Cytopress: Automated Slide Preparation of Cytologic Material From Suspension

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This paper describes a new automated system to prepare slides of cytological material from suspension. The system collects material on a filter tape by filtration and transfers it to glass slides by means of pressure-fixation. Using cervical cells as a model, results show that a well-defined cell number is evenly deposited over a standardized area, while a small number of cells is retained on the tape and a negligible number lost in the filtrate. Contamination is very small. Application of the system to other cytological material (fine needle aspirations, monolayer and cell suspension cultures, agar cultures, and isolated nuclei) is shown. In general, more than one slide can be made from one sample. Several histological staining procedures as well as immunofluorescence labeling protocols can be applied to the preparations obtained in this way. This system thus introduces a method that will standardize specimen preparation, is quick, saves operator time, and can be used for both diagnostic and research applications.

Key terms: Specimen preparation, cervical cells, fine needle aspirations, cell cultures, tumor cell colonies, isolated nuclei

Light microscopical examination of cellular material by human observers has been performed almost exclusively on conventionally prepared and stained slides. Demands on the quality of the preparations were not so extreme because the observer could correct for most preparative shortcomings. To improve this visual and qualitative evaluation, new techniques for cell interpretation are being developed. Our laboratory is involved in this research, developing, among others, specific immunohistochemical stains to detect the tissue origin of tumor cells (18–31), to detect quantitative morphologic and cytochemical changes using image analysis derived techniques (6,30,31), and to assess the malignant behavior of tumor cells in an in vitro model system (5,8,9,27). These new techniques require the preparation of reproducible, well-standardized specimens with homogeneously dispersed, nonoverlapping, single cells or cell groups. Therefore, to complement this research, the development of new cytopreparatory techniques has begun (12,15,16). One of the steps in this specimen preparation procedure is the deposition of cells from suspension onto glass slides. Numerous approaches have been described to achieve this goal. These approaches can be subdivided into three classes: centrifugation (1,2,10,24–26), sedimentation (7,12,23), and transferring the cells through an intermediate filter onto a slide (2,11,16,17,22,23). In the last procedure, the cells either remain on the filter (2,17) or are transferred to the slide by touch (22,23) or pressure (11,16). In a previous paper (16) we have evaluated the latter method. Cells were collected on a polycarbonate filter membrane and transferred to glass slides by simultaneous pressure-fixation. This procedure proved to be relatively robust with respect to parameters that might influence cell recovery such as filtration rate, filter pore size, and pressure time. The present paper describes the use of an automated system based on the above-described principle. Slides are prepared in an easy and quick way competitive with present conventional methods. They are suited for conventional cytology, fluorescence microscopy, and quantitative cytology. Emphasis in this paper has been placed on the determination of cell recovery, distribution, and carry-over, using cervical cells as a model. Applications with other cytological and micellar material are described.

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MATERIALS AND METHODS
The Cytosperm System

The principle of the Cytosperm system is shown in Figure 1. A roll of polycarbonate filter tape (designated B in Fig. 1), 24 mm wide, maximal length 100 m, with 5 μm diameter pores (Nuclopo, Pleasanton CA) is used to collect the cells from suspension and to transport them to a second position, where they are transferred to glass slides. The filter tape is unrolled at one position (A) and rolled up at another (M). At the start of the procedure, a funnel (E) is in position above the tape. Funnels with openings of different form and dimension in the bottom can be used. A fritted glass support (F) presses the tape to the funnel rim from below, resulting in a tight seal. The material in suspension (D) is poured into the funnel and a slight vacuum (−15 cm water pressure; minimal pressure −5.5 cm water, maximal −70 cm water) is applied underneath the filter. The suspension medium is then drawn through, leaving the cells on the tape. After the suspension has been aspirated, the vacuum is disconnected, the funnel is moved away, and the tape is transported to a second position. Here the material on the tape (J) is pressed against a glass slide (H) by a pressure arm (K). A latex rubber sponge moistened with ethanol fixative is mounted on top of this arm. The fixative is applied to the sponge by a tube (L), running through the arm. Through this pressure-fixation step the material is simultaneously transferred and fixed onto the slide. After the pressure application has been terminated, the slide is removed from the device.

