Cell Block Cytology

Improved Preparation and Its Efficacy in Diagnostic Cytology

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Abstract

Cell blocks prepared from residual tissue fluids and fine-needle aspirations can be useful adjuncts to smears for establishing a more definitive cytopathologic diagnosis. They can be particularly useful for categorization of tumors that otherwise may not be possible from smears themselves. A modified cell block technique using an improvised ethanol formalin fixative (Nathan alcohol formalin substitute) followed by a simple paraffin processing schedule is described. This improved preparation offers excellent cytomorphologic features corresponding closely to cells in Papanicolaou-stained smears and ensures optimal preservation of histochemical and immunocytochemical properties. The technique is simple and reproducible and uses routine safe laboratory chemicals. The efficacy of cell blocks also is discussed.

The use of cell blocks in routine nongynecologic cytopathology varies in each institution. Despite the increased use of fine-needle aspiration cytology (FNAC) and immunocytochemistry in the diagnosis of solid tumors, only limited information is available to assess the contribution of cell blocks, although the value of cell blocks has been acknowledged.[1,2] Several techniques also have been reported that vary in scope and the type of fixatives and embedding techniques used, making valid comparison difficult.[1-8]

Most methods previously described are inconvenient or time consuming in a routine active pathology laboratory and include using chemicals that have hazardous potential. In addition, except for mercuric chloride–based fixatives, most do not offer the optimal morphologic characterization of cells compared with corresponding smears.

This article reports a 2-year study of a cell block preparation arising from the need to replace the excellent but highly hazardous B-5 mercury fixative,[9] which had been very successful in our hands. The development for a suitable alternative, aimed at achieving the following basic cytologic expectations, was undertaken: (1) the cells closely resemble corresponding cells in alcohol-fixed Papanicolaou-stained smears; (2) there is adequate clarity and delineation of nuclear and cytoplasmic details; (3) loose cells, cell aggregates, and microscopic tissue fragments are easily recoverable; (4) the cell block sections are suited to a wide variety of histochemical stains and immunocytochemistry; (5) the method is simple, reproducible, and readily adaptable in a routine hospital laboratory; and (6) the final cell morphology in paraffin sections is no less than in B-5–fixed sections. The contribution of cell blocks in the final diagnosis using this method of preparation also was evaluated.

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Materials and Methods

All nongynecologic specimens for cytopathology that consisted of both smears and cell blocks over a 2-year period were reviewed and analyzed. The 1,009 specimens comprised 465 FNAC specimens from various sites and 544 body fluid, washing, and brush washing specimens. The 465 FNAC specimens of solid lesions were as follows: head and neck, 59; salivary gland, 13; lymph node, 41; thyroid, 72; breast, 114; lung and mediastinum, 85; liver, 30; subcutaneous tissue, 10; pancreas, 4; bone and soft tissue, 20; kidney, 4; adrenal gland, 5; ovary, 3; testis, 1; and lesions from the abdominal cavity, 4.

The 544 washings and exfoliative specimens consisted of the following: bronchial washing, 107; bronchoalveolar lavage, 63; sputum, 2; pleural fluid, 182; pericardial fluid, 8; peritoneal fluid, 108; peritoneal washing, 30; ovarian cyst fluid, 15; synovial fluid, 11; urine, 12; brain cyst fluid, 2; and cerebrospinal fluid, 4.

Smearing Technique

The FNAC specimens were obtained as previously described.[10] Two to 4 smears were prepared using precarbowax-coated slides, and these were fixed immediately in 95% ethanol and Papanicolaou stained.[11] Any excessive material, including the needles used in aspiration, was submitted in 50% ethanol (ethanol/water, 1:1) for cell block preparation.

