Original article

Assessment of the diagnostic usefulness of liquid-based cytology. The impact of modifications

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The technique of liquid-based cytology (LBC) is of increasing diagnostic value in non-gynecological cytology. The purpose of the present study was to validate modifications to stages of the LBC process and to assess the diagnostic usefulness of the LBC technique.

Between May 2021 and January 2022, a total of 484 samples were prepared from routine cytology tests carried out in the Department of Tumor Pathology of The Greater Poland Cancer Center. The material was obtained from fine-needle aspiration biopsies and fluid samples. One hundred cases were selected for the research described in the article. The slides were prepared using the Becton Dickinson Totalys SlidePrep device.

Based on the research, it was concluded that predominantly the LBC technique gives better results than a conventional smear because the background, which would otherwise make it difficult to assess preparations, was eliminated. Additionally, in LBC preparations the cellularity was increased, and the cells were arranged in a monolayer system, which improves the image quality. The introduction of modifications to the LBC method facilitated the process of sample preparation, without adversely affecting the quality of the obtained material.

Key words: liquid-based cytology (LBC), SurePath, non-gynecological cytology, fluid from body cavities, fine-needle aspiration (FNA).

Introduction

Gynecological cytology has been used for many years in screening for cervical cancer. The introduction of modifications has increased the diagnostic effectiveness of the testing procedure. Liquid-based cytology (LBC) was first introduced in 1991 and is one of the methods used to improve the diagnostic process of gynecological cytology [1]. This technique can also be used for non-gynecological materials such as fine-needle aspiration (FNA) and body-cavity fluids. The main advantages of LBC over conventional cytology are: partially automated sample preparation (which improves consistency of conditions during material preparation), uniform distribution of material over an area 13 mm in diameter (allowing for a larger number of slides to be analyzed in a shorter time), and convenience in sample transport over long distances [2].

There are currently two methods of obtaining LBC preparations approved by the US Food and Drug Administration: ThinPrep (TP) and SurePath (SP). ThinPrep was approved for cervico-vaginal cytology in 1996 and SP in 1999. Since that time both methods have also been applied to non-gynecological cytology [2].

The liquid-based cytology method makes it possible to obtain a cleaner background, increased cellularity and well-preserved nuclear details through technically improved sample preparation for diagnosis. However, the method remains a diagnostic challenge as the sample processing technique and

preservative fluid may change cell morphology and introduce artifacts [3].

The aim of this study was to explore the impact of modifications to stages of the LBC sample preparation procedure on the diagnostic quality of microscopic preparations. The usefulness of the LBC technique for diagnostic purposes was also assessed.

Material and methods

Materials

Between May 2021 and January 2022, a total of 484 samples were prepared from routine cytology tests carried out in the Department of Tumor Pathology of The Greater Poland Cancer Centre. These are presented in Table I. From these samples 100 cases were selected for the study. In order to objectify the conducted analyses, the selection of 100 samples was completely random and this number was agreed with the pathologists who evaluated the preparations. The material was obtained from FNA biopsies by additional rinsing of biopsy needles in SP medium (404 samples), as well as from unfixed fluid samples (80 samples).

In the Department of Tumor Pathology, in addition to classic cytology smears, a cytoblock is prepared whenever possible. Cytoblocks were made in 80 cases from 484 samples, from fluid from the body cavities. The number of cases from each sample type are presented in Table I.

Methods

Samples delivered in SP media were ready for the subsequent procedures. Fresh material was first rinsed with a preservative solution (Becton Dickinson): CytoRich Red Preservative or CytoRich Blue Preservative (urine samples only) in a 1 : 1 ratio for at least 15 minutes.

Table I. Cytology samples tested in the Department of Tumor Pathology of The Greater Poland Cancer Centre between May 2021 and January 2022

Sample type	Number of samples
Thyroid	119
Lymph nodes	125
Breast	28
Urine	65
Fluid from body cavities	80
Salivary gland	29
Liver	2
Pancreas	27
Skin	5
Stomach	4

Reagents used in the procedure: Becton Dickinson (BD) SP Preservative Fluid (Becton Dickinson), Tris Buffered Saline pH 8.0, prepared according to Becton Dickinson instructions, alcohol-based rinse mixture: Alcohol Blend Rinse 1700 ml (Becton Dickinson)/2-propanol (Chemed), preservative fluid: BD CytoRich Red Preservative and BD CytoRich Blue Preservative (Becton Dickinson), distilled water.

The material was centrifuged for 10 min at 600 rpm. Excess supernatant was decanted and the sediment was shaken for around 2 min at 3000 rpm. Tris solution was added to make the tube volume up to 12 ml. The resulting mixture was then centrifuged again for 10 min at 600 rpm. The supernatant was again decanted, and the remaining sediment was shaken for around 2 min. Unstained slides were prepared using the BD Totalys SlidePrep device, according to the manufacturer's instructions.

Based on the amount of residual sediment, an appropriate sample volume was selected (50–800 μl). Subsequent to slide preparation, hematoxylin and eosin (HE) staining was performed using an automated slide staining system (Leica Histocore Spectra). Alternatively, Papanicolaou (PAP) staining was completed using the BD Totalys SlidePrep system, though this was applied solely to urine cytology slides. The choice of staining method was based on the preferences of the pathologists who routinely examine the cytological preparations. The samples used in this study comprised a part of the routinely prepared preparations for diagnostic purposes.

