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Accuracy of a Low Priced Liquid-Based Method for Cervical Cytology in 632 Women Referred for Colposcopy After a Positive Pap Smear

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The aim of this quality controlling study was to determine the accuracy of liquid-based cytology (LBC) with the Turbitec[®] cytocentrifuge technique. Cervical smears of 632 women, who were referred to our CIN outpatient department, after at least two smears with ASCUS or higher were evaluated and compared with the histological outcome. In 592 cases the smears revealed abnormalities of squamous epithelium, and in 40 cases the abnormalities of glandular epithelium. In the group of squamous epithelium abnormalities, the sensitivity for LSIL was 39.7% and the specificity was 89.2%; for the LSIL+ group, these values were 89.4% and 91.4%, respectively. For HSIL the sensitivity was 68.3% and the specificity 92.8%, for the HSIL+ group 82.3% and 92.3%, respectively. The ASCUS rate was low (2.4%). The Turbitec cytocentrifuge method was proved to be a very good LBC method for cervical smears. Because of a comparable accuracy together with a lower price, this LBC method outweighs commercial alternatives. Diagn. Cytopathol. 2009;37: 579–583. © 2009 Wiley-Liss, Inc.

Key Words: cytology; liquid-based; cervix; low cost

Liquid-based cytology (LBC) methods of preparation of cell suspensions have been introduced to improve the quality of cellular morphology and cytodiagnosis. Several methods are now commercially available, of which the FDA-approved ThinPrep method is used worldwide on a large scale for population screening. A major disadvant-

age of ThinPrep is its price. It is known that the average cost for cervical screening associated with ThinPrep is much higher than with a conventional cervical smear.^{1,2} In 1999, the direct additional disposable cost was estimated \$9.75.³ A recent cost minimization analysis carried out in England showed that the most optimal lowest total processing cost per slide would be £3.68 (\$5.64).⁴ Low-cost LBC methods have been described. One of these alternative monolayer slide preparation methods makes use of a cytocentrifuge and an alcoholic-agar solution, named 3MLBC.^{5–7} Other known systems in Europe using the Hettich cytocentrifuge are CytoSCREEN[®] (Seroa, Monaco, France) and Turbitec[®] (Labonord, Templemars, France). In this quality control study, we determined the sensitivity and specificity of LBC with the Turbitec cytocentrifuge technique in a series of 632 women who recently had an abnormal cervical smear and were referred to our CIN outpatient clinic. These women had either smears with ASCUS, LSIL, HSIL, squamous cell carcinoma ($n = 592$) or a glandular epithelial lesion ($n = 40$). In all cases, histological follow-up was available to determine the accuracy of this LBC method.

Materials and Methods

Cervical cytological smears of 655 women visiting the CIN outpatient clinic of the Department of Gynecology of the University Medical Center Groningen between 2000 and 2007 were included to analyze the accuracy of the Turbitec cytocentrifuge LBC method. This group consisted of women referred after a positive cervical smear of at least two ASCUS-scores. These cytological samples were primarily obtained for a study of the role of tumor suppressor gene hypermethylation in cervical dysplasia and cancer.⁸ As part of that study this LBC method was

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applied to determine the presence and classification of abnormal cells according to the Bethesda classification.⁹ The study was approved by the medical ethics committee of the University Medical Center Groningen.

In brief, the liquid-based Turbitec monolayer technique applied has the following technical steps: cervical cells obtained with a cytobrush are rinsed directly into a vial fixation solution of 50% ethanol with 7% polyethylene glycol 300. This solution is vortexed and cell density of the cell suspension is measured with a photoelectric analyzer (Labonord, Templemars, France). Depending on the cellularity of the sample, up to 6 ml of cell suspension is transferred to a 8 ml Hettich chamber already filled with two drops of an adhesive albumin solution (Stick-on, Labonord, Templemars, France) in 1.5 ml ethanol-polyethylene glycol 300. A cell sediment is obtained using a Hettich cytocentrifuge (Andreas Hettich, Tuttlingen, Germany) at 1,000 rpm for 10 minutes. For this purpose, a cytoinsert with a filter card and a microscopic glass slide already coated with 0.01% poly-L-lysine are used to obtain a slide with a cellular monolayer with a diameter of 17.5 mm. The slides are dried on top for 15 minutes in the Hettich cytocentrifuge and air-dried for 5 minutes. Cervical cells are stained with a modified Papanicolaou stain.

