Technical methods

Urine cytology by a filter imprint method

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The introduction of programmes to detect bladder cancer in people exposed to carcinogens and the increased interest in the value of urinary cytology as a diagnostic procedure have resulted in the need for a satisfactory method of preparing specimens. Ideally, the method must be simple to perform and reproducible, and result in a high degree of cellular clarity and minimal cellular distortion.

Various methods have been used. Smears have been made from centrifuged deposits (Cowen, 1971). The problems encountered with this method have been loss of cells from the slide, partly overcome by using frosted glass slides or coating the slides with albumin, drying artefacts, and the difficulty of obtaining an adequately cellular preparation. Cytocentrifugation, where the cells are spun onto slides, has been introduced more recently, but recovery rates have been found to be poor, and there is also cellular distortion (Barrett and King, 1976).

In many respects membrane filter techniques using polycarbonate or cellulose esters have been found to be superior and are generally regarded as preferable (Tyrkkö, 1972). These two materials differ in their physical, chemical, and optical properties and need to be treated differently (Gill, 1975). Both, however, have their disadvantages. The pore outlines of polycarbonate filters remain visible and are a distraction during microscopy. A method does exist for rendering them invisible, but it is technically exacting and can result in irrevocable damage to the preparation. Although this problem is not present with cellulose filters, the material itself and the mucus and bacterial or other debris, which tend to get trapped within the pores, absorb stain, obscuring the morphological detail of the exfoliated cells.

We have modified the filtration technique by making an imprint onto a glass slide of the cells trapped on a membrane filter.

Method

The urine specimens are preserved with propyl alcohol and concentrated by refrigerated overnight standing in tapering urine flasks. The supernatant is discarded, and the sediment is resuspended in 20 ml urine. Using a Millipore 1225 sampling manifold, 12 samples can be prepared simultaneously. Oxid cellulose ester filters, pore size 0.45 μm , are used and moistened with 5 ml saline. The urine is then filtered, if necessary using a vacuum of not more than 5 mmHg, and the sides of the filtration cup are rinsed with a further 5 ml saline. At no time are the filters allowed to dry out. The filters are then removed, inverted over clean glass slides, and blotted, using clean blotting paper and a single, firm pressure stroke of the hand. The slides with adherent filters are then placed immediately into 3% acetic acid fixative.

The amount of pressure to be applied during blotting is quickly learnt. When correct, the filter falls off the slide after 5 to 10 minutes in the fixative, or is dislodged when the slide on the bottom of the container is tapped. When too great pressure has been used the filter will not be dislodged and has to be peeled off. This can lead to flattening of the cells. Insufficient pressure results in the filter falling off after 5 to 10 seconds and a less cellular preparation.

Fixation is continued for at least 30 minutes; the slides are removed, covered by a thin layer of celloidin, and stained with haematoxylin and eosin.

Results

Recovery studies have been done by counting the cells present in the imprint and those remaining behind on the filter. At least 80% of the cells were found to have been transferred to the imprint. The clarity produced by this imprint method exceeds that obtained using a variety of commercial filters (Figure). There is no cellular distortion and the morphological detail is easily seen without the uneven interference of background staining. In addition, clumps of bacteria and proteinaceous debris remain behind in the filter. Photography for teaching purposes is much easier, and it is possible to use fluorescent staining techniques, such as acridine orange, which are not possible when the filter is still present. Imprints can be kept indefinitely without deterioration, while the stained

Figure Malignant transitional cells in urine. (Haematoxylin and eosin $\times 62$)

filters have sometimes become opaque, because of further drying out and air becoming trapped within the pores.

Comment

This simple modification of the filter technique produces cellular preparations with good preservation of morphological detail unobscured by background staining. The method enables large numbers of urine samples to be screened more easily. Using this method over the past eight years, between 5000 and 6000 urines have been examined. Many have been from surgical patients with recurrent papillary tumours. Of the 1000 industrial wine specimens examined so far, one positive imprint has been found, and the presence of a bladder tumour has been confirmed by cystoscopic biopsy.

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Comparison of radioimmunoassay and enzyme immunoassay for detecting hepatitis B surface antigen in serum from freshly donated blood and selected blood products

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Engvall and Perlmann (1971, 1972) and van Weemen and Schuurs (1971, 1972) pioneered work on enzyme immunoassay (EIA). Solid phase EIA techniques now offer an attractive alternative to the established ^{125}I or fluorescein-labelled-antibody methods for detecting microbial antigens and antibodies.

For some time the solid phase radioimmunoassay (RIA) has been widely accepted as the most sensitive practical assay for HBsAg in the serum of patients and blood donors. Recent reports indicate, however, that a solid phase EIA (Hepanostika) marketed by Organon Teknika may be of comparable sensitivity to the solid phase RIA (Ausria-II) marketed by Abbott Laboratories (Wolters *et al.*, 1976; Ukkonen *et al.*, 1977). Hepanostika is a microplate double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (Voller *et al.*, 1976) in which microplates coated with antibody to HBsAg are reacted with the test samples thought to contain antigen. The HBsAg becomes fixed to such plates and is indicated by means of a reagent consisting of enzyme-labelled antiserum to HBsAg. This reagent converts a colourless substrate to a coloured product, which can be assessed visually.

We have made a direct comparison of Ausria-II and Hepanostika for detection of HBsAg in freshly donated blood, using a coded panel including strongly and weakly positive sera, and factor VIII concentrates previously found to be weakly RIA positive and confirmed by specific neutralisation. Ausria-II was performed, as recommended by the manufacturers, using a Pentawash gun and Filamatic dispenser for bead washing. ^{125}I activity was monitored with a Nuclear Enterprises NE1600 (16-channel) gamma counter. Hepanostika was performed, as recommended by the manufacturer, using semiautomatic washing apparatus and an 11-channel pipette supplied by Organon Teknika. Results (colour change) were read with the naked eye.

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