During the preparation of the cytology slides, the following modifications were made to the procedure:

a) microscope slides, other than those recommended by Becton Dickinson (SurePath PreCoat Slides). We also tested Pathsolutions adhesive microscope slides (ElektroMed – Poland), StarFrost microscope slides (Knittel-Glass – Germany) and Engelbrecht 50 slides (Engelbrecht – Germany),

b) the alcohol-based washing solution was replaced with 2-propanol,

c) HE staining was applied, instead of the PAP staining recommended by Becton Dickinson. Modifications were made sequentially.

After completion of staining, the modified slides were paired with unmodified preparations and assessed by pathologists who were blinded with regards to the type of modification used. All the slides were fully anonymized, and the pathologists were not informed about the modifications. The preparations were evaluated by 4 doctors, including 3 specialists in pathology, each of whom had a minimum of 10 years of experience in the assessment of histopathological and cytological preparations. Routine pathological diagnosis was performed according to standard, routine procedures used in the Department of Tumor Pathology of The Greater Poland Cancer Centre. After comple-

tion of the diagnostic process, additional steps were performed (modifications), which consisted in the separate evaluation of the slides after blinding them.

Pathologists, based on the material taken from the patient, assessed the usefulness of the LBC technique using the methods listed above. For FNA, these were conventional HE staining, May Grünwald Giemsa (MGG) staining, and LBC-based HE staining. For body fluids, these were conventional HE staining, MGG staining, cytoblocks, and LBC-based HE staining. In the case of urine samples, the procedures included conventional HE, MGG, PAP, cytoblocks and, in some cases, an HE-stained slide produced by means of a cytocentrifuge. The assessment of the preparations had no impact on diagnostic decisions or the fate of patients and was performed only to test for technical advantages of the LBC method.

The cytoblock method is a standard procedure used in our unit. Briefly described, the procedure used was as follows: properly labelled specimens were fixed in 95–96% 2-propanol at room temperature, overnight. The resulting fixed material was centrifuged (Centrifuge MPW-223e) for 4 min at RCF 1219 and the supernatant was discarded. Subsequently, the material was combined with a special suspension (Pathsolutions Cell Packing Kit – ElektroMed, Poland), which formed a gel around the cellular material and allowed for the test sample to be placed into a capsule, preventing the material from escaping. The capsule was placed into a marked cassette which was processed to paraffin wax using a suitable method for small specimens.

The term "conventional smear" refers to handmade smears that are created by smearing a drop of the material on the slide. Such smears were made and immediately dropped into 2-propanol for fixation. Following fixation, the slides were stained by the HE method using an automatic slide stainer (Leica Histocore Spectra) and a program dedicated to the staining of cytological preparations.

Pathologists viewed and assessed the slides using optical microscopes. The slides were also scanned (PANNORAMIC P1000 – 3DHistech – Hungary), solely for the purpose of preparing images for this article, and those images were approved by the specialists.

The scanning procedure was as follows: slides were placed into racks and the scanning parameters were selected: (40× objective magnification, automatic selection of the field for scanning). The slides were scanned as a 3-layer z-stack and the resulting data were compressed into a single-layer so-called extended-focus image.

The size of the field of view and final magnification depend on the equipment used to analyze an image. In microscopes the final magnification can be calculated by multiplying the magnification factor of the objective by that of the ocular lenses (for example 40× objective with 10× ocular lens = 400× magnification). When using a scanner, the final magnification is influenced by other parameters including optics in the scanner (objectives, adaptors, and the sensor matrix) and in the display device (type, resolution, and screen size). Therefore, magnification from a microscope cannot be directly compared with digital images. Images shown in this paper were collected whilst using an Iiyama 4K 31.5-inch monitor which gave a rectangular field of view with a 2 : 1 aspect ratio where the area was approximately 960 × 480 μm at a reported magnification of 40×, and approximately 380 × 190 μm at a reported magnification of 100×. These images were collected for illustrative purposes only and were not used in this assessment of samples. The magnification given in illustrations is that reported by the viewing software (3 DHistech CaseViewer).

The microscopic quality of the prepared material was assessed according to the following criteria: cellularity (0–3), the presence of obscuring factors (background staining) (present/none), architectural pattern (tendency to form cellular aggregates: present/none), presence of cells in a monolayer system (yes/no), preparation quality (0–3), and cell size (incomparable/comparable), as presented in Tables II and III.

A semi-quantitative scoring system was used. The individual items were defined as below.

Thresholding for cellularity: cell counting in the preparations was carried out in a semi-quantitative manner. The number of cells from 10 fields of view in each slide was counted. Then, ranges were created (Table II) where scores of 0, 1, 2, 3 were assigned to individual ranges of the number of cells.

Cell size refers to uniformity of cell size within one preparation.

Bioethical committee

There was no objection of the Local Bioethical Committee for the study protocol.

Results

Method modifications

One of the assessed modifications was to change the type of recommended microscope slides used to those from another supplier. Preparations made using slides other than those recommended by Becton Dickinson

Table II. Criteria for assessment of cellularity and quality of preparations

Cellularity*		Preparation quality	
0	Discrete cells	0	Non-diagnostic preparation
1	Up to 20 cells	1	Adequate
2	20–100 cells	2	Good
3	Hundreds of cells	3	Very good

**Cellularity was assessed using a 100× objective.*

Table III. Comparison of selected features in Figure 4 made using the liquid-based cytology technique, and compared with smears made by conventional means, and cytoblock preparations

Compared features	LBC HE	CS HE	Cytoblock preparations
Cellularity (0–3)	3	1	2
Background	None	None	Present (derived from cytoblock reagent)
Cellular architecture	Present	None	Present
Cellular monolayer	Yes	Yes	Yes
Preparation quality	3	1	3
Cell size	Comparable		

CS – conventional smears, HE –hematoxylin and eosin, LBC – liquid-based cytology

showed reduced numbers of cells in the microscopic image (Fig. 1).

