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A New, Simple Method for Intraoperative Fast Diagnosis

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Summary. The suitability of stain-coated, ready-to-use slides (Testsimplets®) for routine staining of intraoperative imprint-cytology preparations is discussed. The microscopic preparations are easily and quickly prepared, may be evaluated immediately and, with a slight loss of quality, may be kept for up to a week after application of paraffin to the cover glass. This method produces interpretable slides from cell-rich benign and malignant tumors. It is by no means a complete substitute for the usual fast-section techniques, but may be used in order to save time.

Key words: Imprint-cytology – Testsimplets® – Intraoperative fast diagnosis.

Introduction

In hospitals without a local histological laboratory, intraoperative histological diagnosis presents an apparently insoluble problem. Intraoperative examination of suspicious tissue may be necessary in the management of some cases.

The present work reports a new technique in intraoperative diagnosis. Neither microtome nor stains are necessary. Ready-to-use, stain-coated slides (Testsimplets®)[1] are used. Testsimplets have been used mainly for routine differential blood counts (Begemann, 1976; Bostjancic, 1977); but in addition produce good results in the spermiogram (Schirren, 1977), in fluid cytology (Kleine, 1977) and in sputum and pulmonary puncture cytology (Böttcher-Randohr, 1977).

Material

Imprint preparations with Testsimplets were prepared in the Pathological-Anatomical Institute of Graz University in recent months during all routine fast-section examinations (see Table 1). The results of this test series are compared with those of frozen and paraffin sections.

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[1] Manufactured by Boehringer-Mannheim

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Table 1

	n	Correct	Doubtfull	Incorrect
Breast				
benign	70	59	11	
malignant	33	31		2
Lymph nodes				
benign	18	16	2	
malignant	4	3	1	
Glioma	11	5	6	
Gastrointestinal				
benign	3	2	1	
malignant	4	4		
Lung	5	5		
Thyroid				
benign	4	3	1	
malignant	1	1		
Other				
benign	21	18	3	
malignant	12	6	6	
Total	186	153	31	2

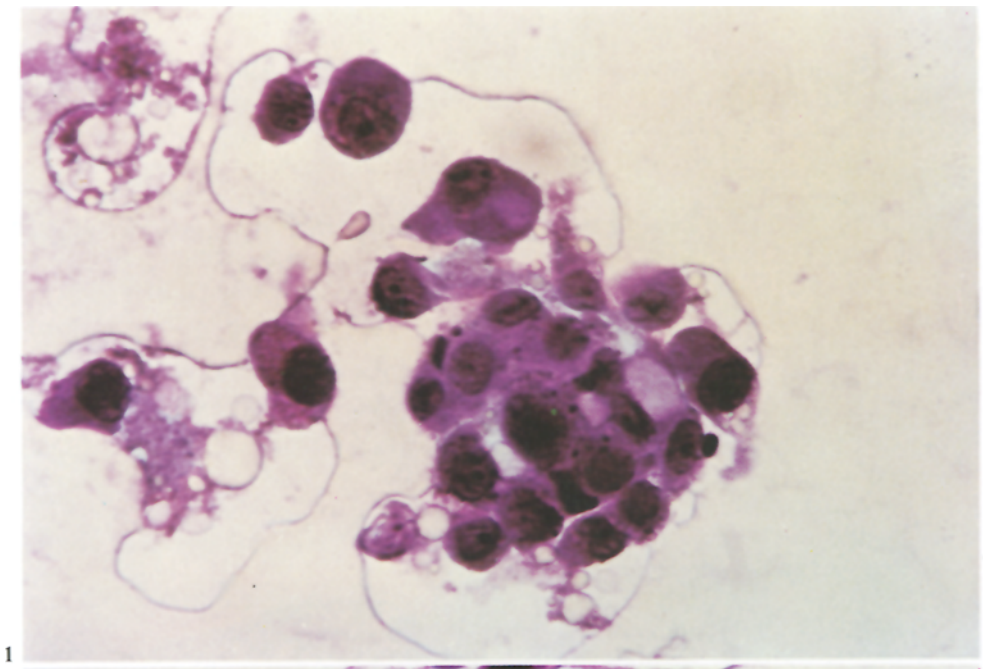
Technique

Testsimplets® are slides which are coated with fixed quantities of the stains new-methylene-blue-N and cresyl violet acetate. They are available in an easily manageable and practical cassette which includes cover glasses. Following macroscopic examination, the suspicious area of the fresh, nonfixed tissue is dabbed *lightly* onto the coverglass. Care should be taken that a small amount of tissue fluid – at most a drop the size of a match head – is applied *evenly* to the cover glass.