Effusion, brushing, washing, and urine specimens were centrifuged at 4,000 rpm for 6 minutes, and direct or cytocentrifuged smears were prepared from centrifuged deposits as previously described.[2] Millipore filters were used for most voided urine specimens and washings wherever appropriate, and the "pick and smear" technique was used for sputum specimens. All smears were fixed in 95% ethanol and Papanicolaou stained. In heavily blood-stained effusions, smears or cytocentrifuged specimens were prepared after removal of excessive blood by Ficoll separation.[2]

Cell Blocking

Following smear preparations, the needles and syringes used to obtain fine-needle aspirates were rinsed in 10 mL of 50% ethanol in a specimen container. Any residual clot or tissue in the hub of needles was removed carefully in the laboratory with the aid of another needle and rinsed in 50% ethanol. The entire material was centrifuged in a 10-mL disposable centrifuge tube at 4,000 rpm for 6 minutes to create 1 or more cell pellets (1 pellet in most cases). The supernatant fluid was decanted and the deposit fixed in *freshly* prepared Nathan alcohol formalin substitute (NAFS) consisting of 9 parts of 100% ethanol (CSR, Sydney, Australia) and 1 part of 40% formaldehyde, HCOH = 30.03 (Ajax Chemicals, Sydney, Australia). Fresh working solution is desired because formalin is capable of oxidizing to formic acid after exposure to air and reacting with blood to form acid hematein pigment artifacts.

Centrifuged deposits of effusions, clots, Ficoll-separated cell deposits, washings, and other fluids, following smear preparations, were fixed similarly for cell blocking. When centrifuged deposits were more than 0.2 mL thick, to facilitate adequate fixation, the deposit was detached carefully from the bottom of the centrifuge tube with the aid of a sharp-edged dipstick. If the centrifuged deposits were too thick, the material was divided into several tubes for multiple cell blocks before fixing in NAFS solution. The fixed cell pellets, at the end of 45 minutes' fixation, were recentrifuged at 4,000 rpm for 6 minutes. These pellets should detach themselves or can be removed easily with a disposable Pasteur pipette following centrifugation. The cell pellets were wrapped in crayon paper, placed in a cassette, and stored in 80% ethanol until ready for processing in the automatic tissue processor using a 13-hour processing schedule as follows: 80% ethanol with 1 change (2.5 hours); 95% ethanol (1 hour), 100% ethanol, 4 times (1 hour each), 1:1 ethanol/xylene (1 hour); xylene, 3 times (1 hour each), paraffin wax, 60°C (1 hour); and paraffin wax, 60°C, vacuum impregnation at 20 lb. for 0.5 hour. The cell blocks were embedded in paraffin and sectioned at 3 µm thickness.

Staining

Routine H&E (Harris H&E) staining was used on all cell block sections. When necessary, histochemical stains for pigments, bacteria, fungi, mucins, and connective tissues were used, and a comprehensive range of polyclonal and monoclonal antibodies was applied in cases requiring identification or phenotyping of tumor cells using the streptavidin-biotin method previously described *without* prior enzymatic digestion.[12]

Method of Analysis

Fine-Needle Aspiration Cytology

All smears and corresponding cell blocks were reviewed separately and categorized into 4 groups based on the final cytologic diagnosis: malignant, suggestive of malignancy, benign, and no malignant cells seen **Table 1**. For this study, the latter group also included cases with insufficient cells for a conclusive diagnosis.

Fluid Specimens

These specimens were grouped similarly in 4 categories: malignant, suggestive of malignancy, benign conditions

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associated with specific infectious cause, and no malignant cells seen.

Results

The cell block sections showed clearly recognizable normal and abnormal cells with minimal shrinkage and aberration **Image 1** through **Image 8**. The cytomorphologic features were well maintained, and staining characteristics of the nucleus, nucleoli, and cytoplasm were sharp and crisp with clear recognition of nuclear and cytoplasmic features closely resembling cells in corresponding Papanicolaou-stained smears (Images 1 and 2). The intracellular details were equally sharp and clearly distinct. The results were also comparable to those evidenced in B-5–fixed sections (Images 3 and 4).

Routine histochemical stains were performed in 64 cases with appropriate positive controls. Periodic acid–Schiff (PAS), diastase-PAS, Alcian blue, mucicarmine for mucins, bacterial and fungal stains and those for *Pneumocystis carinii* (Gram stain, Ziehl-Neelsen, Fite, Gram-Weigert, PAS, Grocott methenamine silver), and others (methyl green pyronin, Verhoeff elastic van Gieson, toluidine blue, Congo red) revealed well-stained positive results with no loss of tinctorial properties in appropriate cases.