The next modification was to change the alcohol-based rinse solution to 2-propanol (Fig. 2). The assessing pathologists noticed no difference in the cytological image of the preparations. The introduction of the described change would not affect the diagnosis.

The last modification tested was to change from the recommended PAP staining method proposed by Becton Dickinson to HE staining. Pathologists in the Department of Tumor Pathology preferred the HE staining to view non-gynecological cytology specimens as it enabled direct comparison with the slides from tissue specimens. It is worth noting, however, that the use of PAP staining made it possible to differentiate between mature and immature squamous epithelium, which is useful in, for example, urine cytology (Fig. 3.)

Figure 4 shows material obtained from body cavity fluids and prepared by various techniques. Selected comparative features are summarized in Table III.

Table III below presents selected features in the preparations from Figure 4 made using the LBC technique and compared with smears using the conventional method and using cytoblock preparations.

Selected preparations obtained during the research carried out in the Department of Tumor Pathology show differences in the purity of the background, the arrangement of cells, and the presence of architectural details (Fig. 5).

Selected urine preparations obtained during the preparation of material with the LBC technique in

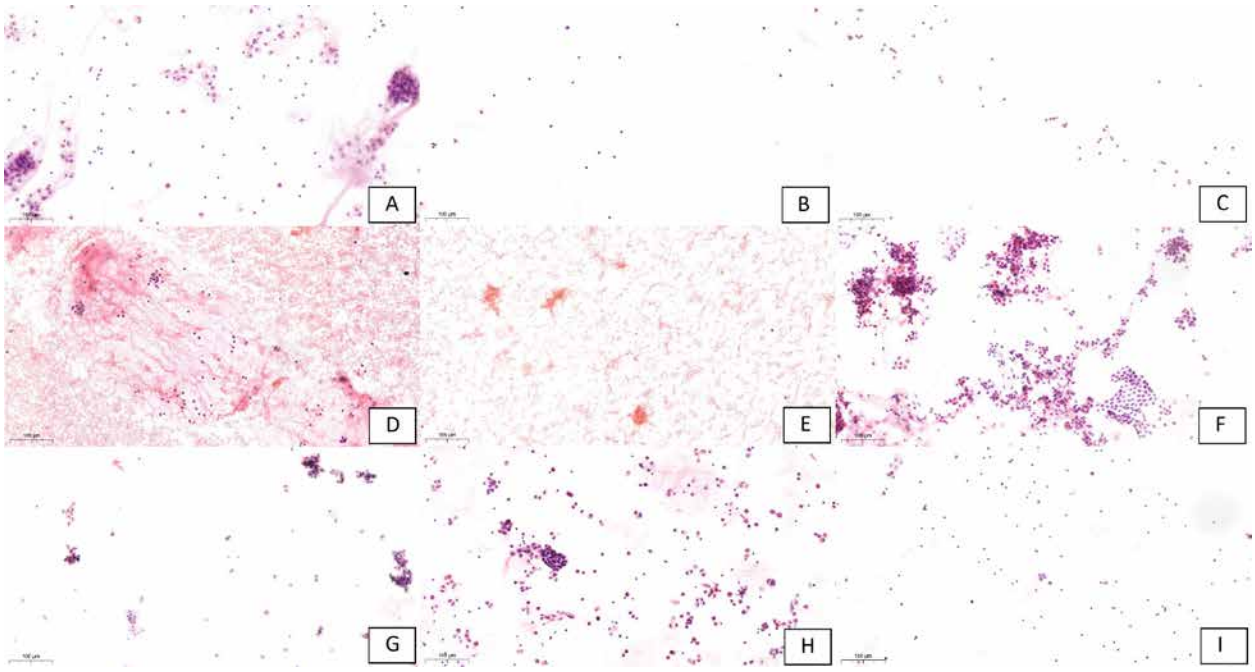

Fig. 1. A) Becton Dickinson (BD) SurePath; B) ElektroMed Pathosolutions; C) engelbrecht; D) BD SurePath; E) ElektroMed Pathosolutions); F) BD SurePath; G) StarFrost; H) BD SurePath; I) StarFrost (fluid from body cavities, 40× magnification, list of slide preparations made on selected types of slides)