Biopsies (large loop excision specimens of the cervix) and hysterectomy specimens were fixed in 4% neutral-buffered formalin and sectioned for light microscopy. Histological sections were stained with hematoxylin-eosin (H&E).

Eighteen Turbitec specimens were excluded from the study because of low cell count, and five specimens were excluded because of inadequate histological sampling, resulting in an inadequacy rate of 4.0%.

The remaining 632 specimens were included for determination of the sensitivity and specificity of Turbitec LBC. Cervical cytological abnormalities were scored by two experienced and LBC trained (CT/IAC) cytotechnicians and cytopathologists according to the Bethesda system. In case of discrepancy, a third experienced cytotechnician scored the smear. These slides were scored in a research setting, not mixed with daily normal cervical population screening practice. The screeners were aware that most of the slides belonged to a population of women with a positive pap smear, but did not know which particular ones, as these were randomly mixed with slides from women who had undergone a hysterectomy for either uterine prolaps or leiomyoma. The cytological results were correlated with histology of subsequent biopsies (large loop excisions) or hysterectomies. The histological diagnosis of the presumed cervical lesion was considered the gold standard.

In 52 cases with discrepancies between cytodiagnosis and histological follow-up diagnosis, smears were reeval-

uated to find out whether this discrepancy was due to either technical shortcomings or due to diagnostic errors made by the cytotechnicians or cytopathologists. These discrepancies were normal cytology with a histology of either CIN I (24 cases), CIN II-III (9 cases), or invasive carcinoma (4 cases), and, in addition, normal histology with cytology scored as LSIL (11 cases) or HSIL (4 cases).

Cytology and histology outcomes were arranged in contingency tables. Sensitivity and specificity were calculated for cervical squamous cell lesions. Rates of diagnostic agreement were analyzed by χ^2 and McNemar tests with SPSS software version 14.

Results

The slides were characterized by a high-quality morphology. Dysplastic and malignant cells were well preserved. Cellular material was evenly distributed on the slide and lacked drying artifact. Erythrocytes were lost due to erythrolysis, whereas neutrophilic granulocytes were loosely arranged instead of lying in thick streaks. As a result, atypical, dysplastic, and malignant cells were easy recognizable. Microbiopsies were present and in invasive carcinomas a tumour diathesis reflected by necrotic background was well preserved, which is in contrast with commercial available LBC methods where it is most often lost. Because of the optimal fixation with a solution of 50% ethanol with 7% polyethylene glycol, 300 nuclear features and chromatin pattern were excellently preserved both for squamous and glandular neoplasms. In addition, infections like *Candida* and *Trichomonas* were easy to recognize.

Cytological examination of the 632 smears revealed abnormalities of the squamous epithelium in 592 cases and abnormalities of the glandular epithelium in 40 cases. All lesions represented primary cervical epithelial lesions; none of the cases represented metastatic lesions.

