

Matters arising

Immediate fixation is cheaper than microwave fixation for obtaining good cytological details in frozen sections

Kennedy and Foulis recently reported the use of microwave fixation in improving the cytomorphological details in frozen sections.¹ Their conclusion was not well founded, however, because they used improper fixation in their controls. As the authors had already noted, air drying at any stage was deleterious to the final cytological details even in the microwave sections, but then they air dried the frozen sections before fixation in Wolman's solution for their controls. Anyone with experience in interpreting cytological smears knows that nuclear details are extremely poor in smears stained with haematoxylin and eosin if there has been air drying.² This is no exception for frozen sections. Air drying "to achieve section adherence before fixation" is totally unnecessary: the difference in temperature between the section (-20°C) and the slide (20°C) is sufficient to attach the section firmly to the slide as well as producing some heat fixation.³ In our laboratory we immediately fix frozen sections in formol-alcohol (equal parts of 10% formalin and 95% alcohol) without allowing them to dry. A fixation period of 20 to 30 seconds is

adequate to achieve excellent cytological details (figure). Besides, we have not experienced problems of detachment of section from the slide.

I do not doubt the value of microwave in speeding up fixation and many staining procedures. And excellent antigenic preservation can be achieved by using microwaves for fixation of surgical specimens.⁴ I disagree, however, with the authors' conclusion that, "improvement in quality of frozen sections alone is sufficient reason to purchase such a relatively inexpensive item (microwave oven)." A hospital administrator should reject such a request if this is the sole justification, because immediate fixation in formol-alcohol is a much cheaper and simpler technique.

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References

- 1 Kennedy A, Foulis AK. Use of microwave oven improves morphology and staining of cryostat sections. *J Clin Pathol* 1989;42:101-5.
- 2 Chan JKC, Kung ITM. Rehydration of air-dried smears with normal saline: application in fine-needle aspiration cytological examination. *Am J Clin Pathol* 1988;89:30-4.
- 3 Bancroft JD. Frozen and related sections. In: Bancroft JD, Stevens A, eds. *Theory and practice of histological techniques*. Edinburgh: Churchill Livingstone, 1982:89.

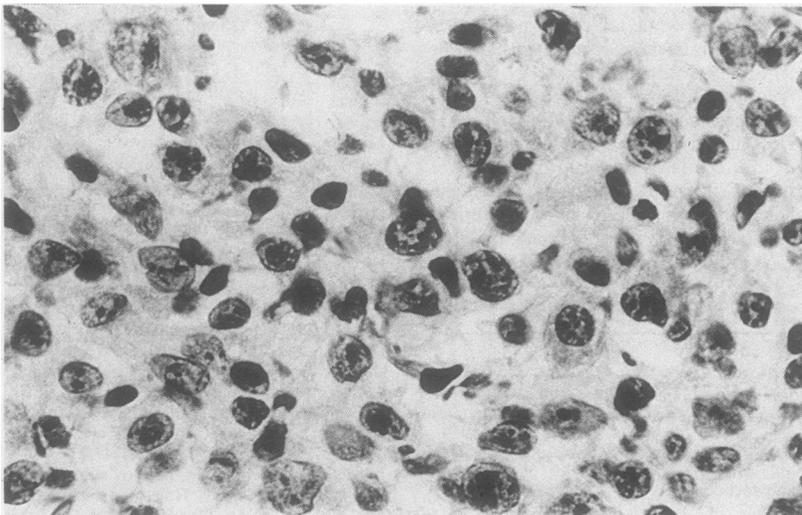


Figure Non-Hodgkin's lymphoma in a frozen section immediately fixed in formol-alcohol. Note crisp nuclear details and the distinct nucleoli. (Haematoxylin and eosin.)

- 4 Leong ASY, Milios J, Duncis CG. Antigen preservation in microwave-irradiated tissue: a comparison with formaldehyde fixation. *Pathol* 1988;156:275-82.

Dr Kennedy comments:

Our paper reflected the change in the quality of haematoxylin and eosin staining frozen sections which occurred when we changed over to microwave assisted fixation. For that reason the only valid controls were smears stained with haematoxylin and eosin produced by our previous method—air dried, then fixed in Wolman's solution.

Although well aware of the problems caused by air drying, particularly for frozen sections of breast, we have found no satisfactory alternative which did not cause a high rate of section detachment. The microwave procedure is the only method which we have found to be satisfactory. Our experience in other departments indicates that we are not alone in this finding.

We have also found the special staining application of the microwave extremely useful, particularly with regard to identifying the resection margins of gastric tumours allowing the pathologist a much greater degree of confidence in classifying tumours from frozen preparations.

When multiple block frozen sections are being processed in our department, we have found that the very rapid (eight second) fixation period greatly contributes to the speed with which a smear stained with haematoxylin and eosin can be prepared.

In conclusion, the above points surely amply justify the very low cost of purchasing a domestic microwave oven.

Effect of heat inactivation of sera on anti-Trichomonas vaginalis IgG ELISA

We recently read with interest the paper by Francis *et al*¹ concerning the deleterious effect of heat inactivation of sera on the results of the anti-toxoplasma IgG ELISA. We have been studying the serum antibody response in human urogenital trichomoniasis using ELISA² and have had the same experience. By screening the antibody titres in a normal population we also included a panel of sera previously tested for toxoplasmosis by complement binding reaction. These sera, unlike the others in our study, were heat inactivated. Surprisingly, they were all positive for anti-Trichomonas vaginalis.