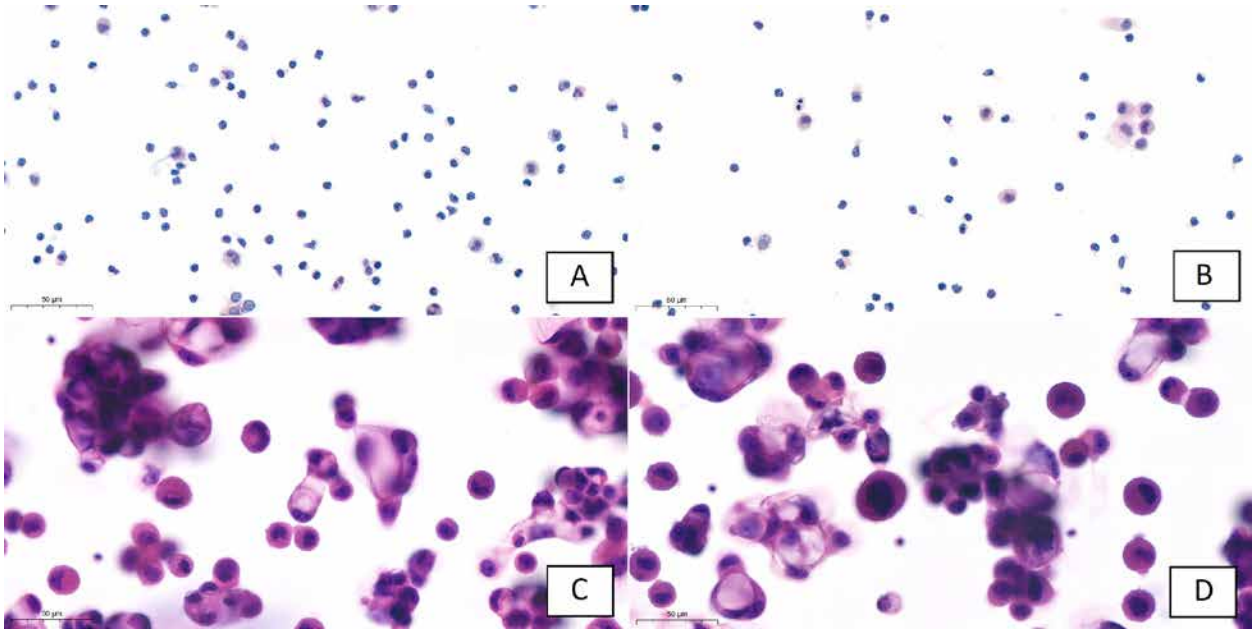

Fig. 2. A) Preparation prepared according to Becton Dickinson recommendations; B) preparation after changing the rinsing solution to 2-propanol; C) preparation prepared according to Becton Dickinson recommendations; D) preparation after changing the rinsing solution to 2-propanol (fluid from body cavities, 100× magnification, comparison of preparations made according to the Becton Dickinson recommendations with those made after changing the rinsing solution)

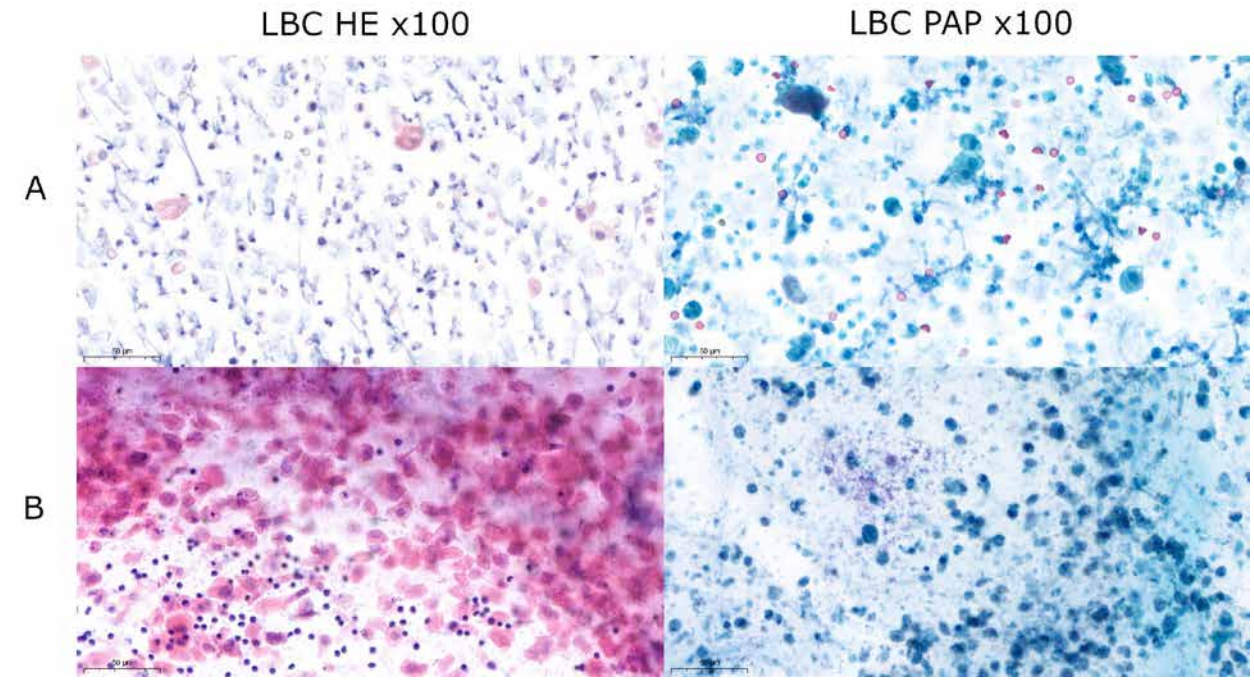

Fig. 3. A) Urine. Individual squamous epithelial cells. Multiple atypical granulocytes; B) urine. Numerous cells of flat and round epithelium. Numerous lymphocytes (HE and Papanicolaou staining, 100× magnification, selected preparations made with the liquid-based cytology technique)

HE –hematoxylin and eosin, LBC – liquid-based cytology

the Department of Tumor Pathology are presented in Figure 3.

Selected urine preparations obtained during the preparation of material in the Department of Tumor Pathology by various techniques are presented in Figure 6.