Squamous Lesions

Cytological diagnoses were correlated with histological outcome translating the Bethesda terminology to the CIN terminology, LSIL corresponding to CIN I, and HSIL corresponding to CIN II and CIN III. Table I summarizes the correlation of cytological and histological diagnoses for squamous lesions. Histology identified 131 squamous cell carcinomas, of which 112 were also diagnosed by cytology. Eight were underestimated as HSIL, seven as LSIL, and four as within normal limits (WNL). Histology identified 202 CIN II-III lesions, 138 of which were also diagnosed by cytology, 16 were overrated as squamous cell carcinoma, 37 underestimated as LSIL, two as ASCUS, and nine as WNL. Histology identified 73 CIN I lesions, 29 of which were the same as the cytology outcome. Sixteen were overvalued as HSIL, four were underestimated

Table I. Cytohistologic Correlations for Squamous Epithelium

Cytology	Histology						%
	Normal	Atypia	CIN I	CIN II-III	Sq ca	Total	
WNL	160	2	24	9	4	199	33.6
ASCUS	4	4	4	2	0	14	2.4
LSIL	11	1	29	37	7	85	14.4
HSIL	4	0	16	138	8	166	28.0
Sq ca	0	0	0	16	112	128	21.6
Total	179	7	73	202	131	592	
%	30.2	1.2	12.3	34.1	22.1		100

WNL, within normal limits; ASCUS, atypical squamous cells of undetermined significance; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; Sq ca, squamous cell carcinoma; CIN, cervical intraepithelial neoplasm. Diagnoses in categorical agreement are in boldface. They compromise 74.8% of all cases.

as ASCUS, and 24 as WNL. Histology identified seven as only atypia, four of which were correctly diagnosed by cytology. One was overrated as LSIL and two were underestimated as WNL. Histology identified 179 specimens as WNL, of which four were overscored as HSIL, 11 as LSIL, and four as ASCUS. The overall histology-cytology correlation rate was 74.8%. The proportions of observations in the different columns show that diagnosis that define the table were significantly related ($X^2 = 905.988$ with 16 df, $P < 0.000$). The sensitivity for LSIL was 39.7% (95% CI: 28.5–50.9%) and the specificity 89.2% (95% CI: 86.5–91.9%), for HSIL these values were 68.3% (95% CI: 61.9–74.7%) and 92.8% (95% CI: 90.2–95.4%), respectively. For squamous cell carcinoma the sensitivity was 85.5% (95% CI: 79.5–91.5%) and the specificity was 92.8% (95% CI: 94.8–98.2%). For the LSIL+ group the sensitivity was 89.4% (95% CI: 86.4–92.4%) and the specificity was 91.4% (95% CI: 87.4–95.4%), for the HSIL+ group the sensitivity was 82.3% (95% CI: 78.2–86.4%) and the specificity was 92.3% (95% CI: 89.1–95.5%), respectively. The ASCUS rate was 2.4%.

Rescreening the discrepancies in the group cytology no abnormalities/histology CIN I (24 cases), eight classified again as no abnormalities, two as ASCUS, four were now rejected because of too low cell count, and 10 were scored as LSIL. In the discrepant group cytology no abnormalities/histology CIN II-III (9 cases), four were classified again as no abnormalities, five as HSIL of which four specimens had only a very few HSIL neoplastic cells, too few to sign it out as HSIL. In the discrepant group cytology no abnormalities/histology invasive carcinoma (four cases), one was reclassified as malignant, one was not properly fixed and should have been rejected, and two were now rejected because of too low cell count. In the discrepant group histology no abnormalities/cytology LSIL (11 cases), 10 were scored as LSIL again and one was rejected because of too low cell count. In 2 of the 10 cases scored as LSIL again, histological follow up after 1 year showed CIN I lesions. In the discrepant group histol-

Table II. Cytohistologic Correlations for Squamous Epithelium After Reevaluation

Cytology	Histology						%
	Normal	Atypia	CIN I	CIN II-III	Sq ca	Total	
WNL	160	2	8	4	0	174	29.8
ASCUS	4	4	6	2	0	16	2.7
LSIL	10	1	39	37	7	94	16.1
HSIL	4	0	16	143	8	171	29.3
Sq ca	0	0	0	16	113	129	22.1
Total	178	7	69	202	128	584	
%	30.5	1.2	11.8	35.5	21.9		100

Abbreviations as in Table I. Diagnoses in categorical agreement are in boldface. They compromise 78.6% of all cases.