The cover glass is then immediately applied to the coated slide. Drying out of the specimen, must be avoided as must shifting of the cover glass on the slide. This should be pressed *lightly* to provide good distribution of the tissue fluid and thus as uniform staining as possible. If a pin is used the fluid is distributed in a star-like pattern from the middle over the entire stain-coated surface. After a staining time of 2–4 min the slide may be evaluated, and will keep at room temperature for at least 4 h. Should a longer preservation be desired, the cover glass may be hermetically sealed with liquid paraffin, which will preserve the preparation for up to a week at room temperature. After one day, however, increasing overstaining and granulation of the cytoplasm develop, often with tearing of the cell membrane. Nuclear staining also is considerably intensified (as in Figs. 1 and 2). This loss of quality is limited so that further evaluation is possible in most cases for up to a week.

Evaluation of Imprint Preparations

In the evaluation of any teased specimen it must be remembered that only those cells which separate easily from the tissue end up on the slide. Epithelial tumors thus provide better material for study than fibrous ones. The staining of the cell nuclei is an intense bluish-red and the nuclear structure, particularly the nucleolus, may be readily examined. The cytosplasmic staining is somewhat variable and is generally redbrown. Fat and mucus do not stain. The usual histomorphological criteria are valid in evaluation.



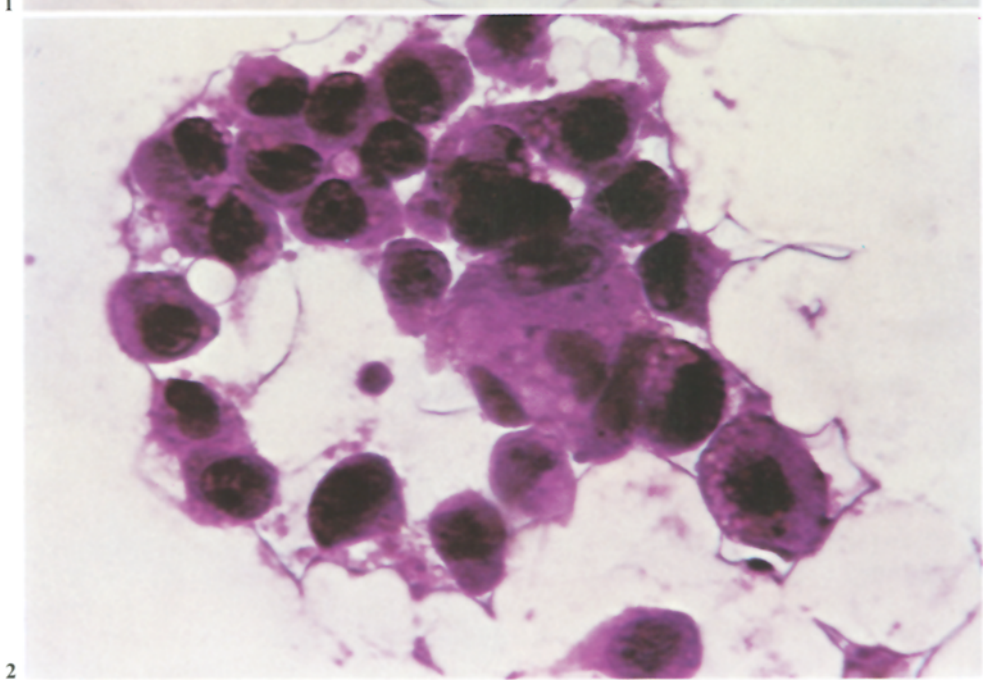


Fig. 1. E. Nr. 22226/78 Cell mass with pronounced polymorphism, from solid scirrhous mamma carcinoma. Photograph 6 days after preparing the imprint (cover glass sealed with paraffin). Testsimplet, $\times 240$

Fig. 2. As Fig. 1: $\times 400$

Sources of Error

1. Crushing. It is not advisable to attempt to squeeze as many cells as possible out of the material by increasing pressure, as nuclear chromatin is rapidly exuded and the cells obtained may no longer be interpretable. Forcible pressing of the cover glass onto the slide is to be avoided for the same reason.

2. Too Little Tissue Fluid. As the steamed-on stain is only effective in an aqueous solution, some liquid is essential. In most cases sufficient tissue fluid is obtained, and application of extra fluid is not advisable, as the even distribution of the stain is then compromised. Better results are obtained by breathing on the cover glass, or by spraying it.

3. Too Much Fluid. With specimens placed in physiological saline during transport to the histological rapid-section examination, too much fluid is found on the cover glass and/or slide.

When the slide is pressed on the cover glass the cells are floated to the edge, and the steamed-on stain solution is diluted. This causes weak central staining and overstaining at the edges.

Insufficient material and lack of experience with imprint preparations on the part of the examiner may lead to diagnostic errors.