Discussion

The introduction of certain modifications to the LBC method may facilitate the process of sample preparation, without adversely affecting the quality of the obtained slides. It is possible to use slides other

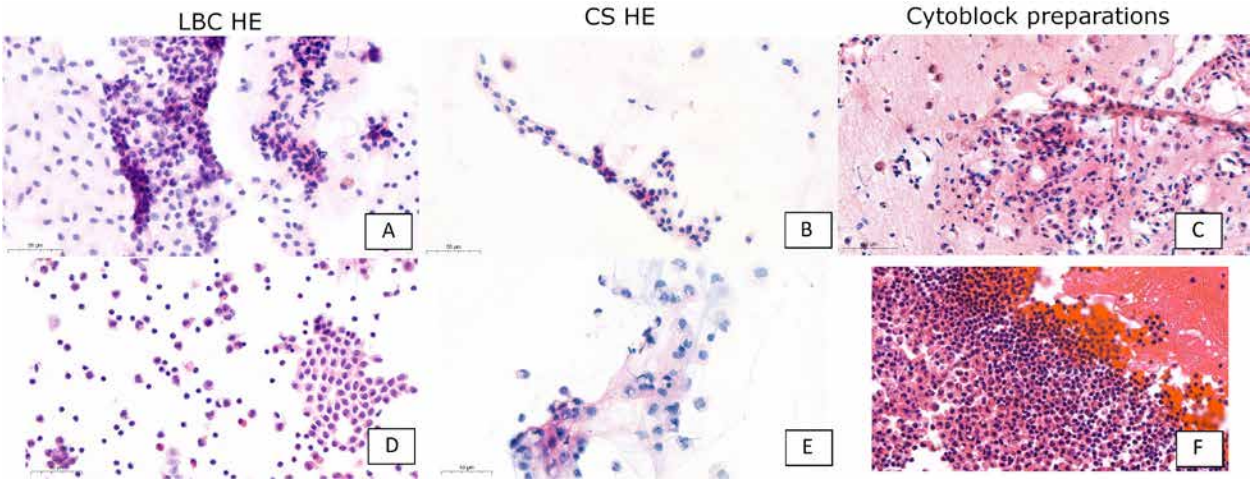

Fig. 4. Liquid-based cytology slides, conventional smears, and cytoblock slides. A, B, C) Body cavity fluid (the preparation shows mesothelial cells and leukocytes, 100× magnification); D, E, F) body cavity fluid (the preparation shows mesothelial cells, macrophages and lymphocytes, HE staining, 100× magnification)
CS – conventional smears, HE –hematoxylin and eosin, LBC – liquid-based cytology

than those recommended by Becton Dickinson, but this was associated with reduced numbers of cells in preparations. Another modification was to change the staining method from PAP to HE, which was found to be acceptable as it became possible to compare the image to the histological picture. The use of 2-propanol as an alternative washing solution is possible and can greatly facilitate the routine preparation of cytological material. For example, in the case of a temporary lack of the rinsing mixture, it is possible to use 2-propanol as a replacement. Before this change is put into practice, the method should be validated, however. Jangsiriwitayakorn *et al*. also tested LBC modifications. In their research, the authors used a different protocol to perform fluid cytology and tested two methods. They used modified LBC performed manually [4].

The priority in the Department of Tumor Pathology in performing these tests was to make a cytological diagnosis, which results from the nature of the workplace. The aim of our research was to modify the technical preparation of the material, to underline the differences obtained by various methods. The modifications and the testing of the diagnostic usefulness of the LBC technique were secondary to our diagnostic aims.

Liquid-based cytology is mainly used in gynecology, but it is also successfully used in non-gynecological cases. Pathologists working in the Department of Tumor Pathology assessed a number of criteria (cellularity, background purity, and architectural pattern, presence of cells in a monolayer system, preparation quality and cell size) in various materials and organs. In LBC, the cells are more clustered. There is homogeneity as to the size of the cells within one preparation. In the case of conventional smears (CS), cells can be "dried" and their size may be altered. In LBC, one may observe more three-dimensional, spatial structures, while in cytoblocks, if used, we see only cross-sections of these structures.

Based on these subjective assessments, it was found that in materials such as fluids from body cavities and urine, the LBC method gives the best diagnostic preparations. When assessing their diagnostic usefulness, it was found that these slides showed increased cellularity of the preparations, reduced background staining, and the presence of cells in a monolayer system. In the remaining cases, such as FNA, the differences between the LBC method and CS did not influence the diagnosis. It is worth performing an additional LBC procedure to expand the possibilities in formulating a pathological diagnosis.

Depending on the type of material, different results were obtained in various studies. The quality of the obtained preparations was better, worse, or comparable, depending on the choice of protocol.

Breast

In a review article, Gerhard *et al*. wrote that, in the case of breast aspirates, LBC preparations show better cell fixation, lower cell overlap and thin-layer cell distribution, as well as improved blood elimination in comparison to conventional cytology. On the other hand, changes were observed in cellular architecture and morphology, as well as the loss of myoepithelial cells and framework elements. The article described the technique of LBC as an automated method for the preparation of thin-layer cytological samples [5]. Another advantage of LBC was described by Osugi, whose work demonstrated the usefulness of the method in immunocytochemistry tests. Tests for p63 were performed to detect myoepithelial cells in LBC preparations and thus the diagnostic accuracy of fine-needle breast aspiration

