Technical methods

A simple method for processing fine-needle aspiration biopsy specimens for electron microscopy

MOHAMMED AKHTAR, M ASHRAF ALI, EMYR OWEN, AND MOHAMMED BAKRY *Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia*

Fine-needle aspiration biopsy is a method whereby a very small quantity of tissue fluid and cells is aspirated from a lesion for cytological examination. This technique has been used for many years in the biopsy of superficial lymph node lesions and breast masses. Biopsy may also be done in this way on prostate, lung, and intra-abdominal organs. In

appropriately selected cases, aspiration biopsy offers a quick, relatively painless, and convenient way of obtaining a pathological diagnosis.^{1,2}

The usefulness and accuracy of fine-needle aspiration biopsy may be enhanced much further if light microscopic examination of the biopsy specimen could be supplemented by electron microscopy. In this paper we describe a simple technique for processing fine-needle aspiration biopsy specimens for electron microscopy.

Material and methods

Fine-needle aspiration is performed in the usual manner, and routine smears are made for cytological examination. The remainder of the aspirated sample is flushed out into a microtube* containing 3% phosphate buffered glutaraldehyde, pH 7.3. The

*Beckman RIIC Ltd, Microtube, Plain, Catalogue No. 31432.

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Fig. 1 Method for processing fine-needle aspiration sample for electron microscopy.

Fig. 2 *Electron micrograph from a fine-needle aspiration biopsy depicting a plasma cell. The biopsy was taken from a mass in the anterior chest wall of a patient with multiple myeloma. $\times 20\,000$.*

specimen is then processed according to the method outlined in Figure 1. The block thus obtained is mounted on an ultra-microtome, and 1 micron thick sections are cut; these are stained with toluidine blue for light microscopic examination. Appropriate clusters of cells are selected for ultrastructural examination, and the block is trimmed accordingly. Ultra-thin sections are stained with uranyl acetate and lead citrate.

Results

In most cases the final block contained representative cells from the lesion in addition to variable numbers of red blood cells and leucocytes. These cells were usually present at the tip of the block and therefore required only a few initial sections for identification. Penetration by the embedding medium was adequate as long as the amount of aspirated material added to

a single microtube did not exceed one drop and the thickness of the cell block after centrifugation was less than 2 mm. In cases where the cell block exceeded 2 mm, the specimen had to be subdivided and processed in more than one microtube. Attempts at processing larger cell blocks always resulted in poor penetration.

Discussion

The method outlined has been used successfully in our laboratory to process more than 50 specimens and has been found to be consistent and reliable. Sufficient numbers of cells were recovered, even in those cases where the yield of aspirated material was relatively scanty. Furthermore, the overall quality of the ultra-thin sections obtained from the blocks was good, and morphological details were well preserved (Fig. 2).

The role of electron microscopy in the field of surgical pathology is now well established. Using the technique described in this report, electron microscopy can now be extended to fine-needle aspiration biopsy specimens. We believe that, in selected cases, electron microscopy of fine-needle aspiration biopsy specimens may provide valuable information, enabling the pathologist to make a much more precise diagnosis than is possible with light microscopy alone.

References

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Requests for reprints to: Medical Affairs, King Faisal Specialist Hospital and Research Centre, PO Box 3354, Riyadh, Kingdom of Saudi Arabia.

An economical, simplified haemagglutination test for mass syphilis screening

JAJ BARBARA, RAJAS SALKER, FATIMA LALJI, TD DAVIES, AND JB HARRIS *North London Blood Transfusion Centre, Deansbrook Road, Edgware, Middx HA8 9BD, UK*

Until recently the only fully automated syphilis antibody screen tests were those employing Venereal Disease Research Laboratory (VDRL) carbon antigen on mass blood grouping machines like the Technicon BG 15 AutoAnalyzer (Technicon Instruments, Basingstoke, Hants) and the Groupamatic 360C (Kontron, Roche Bio-Electronics, St Albans, Herts). We present a semi-automated modification of the commercially available Fujizoki *Treponema pallidum* haemagglutination test (TPHA) marketed by Diamed Diagnostics Ltd (Bootle, Merseyside).

This test uses sheep red cells sensitised with *Treponema pallidum* extracts. Other authors¹ have presented similar methods. However, our modification is based on extensive dilution of the test cells² and the use of a semi-automated mass sampling device³ for diluting donor sera 1 in 8 in one step, in microtitration plates; with this device we can easily transfer 96 samples into microtitre plates using metal loops that can be rinsed between runs. The sera are diluted to a final volume of 25 μ l in the absorption diluent provided in the TPHA kit (with sheep serum added to a final 2% concentration). Extra diluent separate from the kits can be purchased from Diamed. Then 25 μ l of a 0.1% suspension of TPHA test cells in distilled water (with azide, if desired) are added. The plates are kept at room temperature for 15 minutes, centrifuged at 260 g for 1½ minutes, and then sloped at 70°. After approximately 10 minutes positive samples have remained as 'buttons', whereas the negative samples have formed 'streaks'.² Serum samples are used because plasma samples tend to give false-positive results. Inactivation is unnecessary. Screen positive samples are checked by titration with test and control cells and absorbed with control cells if necessary and re-titrated.

To date we have tested 36 500 donor serum samples by both the 0.1% TPHA and the carbon antigen test as used with AutoAnalyzers. Only one or two 0.1% TPHA screen positives are found per plate. Ninety-five per cent of these are quickly verified by titration with test and control cells. The remainder require a repeat titration after absorption. Fifty-five (1 in 700)

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