slide
b: Microscopic preparation of a filter of such a case shows the obstruction of the comma-shaped filter pores by erythrocytes (round components) and mucous (strongly refractile table-like structures)
and cytologists are of the opinion that the IGeL monolayer test should always simultaneously include a conventional smear, as otherwise the requirements of the services outlined in code number EBM 01733 are not fulfilled. Apart from the fact that the purpose of liquid-based tests is thwarted by such procedure, the above-mentioned interpretation is, in the author’s opinion, incorrect. The service legend for EBM code 01733, used for billing cytological examinations according to Section B.2 of the cancer prevention guidelines, stipulates the obligatory content as “cytological examination of one or more smears, also brush smears, of the ecto- and/or endocervix.” Many gynecologists and cytologists automatically equate the expression “smear” with the application of the sample material to the slide. The author cannot follow this line of argumentation: a smear is the removal of bodily material from the surface of a wound or mucous membrane (mouth, urethra, vagina, anus) using a sterile swab, small brushes, or small spatula for the purpose of examination.”

The cancer prevention guidelines (last altered on 01.12.2003) state: “Cytological examination includes the evaluation of material removed for the purpose of cytological examination”. How to preserve the material is not prescribed. Consequently, transferring the sample material to a liquid medium as an alternative to the regular processing of slide-based preparations is not excluded. The latest resolution of the Federal Committee of the 19.12.2006 also does not explicitly forbid the use of liquid-based cytology: “In its meeting on the 19.12.2006, the Federal Committee decided that thin-film cytology and HPV test methods will not be implemented as a cancer prevention screening test for cervical carcinoma.”

The expression “will not be employed” does not indicate that thin-film cytology is not an approved method; in fact, the committee simply decided not to implement the method at present. It is not expressly forbidden to bill liquid-based cytological examinations under the code for preventive examinations. Only the preparation process has got to be billed as a privately paid service (IGeL).
References


PAPANICOLAOU GN, TRAUT HF. Diagnosis of uterine cancer by the vaginal smear. New York: Commonwealth Fund 1943.


Williams A. Liquid based cytology and conventional smears compared over two 12 month periods. Cytopathology 2006; 17: 82–85.


Prof. Dr. med. Reinhard Bollmann, MIAC

Institute of Pathology Bonn-Duisdorf
Heilsbachstraße 15
53123 Bonn

Dr. Reinhard Bollmann studied in Bonn and completed his doctoral studies in Freiburg. While working as an assistant physician at the Institute for Pathology at Freiburg University, Dr. Bollmann was awarded a DFG scholarship to study in London (Department of Histochemistry, Hammersmith Hospital). For seven years he worked as a senior physician in clinically oriented hospital pathology.

In 1980, Dr. Bollmann founded a private institute for pathology that focuses on the field of cytopathology. He is founder of the Leistungserbringungsgemeinschaft für Molekularpathologie (GenOPath GbR – a company providing services in the field of molecular pathology), in which 11 pathological institutes are members. Dr. Bollmann is a member of the board of the Arbeitsgemeinschaft zytologisch tätiger Ärzte Deutschland (AZÄD – a work group of German physicians active in the field of cytology) and the Berufsverbandes Deutscher Pathologen e.V. (professional association of German pathologists). At present, Dr. Bollmann is head of the cytological commission of the Kassenärztliche Vereinigung Nordrhein (Association of Physicians – North Rhein).

Conflict of interest
The author declares that this article was written without the influence of any industrial interests. Dr. Bollmann also declares that he has received a consulting fee from Cytc. However, the payment of this fee has in no way influenced the content of this article.

Manuscript data
Submitted on: 16.11.2007
Accepted on: 16.01.2008
Question 1
What is the most frequent cause of a false-negative result in cancer prevention cytology?
- a. A screening error
- b. An error in sample removal
- c. An error in interpretation
- d. A random error
- e. A distributional error

Question 2
What is the percentage of cells that is discarded together with the sampling device in a conventional smear (Pap smear)?
- a. 20 %
- b. 50 %
- c. 80 %
- d. 0 %
- e. 99 %

Question 3
Which technique is employed to position isolated cells on an optical single plane (monolayer)?
- a. Centrifugation
- b. Single cell preparation (monodispersion)
- c. Filter techniques
- d. Cell enrichment
- e. Ultrafiltration

Question 4
Which technique provides a high contrast between the nucleus, cytoplasm, and the background?
- a. Cell separation and cleansing
- b. Centrifugation
- c. Magnetic enrichment
- d. Phase contrast
- e. Densitometry

Question 5
In the ThinPrep® method, which technique is used for single cell preparation (monodispersion) and cell separation and cleansing?
- a. Shaking
- b. Stirring (in a cylinder) and membrane filter
- c. Cytocentrifugation
- d. Magnetic enrichment
- e. Density gradient centrifugation

Question 6
In the SurePath® technique, which method is used for single cell preparation (monodispersion) and cell separation and cleansing?
- a. Vortexing and density gradient centrifugation
- b. Cytocentrifugation
- c. Filtering
- d. Magnetic enrichment
- e. Ultrafiltration

Question 7
Which sub-step in monolayer preparation methods licensed by the FDA is not included in the PapSpin® method?
- a. Cytocentrifugation
- b. Cell separation and cleansing
- c. Filtration
- d. Cell counting
- e. Densitometry

Question 8
In liquid-based cytological preparation, one phenomenon, which is regarded as an important indication of the preliminary stages of cancer in the conventional Pap smear, is missing:
- a. The “street” phenomenon
- b. The phenomenon of nuclear hyperchromasia
- c. The phenomenon of koilocytosis
- d. Cell overlapping
- e. Dyskaryosis

Question 9
For which method does liquid-based cytology provide an optimal platform?
- a. Long-term documentation
- b. The “molecular Pap”
- c. DNA cytometry
- d. Photographic documentation
- e. Cytological consultation

Question 10
What is the ppv that can be achieved by combining liquid-based cytology with HPV triage and DNA cytometry (risk-adapted multimodal cancer preventive cytology)?
- a. 20 %
- b. 45 %
- c. 88 %
- d. 100 %
- e. 5 %