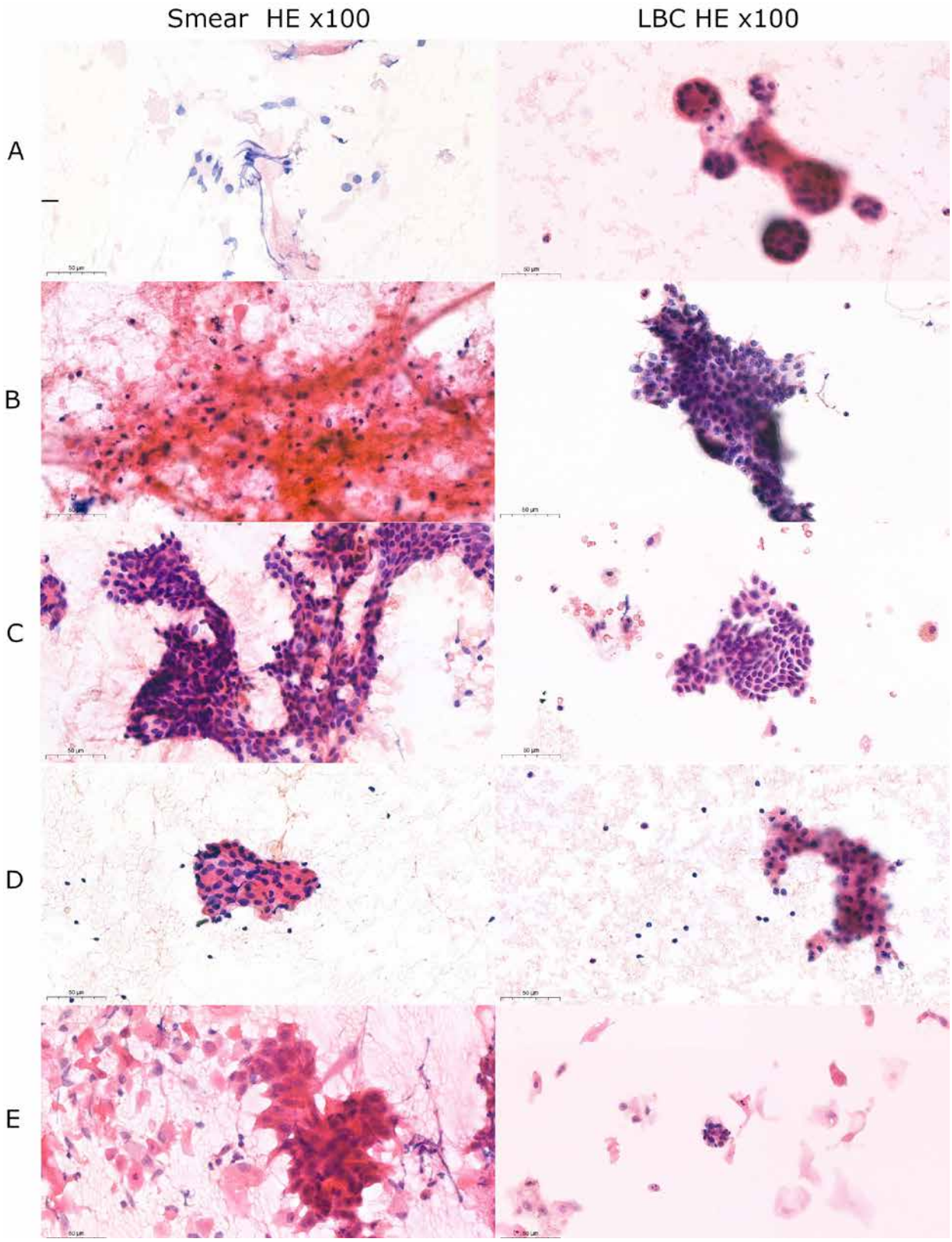

Fig. 5. A) Thyroid, atypical thyrocytes forming numerous small-alveolar systems, the image raises the suspicion of cancer; B) pancreas, cancer cells present; C) breast, atypical glandular epithelial cells, the cytological picture raises the suspicion of highly mature cancer; D) salivary gland, numerous lobes and nests of oxyphilic cells and lymphoid cells in the background, E) lymph node. Squamous cell carcinoma cells present (slides made with the conventional technique Smear and the liquid-based cytology technique, HE staining, 100× magnification)

HE –hematoxylin and eosin, LBC – liquid-based cytology

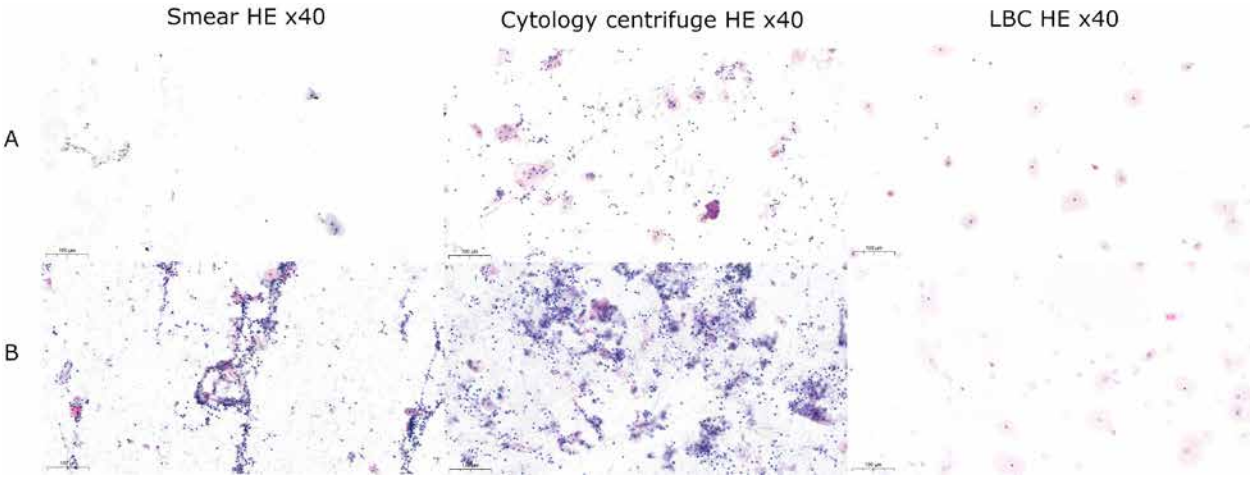

Fig. 6. A) Urine, squamous epithelial cells and a few urothelial cells, some with atypia; B) urine. Squamous epithelial and urothelial cells with no clear signs of atypia. In addition, many lymphoid cells, granulocytes, and bacteria (preparations made by various techniques: conventional technique (Smear), liquid-based cytology technique and by cytocentrifuge, HE staining, 100× magnification)

HE –hematoxylin and eosin, LBC – liquid-based cytology

cytology was increased. The tests were based on BD SP media [6].