Table III. Cytohistologic Correlations for Glandular Epithelium

Cytology	Histology			%
	Dysplasia/AIS	Adenocarcinoma	Total	
WNL	0	1	1	2.5
Dysplasia/AIS	0	3	3	7.5
Adenocarcinoma	4	32	36	90
Total	4	36	40	
%	10	90		100

Abbreviations as in Table I. AIS, adenocarcinoma in situ.

ogy no abnormalities/ cytology HSIL (four cases), rescreening confirmed HSIL. One of them showed a CIN III lesion after 1 year follow up. The results obtained after rescreening are listed in Table II. The overall histology-cytology correlation rate was now 78.6%. The sensitivity for LSIL was 56.2% (95% CI: 44.5–67.9%) and the specificity was 89.5% (95% CI: 86.9–92.0%), for HSIL the sensitivity was 70.8% (95% CI: 64.5–77.1%) and the specificity was 92.7% (95% CI: 90.1–95.3%), respectively. For squamous cell carcinoma the sensitivity was 94.2% (95% CI: 90.2–98.2%) and the specificity was 96.5% (95% CI: 94.8–98.2%). The sensitivity for the LSIL+ group was 95.0% (95% CI: 92.9–97.1%) and the specificity was 91.5% (95% CI: 88.1–95.9%). For the HSIL+ group these values were 84.8% (95% CI: 81.1–88.9%) and 94.1% (95% CI: 91.1–96.9%), respectively. The ASCUS rate was 2.7%.

Glandular Neoplasms

Histology identified 36 adenocarcinomas, 35 of which were endocervical adenocarcinomas and one represented an endometrioid adenocarcinoma. Cytology corresponded in 32 cases, three were underestimated as dysplasia/AIS and one as WNL. Histology identified four cases of adenocarcinoma in situ, all of which had the same diagnosis in cytology. The results are listed in Table III.

Discussion

Optimization of technical steps in cell collection and specimen preparation may translate into increased diagnostic accuracy of cytological diagnosis of cervical epithelial

precursor lesions and result in improved patient care. Conventionally, epithelial cells collected by brushing the cervical transformation zone are smeared directly onto glass slides. Alternatively, cervical cells can be suspended in collecting fixation fluids, after which a thin cell layer can be prepared on a microscopic slide. One of these LBC methods, the ThinPrep method (Cytoc Corporation, Boxborough, MA), is FDA approved. ThinPrep uses a rather expensive automatic laboratory processor for dispersion and filtration of cells from blood, mucus, and debris, after which dispersed cells are collected on a polycarbonate filter and transferred to a microscopic glass slide. Compared to conventional smears, LBC specimens have several advantages. Air drying artifacts seen in conventional smears are not observed with LBC, because cell fixation is rapid and optimal. Moreover, removal of blood, inflammatory cells, and debris results in a clean background and allows easy visualization of atypical, dysplastic, or malignant cells. The area to be screened in an LBC specimen is much smaller than in a conventional Pap smear, which saves reading time. The costs of LBC methods such as ThinPrep, however, are considerably higher than those of conventional Pap smears. Unfortunately, the additional costs of LBC methods like ThinPrep are too high to compensate for a lower number of unsatisfactory samples with optimal morphology or increased sensitivity of detection of ASCUS, LSIL, and HSIL, as may be concluded from the few high-quality evidence-based studies and a single large randomized control study performed to date.¹⁰⁻¹² Importantly, these evidence-based studies have revealed that, although ASCUS/LSIL detection rates may be somewhat higher with LBC, its positive predictive value for CIN2+ is less than that obtained with conventional cervical smears. In terms of cost-effectiveness, the additional cost of LBC may create a problem for small cytology laboratories, in particular if there is no reimbursement for LBC in population screening. Recently, a large split sample study showed that LBC reading with the ThinPrep imager, which selects 22 fields of interest, detected 1.3 more cases of CIN grade 2 or more severe histology per 1,000 women screened than did conventionally read slides.¹³ It remains to be calculated whether the improved reading time and positive predictive value of the ThinPrep imager method in detecting CIN 2+ is cost-effective, allowing funding by national screening programs. For cytology laboratories examining relatively small numbers of specimens LBC, application of a cytocentrifuge monolayer technique may prove to be an attractive alternative. In this study, we tested the accuracy of one of these cytocentrifuge monolayer methods, the Turbitec cytocentrifuge method, in 632 women referred to our colposcopy CIN outpatient clinic after a positive cervical smear.