Immunohistochemical staining using streptavidin-biotin with the DAKO Quick Staining alkaline phosphatase labeled with new fuchsin chromogen (DAKO, Carpinteria, CA) or peroxidase labeled with diaminobenzidine as the chromogen were applied in 65 FNAC cases and 31 fluid specimens to identify or categorize the cell types.[12] A comprehensive range of antibody markers was used: B72.3 (Biogenex, San Ramon, CA), AE1/AE3 (Boehringer Mannheim, Castle Hill,

Table 1
Number (Percentage) of Specimens With Cell Blocks

	Final Cytologic Diagnosis				
Specimen	**Malignant**	**Suggestive of Malignancy**	**Benign**	**No Malignant Cells Seen**	**Total**
Fine-needle aspiration cytology	196 (42.2)	24 (5.2)	29 (6.2)	216 (46.4)	**465**
Fluids	95 (17.5)	2 (0.4)	11 (2.0)	436 (80.1)	**544**
Total	**291 (28.8)**	**26 (2.6)**	**40 (4.0)**	**652 (64.6)**	**1,009**

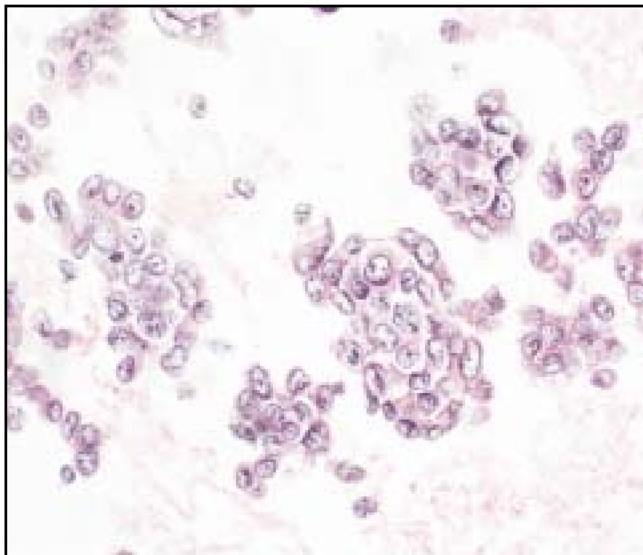

Image 1 Fine-needle aspiration cytology of breast. Nathan alcohol formalin substitute (NAFS)-stained cell block section compared with smear. Single cells and clusters present in the multiple minute NAFS-fixed tissue fragments of breast carcinoma show minimal shrinkage and distortion compared with the smear from the same tumor shown in Image 2. Granular precipitated background is noted (H&E, ×130).

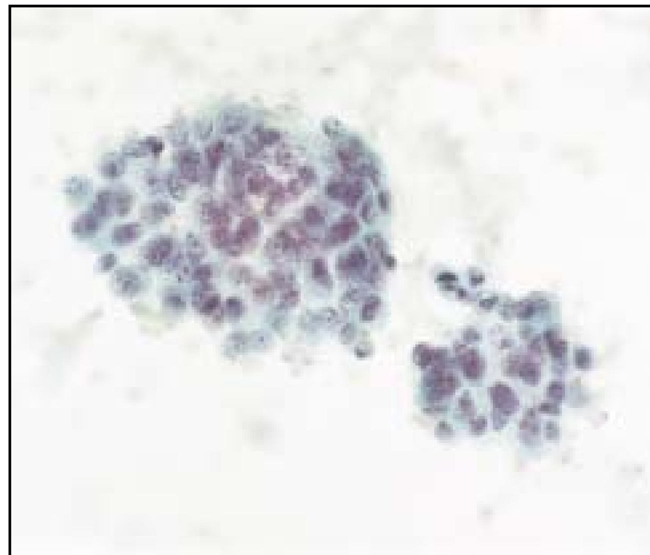

Image 2 Fine-needle aspiration cytology of breast. Corresponding alcohol-fixed smear of same lesion shown in Image 1 (Papanicolaou, ×130).