Liquid-based cytology has also been found to be applicable to the accurate assessment of Her-2 gene amplification, including by means of situ hybridization. Liquid-based cytology was performed using the TP technique [7].

In the present study, no differences were noted between preparations made with the LBC technique and those made using conventional cytology (smears). This may be due to the limited number of tested samples and low variety of the analyzed materials – most of the cases were from cysts in cases of mastopathy. But it is precisely in this type of material that it is possible to make preparations using the LBC technique, which is useful.

Lymph nodes

Lymph node tissue has also been the subject of testing. Budhwar *et al*. found no statistically significant difference between the quality of LBC preparations and CS in terms of cellularity and cyto-architectonic patterns, nor in the presence of a cellular monolayer. It was noted that nuclear and cytoplasmic details are well visualized in LBC. Based on their observations, the authors concluded that LBC was advantageous in comparison to conventional cytology in the diagnosis of metastatic squamous cell carcinoma. The material was developed and stained by the PAP method with a BD PrepStain slide processor (SP) [3]. In diagnosing cervical lymphadenopathy, Bandoh *et al*. found LBC from FNA material from lymph nodes to be useful in the identification of metastases – largely through greater diagnostic accuracy by reduction of non-diagnostic preparations. In their research, the authors performed LBC using LBC PREP 2 according to the manufacturer's instructions. Slides were stained by the PAP method [8].

Most studies, such as that of Kim *et al*., have shown LBC quality to be better than that seen in other methods due to the elimination of background, thus obtaining pure cytological material. However, it is worth noting that background composition is important for the correct diagnosis of some disease entities. In their study, the authors performed LBC according to the SP NON-GYN protocol on the BD PrepStain Slide Processor. Slides were stained by the PAP method [9].

The research carried out in the Department of Tumor Pathology showed that the LBC method could be utilized in lymph node pathology, particularly in cases of cancer of unknown primary where it was helpful in identification of the source of metastases. Non-lymphatic cells were found to be more easily visible, which was helpful in making the diagnosis. Often the material from a conventional smear was insufficient, and thus the preparation became non-diagnostic.

Salivary gland

The liquid-based cytology technique may be helpful for changes in the salivary glands. The results of one study showed that when assessed together with clinical and radiological data the BD SP technique is a reliable and feasible technique for the evaluation of salivary gland lesions. The material used for that study was obtained from FNA biopsies by additional rinsing of the needles in SP medium (BD Diagnostics-TriPath, Erembodegem, Belgium). The samples were processed using the SP technique. Liquid-based cytology slides were stained with PAP stain [10].

Kumar *et al*. used the SP LBC technique in accordance with standard procedures from the manufac-

turer. Liquid-based cytology slides were stained by the PAP method. The authors found that, in FNA biopsies of the salivary glands, both CS and LBC were of similar diagnostic value. In the case of pleomorphic adenoma, the interpretation of CS was easier than LBC due to abundant chondromyxoid stroma. The reduced cellularity observed in the LBC samples may be due to a loss of material through decantation, a step in the LBC method, leading to reduced cellularity of the sample. However, it is worth performing LBC additionally. This technique took less time and the cells had well-preserved cytological features [11].

The research carried out in the Department of Tumor Pathology showed that, in the case of the diagnosis of solid tumors of the salivary gland using the defined criteria, LBC is a better tool for pathological diagnosis than CS. This was due to the greater concentration of material on the slide – there were more cells in the field of view and the background was devoid of obscuring artifacts.

Thyroid

Between 3% and 7% of thyroid nodules are malignant and the only method that allows for a preoperative (PO) qualification for this malignancy risk group is FNA [12]. In the case of materials derived from the thyroid gland, the results of research concerning the usefulness of LBC are inconclusive.

Based on the results of studies by Chong *et al*., it was found to be possible to replace CS with FNA of thyroid lesions and LBC. The fact is that there are advantages and disadvantages to LBC in thyroid FNA though, more than likely, it makes little difference to the formation of a diagnosis. Thus, the results of TP and SP tests are reliable, even when performed without additional CS. The authors noted, however, that more data and studies are needed to confirm their results. The article was a database overview. The analyzed LBC methods for thyroid fine-needle biopsy used by the authors of the article were: TP, SP, Liqui-PREP, Cell & Tech, EasyPrep and HuroPath [13]. Sharma *et al*. prepared LBC samples using a semimanual procedure, as in our case. Tubes with material were placed into the BD PrepStain processor, using fully automated software. For each sample, one PAP-stained LBC smear was obtained in this way.