Using a threshold of ASCUS, the sensitivity and specificity of LBC with the Turbitec method for LSIL is

39.7% (95% CI: 28.5–50.9%) and 89.2% (95% CI: 86.5–91.9%), for HSIL 68.3% (95% CI: 61.9–74.7%) and 92.8% (95% CI: 90.2–95.4%), respectively. For the LSIL+ group these values were 89.4% (95% CI: 86.4–92.4%) and 91.4% (95% CI: 87.4–95.4%) respectively, whereas the sensitivity and specificity for the HSIL+ group was 82.3% (95% CI: 78.2–86.4%) and 92.3% (95% CI: 89.1–95.5%). In this series of women with a previously positive cervical smear, the ASCUS rate was very low (2.4%). After reevaluation of the 52 cases with discrepancies between cytodiagnosis and histological follow-up, sensitivities and specificities were higher, as might be expected. For LSIL the sensitivity was 56.2% (95% CI: 44.5–67.9%) and the specificity was 89.5% (95% CI: 86.9–92.1%), for HSIL 70.8% (95% CI: 64.5–77.1%) and 92.7% (95% CI: 90.1–95.3%), respectively. For the LSIL+ group, we found a sensitivity of 95.0% (95% CI: 92.9–97.1%) and a specificity of 91.5% (95% CI: 88.1–95.9%) and for the HSIL+ group these values were 84.8% (95% CI: 81.1–88.9%) and 94.1% (95% CI: 91.1–96.9%), respectively. The ASCUS rate was 2.7%.

The figures in the glandular neoplasm group were too low to calculate an accurate reasonable sensitivity and specificity, but showed comparably good results.

Meta-analysis has shown that conventional Pap screening has a sensitivity of approximately 50% and specificity at about 80%.¹⁴ The sensitivity in the present study is much higher. This is mainly the result of the study design. Without doubt, the fact that cytotechnicians were aware that the majority of the slides belonged to a population of women visiting our CIN outpatient clinic has resulted in this very high sensitivity. In fact, it is to be expected that the sensitivity reflects the true sensitivity of the Turbitec technique, sampling error being responsible for the cases not detected with this monolayer technique.

The Turbitec technique slides provide a high-quality morphology. Atypical, dysplastic, and malignant cells are very well preserved because of optimization of ethanol-based fixation, lack of drying artifact, equal distribution of the cell material, reduction of inflammation, and preservation of a tumor necrotic background in invasive carcinomas.

A disadvantage of this technique is that it is a more labor-intensive method. Preparation of one slide costs 45 minutes in addition to the time needed to make a conventional slide. Nevertheless, this method is considerably cheaper when compared with the commercial LBC methods for cervix smears and therefore could be a good alternative. The cost are low because of a relatively low price of the centrifuge (around \$8,000) and funnel assemblies (for 12 pieces around \$1,500) that could be washed and reused over and over again. The only disposable that is necessary is the filter card (around \$0.30).

In conclusion, the Turbitec cytocentrifuge technique is a very good LBC method for cervical smears with a high

accuracy for a relatively low price and is a good alternative for the more expensive commercial LBC methods.

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