Figure 2a shows a front view and figure 2b a top view of the Cytosperm system. Almost all handling has been automated. Before the procedure is started, both vacuum time and pressure time are set on the front panel (see Fig. 2a). A numeric display in the center shows the chosen times. Vacuum time can also be regulated by hand using the central knob on this panel. A vacuum time of about 15 s and a pressure time of 20 s have been used in this study. Two different funnels have been used, one with a 26 × 35 mm rectangular opening in the bottom, and one with a 10 mm diameter round hole. The choice of funnels is made by determining the number of cells one wants to inspect on the slide. For cervical cells, fine needle aspirations, cultured bladder tumor cells, and tumor cell colonies, the funnel with the rectangular opening is used. For cultured lung carcinoma cells and isolated nuclei, the funnel with the round hole is chosen.

The first step in the operation is the simultaneous movement of the funnel and the positioning of a glass slide above the tape. Next, the glass support presses the tape against the funnel and the device waits for the operator to indicate that the sample has been poured into the funnel to start the vacuum. When the vacuum is terminated, the glass support is lowered and the cells on the tape are transported to the "pressure position" (G in Fig. 2b). Then the funnel is moved away from the tape. During this movement, 80 μl of ethanol is pumped into the sponge by a peristaltic pump (P, Fig. 2b), ensuring a constant amount of fixative for every sample. When the funnel is completely moved back, it is rinsed with tap water (through the tube N), while the tape with the cells is pressed against the slide. After termination of the pressure, the funnel is moved above the tape again. Concomitantly, the metal plate pushes the bottom slide from the stack of blank slides (O) to a position above the tape, and the slide with cells already transferred moves forward on a guide rail, where it can be manually removed.

Clinical and Biological Material
Cervical cells. Cervical cells were collected with a plastic spatula, suspended in phosphate-buffered saline (PBS) containing 20% ethanol as a preservative (12) and stored at 4°C. One to six days after collection, the cells were disaggregated by hydrodynamic forces induced by a rotor device as described elsewhere (15). Cell concentration was determined using a Coulter Counter Model ZB1 (Coulter Electronics, Hialeah, FL) with a threshold setting that discriminated epithelial cells from most leukocytes present. Nine-milliliter cell dilution were made with PBS-20% ethanol containing approximately 36–72 × 10⁵ epithelial cells. On the average, four dilutions (range: one to 42) per sample could be made. The slides were postfixed for 30 min in ethanol and then Papanicolaou (Pap) stained.

Fine needle aspirations. Collection and processing of fine needle aspirations from the breast and the lung were performed in the same way as described for the cervical cells, but processed the same day. They were methanol postfixed and stained for intermediate filaments (cytokeratin 18, vimentin) using an immunohistochemical method (18,20,21).

Cell cultures. Cells from a bladder carcinoma cell line (T24) were cultured as described elsewhere (29). For harvesting they were incubated briefly with trypsin (0.25%)–EDTA (0.1%), centrifuged (10 min × 400g), and
Fig. 2. The Cytopress system. a, front view; b, top view. For the explanation of letters A–M, see Figure 1. N, tube through which the tap water rinse flows to the funnel; O, stack of glass slides; P, peristaltic pump. For further details, see the text.
resuspended in PBS with 40% ethanol. After cell counting, appropriate cell dilutions were made in PBS-40% ethanol and processed on the Cytopress. The slides were postfixed for 30 min in ethanol and PAP stained, or fixed in a methanol, formalin, acetic acid mixture (85:10:5 w/v/v) and stained quantitatively for DNA and protein using Feulgen-Pararosanilin (SO2)-Light Green (14).

Cells from a line of a small cell carcinoma of the lung were cultured as described elsewhere (3). These cells, growing as free-floating aggregates, were centrifuged (5 min × 500g), resuspended in PBS, and processed on the Cytopress. After post fixation in methanol (−20°C, 1 min) and acetone (three times 20 s), they were stained for vimentin (12).