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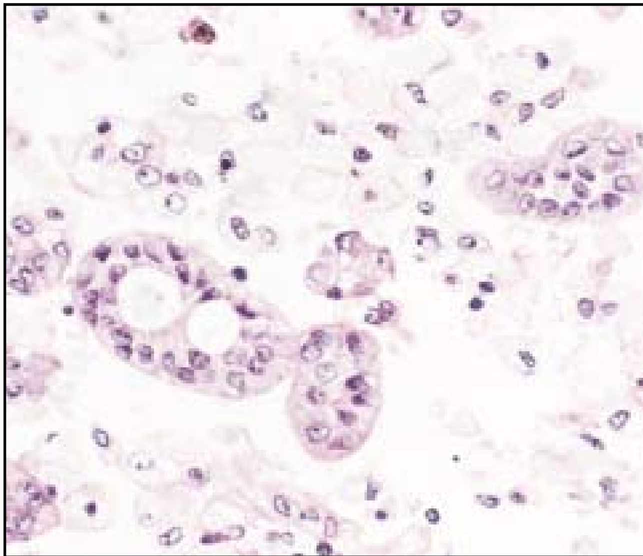

Image 3 Pleural fluid. Nathan alcohol formalin substitute–fixed section. Malignant cell clusters from a metastatic adenocarcinoma with cell morphology and details similar to those from same B-5–fixed specimen shown in Image 4 (H&E, ×130).

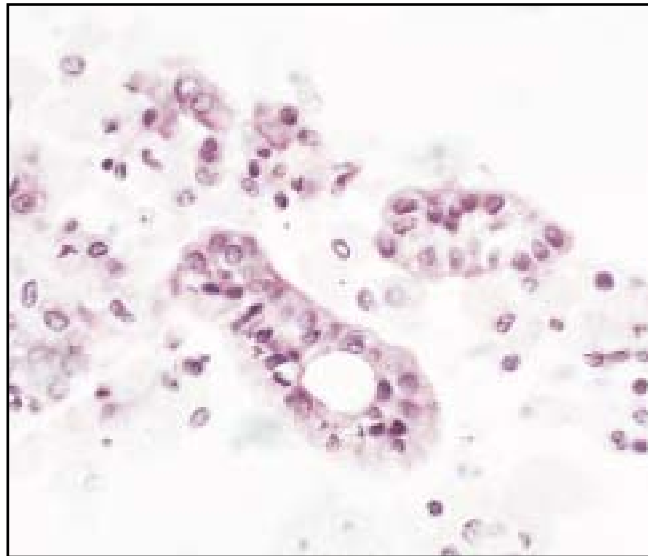

Image 4 Pleural fluid. B-5–fixed section of same specimen shown in Image 3 (H&E, ×130).

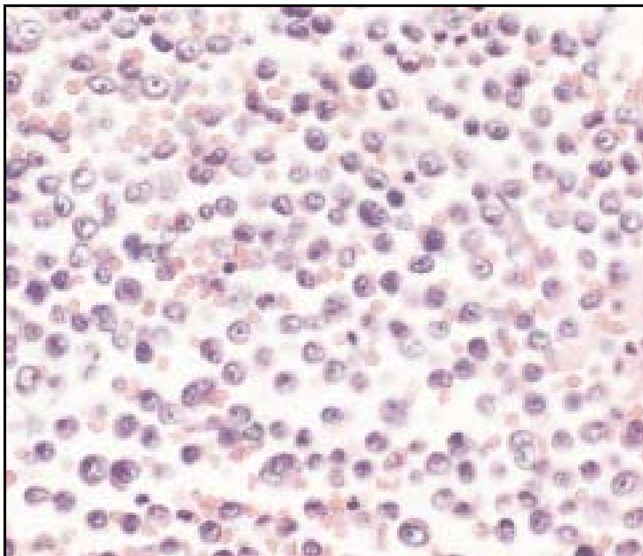

Image 5 Pleural fluid. Nathan alcohol formalin substitute–fixed cell block section showing round pleomorphic single cells with clear crispy nuclear and cytoplasmic features of plasmacytoma in proteinaceous background (H&E, ×130).