Results and Discussion

From a total of 186 surgical specimens, imprint preparations were made with Testsimplets and compaired with frozen sections (thionin stain) and paraffin sections (hematoxylin-eosin and other stains) (see Table). Imprint specimens from the breast offered the possibility of accurate evaluation of the technique as allready emphasised. The number of cells obtained depends on the imprint technique and the histological type of tumor, few cells may be obtained from scirrhous carcinomas whilst cells are plentiful in solid or medullary carcinomas. The arrangement of cells in malignant tumors is typical: cohesion of the tumor cells is slight a valuable diagnostic point (Figs. 1 and 2). Nuclear and cellular polymorphism is variable, and may not be pronounced in scirrhous carcinoma. Tumor cells have one or more, oft reddish-stained nucleoli. The poor cohesion of tumor cells is a very important differential-diagnostic criterion in distinguishing malignant tumors of the breast from fibroadenomas (Godwin). The cells of fibroadenomata are often found in dense nests, but are likely to be small, tailed, and show not notable polymorphism (Fig. 3). Tailed cells with no pleomorphism which occur occasionally in isolation, or in small nests, indicate a (fibrous) mastopathy. In these cases a negative result does not exclude malignancy. With Testsimplets® 31 of 33 breast carcinomas were diagnosed without ambiguity. In two cases no tumor cells were found. These were distinctly scirrhous carcinomas which were clearly diagnosed as carcinoma on macroscopic

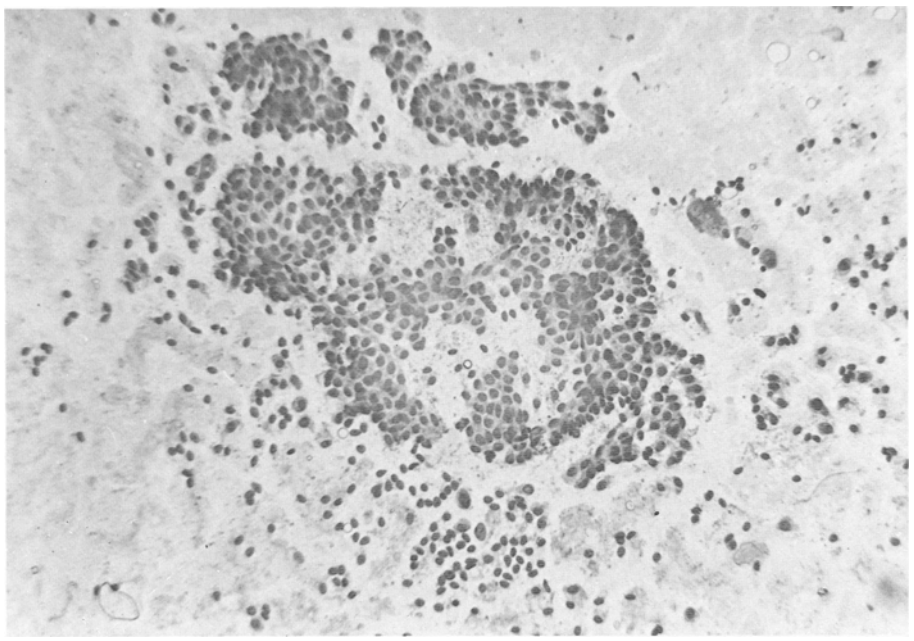


Fig. 3. E. Nr. 26299/78 Intracanalicular fibroadenoma of the mamma. Distinctly spindle-shaped cells are found in groups and bands. Photgraph 24 h after preparation of the imprint slide. In the center the cells are not so sharply stained. Testsimplet, × 80

examination. For border line cases (duct papilloma, proliferating mastopathy or lobular carcinoma in situ) the method is inadequate.

Good results may also be obtained in the diagnosis of metastases in lymph nodes and with epithelial tumors of the lung and thyroid. The method appears to be less suitable for diagnosis of brain tumors, with the exceptions of polymorph-cellular glioblastomas and metastases, and for lymph-node diseases, such as specific inflammation or lymphoma. Suen came to similar conclusions from his examination of imprint preparations and hematoxylin-eosin staining in 1258 cases.

This simple method permits rapid diagnosis of unfixed tissue which is valid only in the case of clearly positive findings. The reliability increases with the examiner's experience, and the technique should be appraised as a supplement to rapid – section diagnosis. It produces clearly positive results in a large percentage of solid carcinomas, and in surgical departments without a nearby histology laboratory, saves the time necessary for the results of a frozen or paraffin section to arrive. It must, however, be stressed that the usual routine rapid-section technique is preferable in some cases. Preservation of imprint preparations over a longer period of time is not possible and photography offers the only means of documentation. We propose to test the technique further with a larger amount of material.

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