**Tumor cell colonies.** Material derived from a Grazit tumor was cultured in double-layer soft agar according to a method described by Hamburger and Salmon (4). After 28 days in culture, the upper agar layer was carefully separated from the lower layer. Two percent polyethylene glycol 1500 in 50% ethanol and PBS (1:1, v/v) was added to the dish and the separated layers were incubated for at least 24 hr to soak off the colonies. The suspension was collected and the two layers were rinsed with extra volumes of the mixture described above. From these pooled suspensions, slides with cell colonies were prepared on the Cytopress. The slides were postfixed for 5 min in methanol and PAP stained.

**Endometrium tumor cell nuclei.** Endometrium tumor cell nuclei were obtained by thawing a liquid nitrogen stored sample (less than 0.2g) of endometrial tumor in PBS with 1 mM EDTA and 1 mM phenylmethylsulphonylfluoride. The material was kept at 4°C and homogenized with three strokes of a tight-fitting pestle in a Dounce homogenizer. The nuclear suspension was injected into the methanol, formalin, acetic acid fixative (see above), the nuclear concentration determined, and approximately 40,000 nuclei introduced into the funnel with the round opening. To eliminate extranuclear material, the nuclear suspension was rinsed several times in the funnel with extra volumes of fixative. The extranuclear material disappeared through the pores of the filter tape into the filtrate. Preparations were postfixed for an additional 30 min in the same fixative mixture and stained quantitatively for DNA and protein using Feulgen-Pararosanilin(SO2)-Light Green (14).

**Cell Counting**

The number of cervical epithelial cells found on the slide and the filter tape was calculated from counting 24 18 × objective fields (covering approximately 22.5 mm²). This counting was performed in six circular areas (3.5 mm diameter, four nonoverlapping 18 × fields per circle) that were drawn in a regular pattern (see Fig. 3), using a standard template that exactly matched the cell area.

**Fig. 3. Schematic drawing of three slides with different counting patterns.** Four nonoverlapping 18 × objective fields were counted in each circle. The pattern on slide A was used in this study.
To count the numbers of cells remaining on the filter tape, the part of the tape on which the cells were deposited was marked in advance and cut after the slide was made. It was attached to a glass slide and Papanicolaou stained.

In abnormal samples, the abnormal cells and total epithelial cells (normals and abnormalities) were counted. Abnormal cells were defined as cells consistent with a slight dysplasia or a more severe epithelial abnormality. In cases in which the number of these cells was low (less than 100 per 24 fields), a 3 × 1 cm field (indicated by a second template) was counted. The number of abnormal cells expected in the entire (18 × 35 mm) cell area was then calculated.

The number of cells that passed through the filter tape was examined in pooled filtrates as described elsewhere (16).

RESULTS

Figure 4a shows two slides of cytologic samples that have been prepared on the Cytospin. Note that the borders of the areas are well defined. The areas exactly match that of the rectangle opening in the funnel used for the deposition of the cells on the tape. Figure 4b shows a microscopic picture at low magnification of a cervical specimen in order to illustrate the cell distribution patterns found in Cytospin preparations.

Cervical samples were counted to compare the number of cells recovered on the slide with those retained on the filter tape. First, the slide-counting procedure itself was evaluated. Three different regular patterns, evenly distributed over the cell area (indicated on slides A, B, and C in Fig. 3), were counted on three slides. The results in Table 1 show that none of these patterns resulted in a consistently higher or lower count. This suggests that the chosen procedure (pattern A in this study) gives a reasonable estimate of the cell number for the entire specimen. In previous studies (15,16), this type of counting has proven to be highly reproducible. After this initial evaluation of counting patterns, cervical samples were processed and a random selection covering a broad range of cytologic diagnoses was evaluated during a 9-month period. The total epithelial cell number and the number of abnormal epithelial cells found on the slide and the filter are shown in Table 2. These numbers reflect the complete distribution of the suspended cells since previous studies (16) have shown that virtually all cells from suspension were recovered on the slide and the filter. From these counts, the percentage of cells retained on the filter has also been calculated. The total epithelial cell number found on the slides remains fairly constant for all cervical specimens. The number of cells found on the filter, however, rises for carcinoma in situ and particularly for invasive carcinoma specimens.