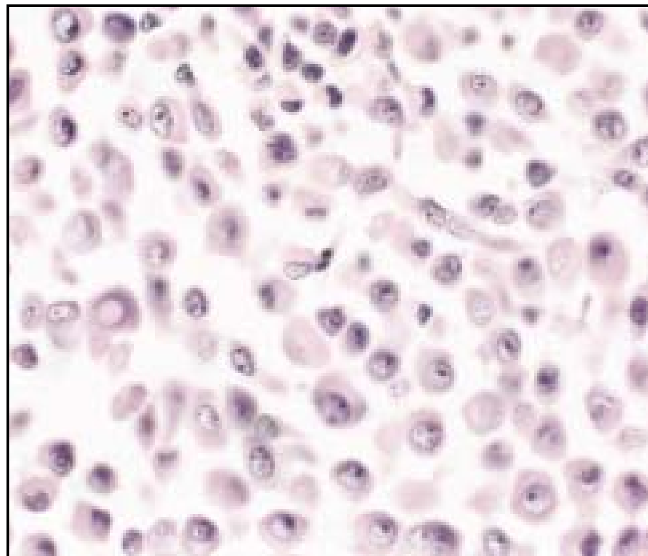

Image 6 Fine-needle aspiration cytology of lymph node. Nathan alcohol formalin substitute–fixed cell block section containing epithelioid malignant cells with poor cell cohesion, sharp clarity and definition of nucleoli, nuclei, nuclear inclusions, and cytoplasmic features of metastatic melanoma (H&E, ×130).

Australia), CAM 5.2 (Becton Dickinson, San Diego, CA), chromogranin (Immunon, Pittsburgh, PA), and from DAKO, alpha-fetoprotein, carcinoembryonic antigen, epithelial membrane antigen, kappa and lambda light chains, MIC2 (CD99) gene product, prostatic specific antigen, prostatic acid phosphatase, synaptophysin, neuron specific enolase, chromogranin A, thyroglobulin, calcitonin, vimentin, S-100, HMB 45, leukocyte common antigen (CD45), and CD20. The DAKO B-HCG and Ber-EP4 also were used in later cases that are not part of this review. All markers were applied in selective cases *without* preenzymatic digestion. NAFS fixation and processing had

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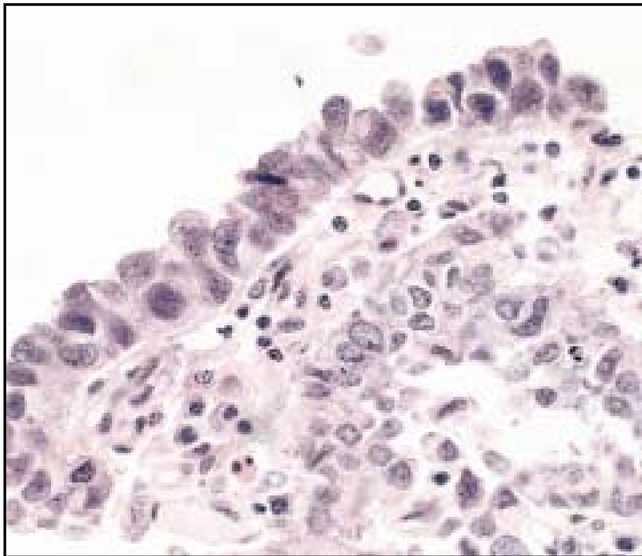
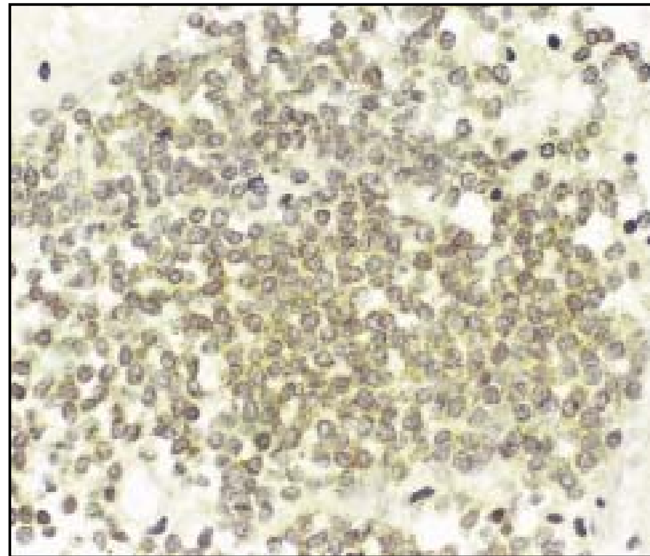