The studies conducted by Sharma *et al*. showed that LBC had a higher non-diagnostic index than CS, making it impossible to replace traditional smears with LBC. Cytological differences included: higher frequency of single naked nuclei, smaller nuclear detail, and cytoplasmic, three-dimensional structures, a decrease in the amount of blood in the preparation, and an increase in the number of macrophages. It was concluded that it was easier to diagnose malignant cases using CS; however, studies on a larger group were deemed necessary [14].

Based on the research of Saleh *et al*., it was found that the TP method was associated with a lower percentage of non-diagnostic preparations, similar diagnostic percentages for chronic thyroiditis and atypical/neoplastic changes, and a slightly better diagnostic percentage for colloidal nodules. It was found that both methods were worth using in cases of thyroid FNA. The authors performed LBC using the TP method, semi-manual sample preparation and the TP Processor 2000 apparatus (Cytyc Corporation). The resulting LBC smears were stained by the PAP method [15].

During the research carried out in the Department of Tumor Pathology, improved preparation quality was noted using SP media. When preparing samples from thyroid FNA, large amounts of blood made it difficult for the material to adhere to the slide. A significant number of cells remain in the needle hub and rinsing in the SP medium allowed for the maximum possible amount of material to be obtained, and for its immediate fixation. Conversely, when performing a conventional smear, the cells are often dry or even crushed, which is caused by the needle "tapping" on the glass slides.

Urine

Urinary cytology plays an important role in the diagnosis and monitoring of urothelial carcinoma patients, including asymptomatic cases. Lu *et al*. used a semi-automated LBC method, semi-manual sample preparation and the AutoCyte Prep system. PAP stained smears were obtained. In their study the authors found that 80% of high-grade aggressive urothelial tumors could be detected using urine cytology [16]. Urine cytology tests are characterized by high sensitivity for high-grade tumors and low sensitivity for low-grade tumors [17]. Liquid-based cytology showed that cells tended to be in three-dimensional clusters. A number of morphological features were taken into account, including cell pleomorphism, enlargement of the cell nucleus, hyperchromasia of the cell nucleus, and mitotic activity. The authors concluded that SP exhibits greater cellularity and a cleaner background character for the detection of high-grade urothelial carcinoma. In addition, a reduced number of background artifacts was noted and the overall preservation of cytological features allowed for an accurate diagnosis. Conventional smears, on the other hand, had more pronounced nuclear detail, which could be helpful when diagnosis based on SP was ambiguous [16].

Ren *et al*. described the TP and SP techniques but used the TP technique for urine samples in the laboratory. Theirs was a review article. No information was given as to whether the method was automatic, semi-automatic, or manual. The liquid-based cytology smears were stained by the PAP method [18].

Son *et al*. used a semi-automatic LBC method, semi-manual sample preparation and then the CellPrepPlus system [19].

In the case of high-grade urine evaluation, TP and SP techniques allowed for more accurate diagnosis of urothelial cancer, cancer in situ, or dysplasia [18, 19].

The usefulness of the LBC method for the development of urine samples can be confirmed by the studies conducted in the Department of Tumor Pathology. Preparations made using a cyto-centrifuge and LBC show very good quality. Conventional smears, on the other hand, were often impossible to use in making a proper diagnosis, owing to insufficient material on the slide.

Liquids

Body cavity fluids account for the highest proportion of LBC samples. These samples are less susceptible to contamination or artifacts due to the addition of the preservative fluid, which allows the cells to maintain their optimal appearance as they are instantly fixed. Additionally, fixed samples can be stored at room temperature for up to 4 weeks from the date of collection (information from Becton Dickinson).

Dadhich *et al*. prepared samples for their research using the SP technique and the slides were stained using BD reagents. The study showed that the LBC method gave better results compared to CS due to a reduced examination time and a more accurate background image in the cytology of body fluids. However, in terms of staining quality and morphological evaluation of various cellular components, CS remained their preferred approach [20].

The research of Tyagi *et al*. showed that, in the case of the cytological analysis of peritoneal washings, LBC techniques led to improved concentration of tumor cells and therefore to a reduction in false negative cases, thus improving diagnostic accuracy. This was helpful in the treatment of gynecologic specimens. The samples were processed according to the manufacturer's instructions for the SP LBC technique [21].

Based on several modifications introduced during this study, it can be concluded that, in the case of liquids, preparations made using the LBC technique gave better results than those from CS. This was due to the reduction of background, as well as greater cellularity and, simultaneously, a monolayer of cells. Cytoblock preparations were also found to be suitable for the analysis of fluids.

Conclusions

The liquid-based cytology technique is of increasing diagnostic value in cases of non-gynecological cytology.

Based on our research, it can be concluded that, in most cases, the LBC technique gives better results than CS because the background, which would otherwise make it difficult to assess preparations, was eliminated. Additionally, in LBC preparations the cellularity was increased, and the cells were arranged in a monolayer system, which improves the image quality.

The introduction of modifications to the LBC method facilitated the process of sample preparation, without adversely affecting the quality of the obtained material. The use of 2-propanol as a modification to the procedure, as part of the washing solution, can greatly facilitate the routine preparation of cytological material.

The liquid-based cytology technique allows for the processing of all available material and gives a wider range of diagnostic possibilities such as further immunocytochemical tests. An important aspect is the standardization of the method. Many preparations can be obtained which are relatively homogeneous in cell content.

Use of both LBC and CS can help to expand diagnostic possibilities, and the available material can be tested in its entirety.

The use of a scanner to present images of preparations improves the quality of the images obtained, while allowing for more images to be obtained in a shorter time. In addition, digital pathology allows for nearly instantaneous transfer of images, so that they may be consulted remotely. Image analysis software offers more possibilities than a classic microscope.

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The authors declare no conflict of interest.

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