For the abnormal cell counts, the same tendency for cell distribution between slide and filter is found as in the total epithelial cell number. However, for the abnormal cells, the percentage of cells retained on the filter was less than that for the total epithelial cell number. Cytologic evaluation of the abnormal cells on the filter did not show any specific preference of particular abnormal cell types for the filter.

The coefficient of variation (CV) of the cell counts of the 24 sampled fields per slide is a good measure of the cell distribution. Table 3 summarizes these CVs and shows that the cell distribution becomes more uneven with increasing grade of abnormality. Part of this increase is caused by a large proportion of the cells present in sheets or clusters being abnormalities.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Cervical Cell Number (Epithelial Cells/mm²) on Three Cytospin Slides Determined Using Three Different Counting Patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slide 1</td>
</tr>
<tr>
<td>Pattern A</td>
<td>60.1</td>
</tr>
<tr>
<td>Pattern B</td>
<td>55.6</td>
</tr>
<tr>
<td>Pattern C</td>
<td>50.1</td>
</tr>
<tr>
<td>Mean</td>
<td>55.3</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td></td>
</tr>
<tr>
<td>Slide 1</td>
<td>8.1</td>
</tr>
</tbody>
</table>
AUTOMATED SLIDE PREPARATION

Table 2
Cervical Cell Number on Slide and Filter Tops, Found After Using the Cytospin Procedure

<table>
<thead>
<tr>
<th>Cytological diagnosis</th>
<th>Total epithelial cells/mm²</th>
<th>Total no. of abnormal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slide</td>
<td>Filter</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>88.9 ± 21.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(54.8–139.3)</td>
</tr>
<tr>
<td>Slight dysplasia</td>
<td>8</td>
<td>64.2 ± 26.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(35.5–111.7)</td>
</tr>
<tr>
<td>Moderate dysplasia</td>
<td>6</td>
<td>53.8 ± 28.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(17.1–75.9)</td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>6</td>
<td>59.6 ± 28.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(32.0–94.4)</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>6</td>
<td>63.2 ± 39.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25.8–130.3)</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>5</td>
<td>51.6 ± 11.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(32.1–61.5)</td>
</tr>
</tbody>
</table>

*Total cell number is shown as epithelial cells/mm², whereas the number of abnormal cells is shown for the entire slide or filter. The numbers are shown with standard deviation and range (in parentheses).

During the same 9-month period of Cytospin evaluation, pooled filtrates were analyzed for possible cell loss on 8 different days. The results, summarized in Table 4, show that the loss of normal cells was negligible and that no abnormal cells were found in the filtrates.

Possible cross-contamination was studied in a series of seven normal cervical samples. After each processed sample, a “blank” containing only PBS-20% ethanol was processed and the number of cells present on the resulting slides was counted. This solution, in which the cells were suspended after collection, is meant to act as a preservative to allow storage of the cells for several days (12). Therefore, the counting was performed at three different storage times, with the same seven specimens. The results in Table 5 show that half of any cells were carried over. These cells were mostly found at the edges of the slides.

Figure 5 shows examples of cellular material processed on the Cytospin. Figure 5a shows cervical cells of an invasive carcinoma specimen. Figure 5b,c shows cells from fine needle aspirations of the breast. The cells in Figure 5b were Pap stained. Those in Figure 5c were stained for one of the intermediate filament proteins (cytokeratin 18), using an immunohistochemical method (20). Figure 5d shows Pap-stained cells from a fine needle aspiration of the lung. Cells from a small cell lung cancer cell line stained immunohistochemically for the intermediate filament protein vimentin are shown in Figure 5e. Figure 5f,g shows cells from a bladder carcinoma cell line (T 24) that were Pap stained (5f) and quantitatively stained for DNA and protein (5g), using Feulgen-Pararosanilin/SSO₂PyLight Green (14). A mitotic figure is clearly seen in one of the central cells in Figure 5f. A tumor cell colony of a Grafton tumor grown in a double-layer soft agar medium and Pap stained is shown in Figure 5h. Nuclei from an adenoacanthoma of the endometrium, quantitatively stained for DNA and protein, are shown in Figure 5i.