▌Image 7▌ Ureteric washing. Nathan alcohol formalin substitute–fixed cell block section depicting atypical cells with crisply stained nuclear and cytoplasmic features from a transitional cell carcinoma in a microscopic tissue fragment in saline washing (H&E, ×130).



▌Image 8▌ Fine-needle aspiration cytology of lymph node. Strong MIC2 (CD99) spotty immunoperoxidase reaction in Nathan alcohol formalin substitute–fixed cell block section with sharp recognizable atypical nuclear features of the small malignant cells from Ewing sarcoma (×130).

▌Table 2▌
Sensitivity of Smears and Cell Blocks*

Specimen/Category	Smears	Cell Blocks
Fine-needle aspiration cytology		
No malignant cells seen (n = 160)	131 (81.9%)	86 (53.7%)
Benign (n = 29)	12 (41%)	19 (66%)
Suggestive of malignancy (n = 24)	21 (88%)	21 (88%)
Malignant (n = 196)	183 (93.4%)	174 (88.8%)
Subtotal (n = 409)	347 (84.8%)	300 (73.3%)
Fluids		
Malignant (n = 95)	95	93
Benign (n = 11)	11	10
Subtotal (n = 106)	106 (100.0%)	103 (97.2%)
Total (n = 515)	453 (88.0%)	403 (78.2%)

* Data are given as number (sensitivity).

no deleterious effect in the staining of normal or neoplastic cells (Image 8).

Sensitivity of Cell Blocks and Smears

Fine-Needle Aspiration Cytology

Sensitivity information is given in **▌Table 2▌**. Cell blocks contained diagnostic cellular material in 300 (73.3%) of 409 lesions aspirated in which a conclusive diagnosis of benign lesions, no malignant cells present, suggestive of malignancy, or malignant cells present was possible; this was less sensitive than smears in which diagnostic cells were present in 347 cases (84.8%). There were also 56 cases with insufficient material in smears and cell block sections.

This series of 160 cases in which precise reporting of "no malignant cells present" included 131 smears (81.9%) and 86 cell blocks (53.8%). The cell blocks nevertheless were instrumental in improving the overall negative results in 29 additional cases (18.1%).

In a further 29 benign lesions (pleomorphic adenoma, 6; fibroadenoma, 7; hyperplasia, 3; follicular lesion, 5; goiter, 7; and Hashimoto thyroiditis, 1), the smears were diagnostic of the true nature of the lesions in 12 (41%) of the cases. Diagnostic material was more apparent in 19 (66%) of the cell blocks, and overall sensitivity was improved in 10 cases (34%).

There were also 24 cases in which the final diagnosis was equivocal in both the smears and cell blocks. The atypia was apparent in 21 smears (88%) and 21 cell blocks (88%), improving the overall sensitivity by 3 (12%).

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There was unequivocal malignant cellular material in 196 cases (squamous cell carcinoma, 38; adenocarcinoma, 83; adenosquamous carcinoma, 2; non–small cell carcinoma, 33; small cell carcinoma, 6; transitional cell carcinoma, 3; papillary carcinoma of the thyroid, 2; carcinoid tumor, 2; hepatocellular carcinoma, 4; lymphoma, 4; metastatic melanoma, 16; Ewing sarcoma, 1; Hodgkin disease, 1; and plasmacytoma, 1) in smears, cell blocks, or both. The smears were positive in 183 (93.4%) cases and 174 cell blocks (88.8%), with an overall improvement in the final diagnosis by 13 (6.6%) when smears were complemented by cell blocks.

