Technical methods

Colposcopic biopsies of the cervix: use of Bouin’s fluid, multicompartment wax rafts

G PHILIP, AH SPICER Department of Pathology, Kingston Hospital, Kingston upon Thames, Surrey

The superior fixation of nuclear chromatin by fixatives containing acetic acid is well known and, like others, we have found that for biopsies of the cervix Bouin’s fluid (saturated aqueous picric acid 75 cm³, formalin 25 cm³ and glacial acetic acid 5 cm³) gives better fixation than formaldehyde in saline. The main difference is seen in the improved clarity of the epithelial cell nuclei, which appear very much as they do in alcohol-fixed cervical smears stained by the Papanicolaou method. Red cells are lysed, but this is of no consequence.

Fixation of small biopsies in Bouin’s fluid is complete in 6–12 h but they are conveniently left in fixative overnight since no problem has been encountered with excessive hardening of the tissue. Processing is done by machine on a 17 hour schedule, the first transfer being from Bouin’s fluid to 70% alcohol. The clearing agent is chloroform. The same technique is used for cutting sections as for formalin-fixed material and only minor alterations are needed in the staining times with haematoxylin and eosin. There is no need to remove the yellow picric acid staining from the sections, although this can be done by treatment with 2-5% sodium thiosulphate followed by alcohol after the sections have been taken to water.

The main disadvantage of Bouin’s fluid is its tendency to stain almost anything it touches bright yellow. We have devised a composite container consisting of four glass bijou bottles fixed to a metal base of suitable size and have found this convenient in use and not easily upset. The base used is a metal lid of convenient size (approximately 5·5 cm in diameter) and the bijou bottles are fixed to it with epoxy resin glue (Figure). This assembly fits inside a larger screw-top jar and a piece of plastic sponge in the bottom of the jar prevents rattling and absorbs any spillage. The individual bijou bottles are labelled “A, B, C & D” with waterproof ink, but one label on the outer container serves to identify the group of biopsies with the correct patient.

These multiple containers are sent out from the laboratory with approximately 5 cm³ of fixative already dispensed into each bijou bottle. In the clinic the biopsies are picked off the biopsy forceps with a fine disposable needle and transferred to small rectangles of dental wax sheet (“Tenatex” toughened red dental modelling wax, Associated Dental Products Ltd, Swindon). The wax is then “speared” with the needle and transferred with the biopsy to the appropriate bijou bottle. The wax raft provides a quick and easy method of transferring the biopsy but is not intended to maintain it in any particular orientation; indeed the biopsy frequently becomes detached from the wax as soon as it enters the fluid. In any case, the biopsy remains below the surface of the fixative even if attached to the raft. Wax is preferred to card or blotting paper because of this lack of adhesion, which has the effect that small detached groups of abnormal epithelial cells are more likely to remain with the biopsy and appear in the finished sections.

Requests for reprints to: Dr G Philip, Kingston Hospital, Kingston upon Thames, Surrey KT2 7QB, England.
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G Philip and A H Spicer

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