DISCUSSION

The cytological specimen preparation procedure outlined in this paper is a reliable method to deposit cellular material on glass slides. Cells or nuclei are deposited on a standardized area relatively easily and within a minimum of time. For most specimens in this study, a filtering time of 15 s and a pressure time of 20 s have been used. These times were chosen as a result of experience with an earlier hand-based system (16). Together with various intermediate steps, this results in a total processing time of 1 min per specimen. Combined with a 15-s disaggregation time (15), cell counting, and dilution, the total processing time from patient to slide can be kept within a few minutes.

The system, as it has been tested with cervical cells, results in slides with a well-standardized cell number and with a minimum of cells remaining on the filter tape. A negligible number of cells is lost in the filterate. Recovery rates from normal up to carcinoma in situ specimens are comparable to those found by Barrett and King (2) for collecting cells from body cavity fluids on Millipore filters. They are somewhat less than those found by Nielsen et al. (11) for collecting cells from urine specimens on glass slides by a comparable Millipore filter imprint technique. In the latter studies, however, the slides had to be coated with gelatin-chrome alum. In the present study, uncoated slides could be used, and precoating of the slides was unnecessary. The higher
Table 4
Total Number of Cervical Cells Found in Pooled Filtrates After Using the Cytopress Procedure

<table>
<thead>
<tr>
<th>Day no.</th>
<th>No. of samples processed</th>
<th>Cytological diagnosis of the individual samples</th>
<th>No. of cells in filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>10 normals</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 normals</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>1 moderate dysplasia</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 slight dysplasia</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>3 moderate dysplasia</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 severe dysplasia</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 carcinoma in situ</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 slight dysplasia</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 severe dysplasia</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 carcinoma in situ</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 invasive carcinoma</td>
<td>0</td>
</tr>
</tbody>
</table>

*Filtrates were analyzed at 8 different days during the 9-month Cytopress evaluation.

Table 5
Determination of Specimen Carry-Over in a Series of Seven Normal Cervical Samples, Determined After Three Different Periods of Sample Storage*

<table>
<thead>
<tr>
<th>Days of storage</th>
<th>No. of cells carried over</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.4 ± 8.1 (1-25)</td>
</tr>
<tr>
<td>3</td>
<td>5.1 ± 6.6 (2-20)</td>
</tr>
<tr>
<td>6</td>
<td>8.5 ± 9.5 (1-31)</td>
</tr>
</tbody>
</table>

*Numbers are shown as total number of cervical cells ± standard deviation, with the range in parentheses. For further details, see the text.

number of cells retained on the tape for invasive carcinoma specimens could be caused by the more unfavorable conditions (more damaged cells caused by the neoplastic process, cell debris, and blood cells present) the cells were exposed to, which made them more "sticky" to the filter. Still, we feel that the number of cells, both total and abnormal, found on the slide is sufficient for a reliable diagnosis, especially since there was no preferential cell loss of abnormal cells. Part of the extracellular material, such as protein and cell debris, is found in the filtrate. Therefore the procedure results in cleaner slides than with conventional methods. The cell distribution is fairly homogeneous (see Fig. 4b and Table 3).

Few numerical data on cell distributions with cell preparation procedures have been published so far. Watts and co-workers (28) have shown data for two cervical cell samples using their sedimentation technique on polystyrene-coated slides. With the introduction of quantitative cell analysis and especially automated methods of cell measurement (30), a well-standardized cell distribution with almost equal cell densities per measured field is very important to make these measurements efficient. Cells in these slides can be more easily observed than in routine (cervical) slides because a monolayer cell layer is created with a majority of the cells and cell groups lying singly.

More than one slide per sample can be prepared in most cases, allowing several stains to be applied. For instance, stains for quantitative cell measurement (13,14) or immunofluorescent stains (15,20,21) can be used in addition to conventional cytology stains.

With respect to cross-contamination of cells, we have found that about five to ten cells per 36–72 × 10³ normal cervical cells may be carried over onto the next slide. When samples of cells of different origin are processed successively, one should be aware of this possible contamination from the preceding preparation. However, these cross-contaminated cells are found mostly at the edges of the slide, which makes recognizing them easy. A matter of concern might be the eventual cross-contamination of benign samples with malignant cells. Present studies are underway to investigate this possibility.