Fluid Specimens

Cytologic examination of 544 fluid specimens revealed 95 malignant cases (17.5%), with smears positive in all cases and almost identical findings in the cell blocks (93/95 [(98%]). There were also 12 cases (13%) in which neither smears nor cell blocks were equivocal. In 11 benign conditions with evidence suggestive of an infective cause (*Cryptococcus* species, 2; *P carinii,* 6; *Aspergillus* species, 2; and *Strongyloides* species, 1), these were identifiable in all cases in the smears and in 10 cell blocks (91%).

Discussion

A new fixation and processing module was established in a study of 1,009 specimens in which cell blocks were prepared simultaneously from residual material after smear preparation. The contribution of cell blocks to the diagnosis also was documented. There is sparse corroborative study in the literature on the routine use of cell blocks, probably because of differing emphasis placed on them in various institutions. Different fixing and processing techniques to maximize the recovery of cellular material from washings, tissue fluids, or fine-needle aspirates with various degrees of expectations make valid comparison difficult. As indicated in this series we share the view of Zito and others[1] that FNAC sometimes does not yield information for precise diagnosis, and the risk of false-negative diagnosis and indeterminate diagnosis is always present. They too observed that although various authors have reported the use of cell blocks, few have given sufficient attention to the development of a satisfactory technique.[1]

A wide range of histologic fixatives has been used for cell blocks, primarily buffered formalin, neutral buffered formaldehyde solution, Bouin solution, picric acid fixative, Carnoy fixative, and ethanol.[1,2,3,7] Formalin, an acceptable tissue fixative, also has been used widely for cell blocks by most researchers. In our experience, it is by far the least satisfactory fixative for preservation and discrimination of nuclear and cytologic details from a cytologist's perspective. The others mentioned are inconvenient for routine use and time consuming, and they require special attention and care when the material is not easily recognizable. The difficulty of recovery and processing of small tissue fragments also has resulted in several unconventional approaches using bacterial agar, plasma-thrombin clot, acetone-melted paraffin technique, gelling and setting agents, and gelatin capsules.[2,3,5-7]

Lillie modified B-5 fixative (saturated mercuric chloride, 9 parts; formaldehyde, 1 part) is a protein precipitant fixative initially recommended for lymphoid and hematopoietic cells of lymph nodes and bone marrow trephine biopsy specimens, respectively, because of the excellent morphologic presentation and staining properties induced by this fixative.[9] It also has been the fixative of choice in immunohistochemistry.[12] We used B-5 extensively in cytology in all types of specimens, with remarkable clarity in the display of cell morphology and cellular details, until the toxic effects of mercury chloride and its disposal became important considerations.

In the present study, we were able to replace the toxic mercuric chloride component in B-5 with the equally efficient protein precipitant and tolerant fixative, absolute ethanol. In conjunction with formalin, it consistently offers results that are equally comparable with B-5 (Images 3 and 4). In this initial trial of 1,009 cell blocks representative of FNAC and all forms of fluid specimens, no special congealing techniques were found necessary. The only noticeable difference from B-5 introduced by NAFS is the resulting deeper H&E staining, which is adjustable with a shorter staining schedule if desired. Centrifugation of the precipitated surrounding proteinaceous substances, blood, and plasma also resulted in sizable, firm cell pellets that enabled the recovery of cells and tiny microscopic cell aggregates, as well as facilitated subsequent easy handling, embedding, and sectioning of these paraffin blocks (Images 1 and 5).

Both the absolute alcohol and formalin solutions in this technique are general purpose grade reagents that are used routinely in cytology and histology laboratories. We have not used 95% ethanol in place of absolute alcohol, as there is no cost advantage with the minuscule amounts required compared with the large volumes needed routinely for final dehydration during staining and tissue processing. The method also takes advantage of the short 13-hour processing schedule routinely used for small biopsy specimens, such as bone marrow and true cut biopsies. The limited exposure to alcohol, xylene, and paraffin baths minimizes the artifacts of cellular shrinkage and loss of cytologic details. For the same reasons, NAFS fixation and the short processing schedule also enhance the immunohistochemical staining, requiring no protease pretreatment for the exposure of antigenic sites. Immunohistochemistry was advantageously applied in 65