Fine needle aspirations are processed in the same way as cervical specimens and immunofluorescent labeling for intermediate filament proteins (Fig. 5c) (15,20,21) has been applied. Together with conventional Pap staining, this immunocytochemical method allows the determination of the tissue of origin of the tumor cells. For the latter procedure, the samples must be processed on the day of collection.

Cytopress preparations can also be made from cultured cells. Cells cultured in monolayer (the bladder carcinoma cell line T24) or as free-floating aggregates (a small cell carcinoma cell line) have been processed and Pap stained (Fig. 5b), quantitatively stained for DNA-protein (Fig. 5g), and stained for intermediate filament immunofluorescence (Fig. 5e). For both fine needle aspirations and cell cultures, the staining determines the
Fig. 5. Examples of cells from slides prepared on the Cytopress. a, cervical cells from an invasive carcinoma sample (Thionine-Feulgen-Congo Red, ×170). b, cells from a fine needle aspiration of a large cell ductal carcinoma of the breast (Papanicolaou stain, ×170). c, cells of a fine needle aspiration of a ductal carcinoma of the breast, immunohistochemically stained for cytokeratin 18 (×370). d, cells of a fine needle aspiration of a squamous cell carcinoma of the lung (Papanicolaou stain, ×170). e, cells of a cell culture of a small cell carcinoma of the lung, immunohistochemically stained for vimentin (×280). f, cells of a monolayer cell culture of the bladder carcinoma cell line T24 (Papanicolaou stain, ×270). g, the same cells as in f, now quantitatively stained for DNA and protein (Feulgen-Pararosanilin (SO4)Light Green, ×340). h, a tumor cell colony of a cervical tumor, grown in double-layer soft agar (Papanicolaou stain, ×650). i, model of an adenocarcinoma of the endometrium, quantitatively stained for DNA and protein (Feulgen-Pararosanilin (SO4)Light Green, ×270).
medium in which the cells are suspended and processed in the Cytopress system. For morphological examination using the conventional Pap stain or for quantitative DNA and protein staining, a PBS solution with 20 to 40% ethanol concentration is needed to preserve optimal morphology. For immunofluorescent intermediate filament staining, PBS without ethanol can be used.

New methods have been developed to assess the aggressive behavior and chemotherapeutic sensitivity of tumors, using a double-layer soft agar system (4,8,9,27), in which single tumor cells are cultured in vitro. The evaluation of the morphology of the tumor cell colonies is difficult in the agar, but with the Cytopress system it has been found that the evaluation of these colonies on slides is much easier.

For more in-depth studies in which isolated nuclei are measured to quantify DNA and nuclear protein, the Cytopress offers two advantages: slides are easily prepared, and most extracellular material is lost through the pores of the filters, thus resulting in cleaner slides. Unfixed nuclei in PBS can be processed, but tests resulted in several cases in which nuclei were damaged or deformed. Fixing the cells in suspension resulted in nuclear preparations of excellent morphology, while no obvious change in recovery on the slide was noted.

In all cases, an additional fixation of the cells or nuclei on the slides has been performed after Cytopress preparation. These fixations varied, depending on the staining to be applied. Studies are underway to determine whether the additional ethanol postfixation for routine Pap staining can be omitted. In this way, slides for routine cytology can be immediately stored for later staining.

Studies are underway to extend the capabilities of the Cytopress system to cytological samples that contain large amounts of extracellular material, such as pleural and ascitic fluids. Since this extracellular protein may possibly obstruct the pores in the filter, techniques such as altering the suspension medium, changing vacuum pressure and time, and using alternative pore sizes are being explored.

In conclusion, the described system is a highly useful device for the preparation of slides from a variety of cytological material. Because in most cases more slides per sample can be made, the diagnostic capabilities can be extended by using multiple stains including newly developed, more specific ones. For large-scale processing, the technique offers higher speed and easier automation than other methods. In addition, the quality of the specimen is superior for both visual and machine interpretation.

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LITERATURE CITED