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Table 3
Sensitivity of Cell Blocks

No. of Cases	(Sensitivity)
Axe et al,[14] 1986 (n = 22)	16 (73%)
Kern and Haber,[15] 1986 (n = 393)	237 (60.0%)
Wojcik and Selvaggi,[16] 1991 (n = 84)	71 (84%)
Leung and Bedard,[6] 1993 (n = 63)	54 (86%)
Present study (n = 331)	296 (89.4%)

(22.3%) of the 291 malignant specimens diagnosed in FNAC and fluid specimens. Histochemical stains for cell products and microorganisms were successfully applied in 64 (6.3%) of 1,009 cases.

Few studies have compared the value of cell blocks with smears.[6,13-16] Keyhani-Rofaga et al[13] reported that in a study of 85 cases, 55% of the original smear diagnoses were improved after the cell block was examined. The sensitivity of cell blocks varies from 60% to 86%, depending on sampling type and size, type of specimens, and aspiration techniques used **Table 3**.[6,14-16] Axe et al[14] showed that the sensitivity of Papanicolaou-stained smears (79%) was slightly superior to cell blocks (73%). Kern and Haber[15] studied 393 cases of cell block preparation. In 237 (60.3%), the findings were confirmatory, and in 103 cases (26.2%), cell blocks provided additional information for diagnosis. Wojcik and Selvaggi[16] showed that 84% of the cases had identical results on both smears and cell blocks. Leung and Bedard[6] found that all cases with adequate material could be diagnosed on a cell block preparation. Our study shows that conclusive diagnostic material was available in 296 (89.4%) cell blocks from 331 cases.

In this series of 409 FNAC specimens (Table 2), a definitive cytologic conclusion of malignancy, suggestive of malignancy, benign neoplasm, or no malignant cells, was possible from smears alone in 347 cases (84.8%) and cell blocks alone in 300 (73.3%). The overall improvement, when both smears and cell blocks were studied together, was 15.2%, or 62 cases. This does not include the improvement in specificity resulting from the use of special stains and immunohistochemical markers that were available in cell blocks from 73.3% of cases. In our experience, cell blocks are a necessity arising from variations in technique and experience of the attending physicians aspirating the lesions that may result in diagnostic material left clotted or trapped in the needle and poor quality smears.

The overall sensitivity of Papanicolaou-stained smears (87.9%) was slightly superior to cell blocks (78.2%). In relation to all tumors, benign and malignant, precise diagnosis on FNAC specimens was made in 225 cases; the smears (195 cases [86.7%]) were marginally superior to cell blocks (193 cases [85.8%]). When combined with the 95 malignant fluids, or 320 cases in all, the sensitivity of smears was 90.6% (290 cases) and cell blocks 89.4% (286 cases), which reinforces the contribution that cell blocks can make to the final diagnosis.

Decisions about the remaining 56 FNAC specimens that had insufficient cells in the smears also were supported by the cell blocks containing largely acellular proteinaceous material, which further demonstrates the technical merit of this preparation to trap microscopic tissue particles, if present, by precipitation of the tissue fluids.

In the fluid specimens, the results of smears and cell blocks were almost identical; the smears were marginally superior by 2.8%. The effectiveness of the cell blocks lies in the availability of diagnostic material for further histologic examination, histochemistry, and immunohistochemistry for better classification of the tumor and identification of the infectious cause with microbiologic stains. Specific organisms were demonstrable in 10 of the 11 positive smears in these cases.

Not all specimens are suitable for cell blocks following smear preparation. In the present series, cell blocks were possible only in cases in which some tissue material or blood was visible to the naked eye in the specimen container or in the hub of the aspirating needle. In many cases, often in benign subcutaneous lesions, the needle washings barely contained visible material, and Millipore filtration was used successfully to recover the cellular material.

Conclusion

A new simple and reliable cell block technique that is suitable for all types of cytology specimens is presented. The contribution of cell blocks to the final cytologic diagnosis supports the view that cell blocks should be considered in all fine-needle aspiration specimens whenever possible and in selective cases of exfoliative cytology specimens after review of the smears.

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