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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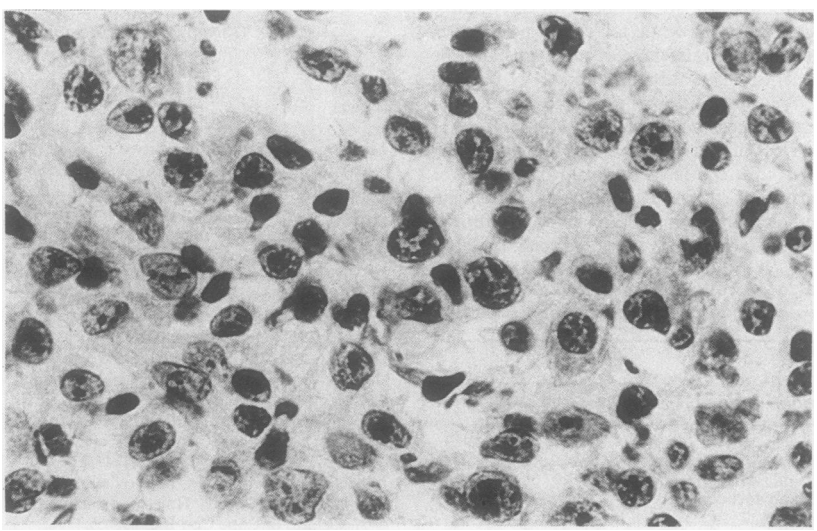

Figure *Non-Hodgkin's lymphoma in a frozen section immediately fixed in formol-alcohol. Note crisp nuclear details and the distinct nucleoli. (Haematoxylin and eosin.)*

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 of 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 ELISA[2] 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, these were all positive for anti-*Trichomonas vagin*

Table *Influence of heat inactivation of human sera on results of anti-T vaginalis IgG ELISA*

Serum designation*	Absorbance†		Titre IgG	
	Unheated	Heated	Unheated	Heated
096	0·03	0·45	0	4000
103	0·00	0·30	0	4000
104	0·02	0·30	0	4000
106	0·02	0·54	0	8000
020	0·07	0·99	0	>8000
034	0·03	0·88	1000	>8000
015	0·05	0·65	2000	8000
017	0·03	0·39	2000	4000
032	0·06	1·26	1000	>8000
027	0·00	0·32	0	4000
091 T	0·29	0·74	4000	8000
105 T	0·09	0·38	1000	4000
062 T	0·03	1·52	1000	>8000
078 T	0·10	1·35	1000	>8000
066 T	0·07	0·99	2000	>8000
091 T	0·07	0·93	2000	8000
028 T	0·07	0·98	1000	>8000
034 T	0·28	1·41	4000	>8000
099 T	0·05	1·07	0	>8000
075 T	0·04	1·27	2000	>8000

*Sera designated T are from women with positive culture for *T vaginalis*.
†At a serum dilution of 1/4000.

alis antibodies by ELISA. The effect of heat inactivation on the results of ELISA was therefore investigated.

Twenty sera, 10 from women positive by culture for *T vaginalis*, and 10 from unaffected patients, were tested by ELISA as previously described[2] before and after heat inactivation (56°C, 30 minutes). The results are given in the table. The titres of all tested sera became positive after inactivation. Francis *et al* consider that the non-specific attachment of heat-aggregated immunoglobulin to the solid phase is the possible cause of increased absorbancies.[1] According to our results, heat inactivation of sera should also be avoided in anti-*T vaginalis* IgG, and possibly, when determining specific antibodies of any kind by ELISA.

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Dr Joynson comments:

We first brought attention to the problem of false positive results occurring with heat-inactivated sera in an *indirect* ELISA for toxoplasma Specific IgG in 1987[1] and suggested that it could occur with indirect ELISA's for other agents. The work of Gombošová and Valent substantiates this. We would, however, point out that the problem seems to be confined to *indirect* ELISA methods—that is, where antigen is coated on to the solid phase. In our experience false positive results do not occur with heat inactivated sera when tested for toxoplasma specific IgM by an antibody class capture type assay.

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Manual and automated prothrombin time tests

I was interested in the paper by Poller *et al* and a little surprised by their finding that manual prothrombin time tests show greater precision than automated tests in the National External Quality Assessment Scheme (NEQAS) survey.[1] Intuitively, I would have expected the opposite. Their findings seem to contrast greatly with the situation in North America.

Unfortunately, the international normalised ratio has not yet been widely adopted here and the international sensitivity index of reagents commonly used is quite different, so that a direct comparison cannot be made. On the basis of the College of American Pathologists (CAP) survey,[2] however, in which a large number of laboratories in the USA and Canada participate, there seems to be a much higher degree of precision in automated prothrombin time results compared with that shown by the NEQAS results.

In the CAP survey, participants are grouped according to the reagent/analyser combination used. Review of the results for 1988 shows the most common combination to the Dade Thromboplastin C with the MLA Electra 700. This was used by more than 250 laboratories to analyse 15 survey samples with prothrombin times in the range 10–24 seconds. In no case did the coefficient of variation (CV) exceed 4%. The Coagamate X-2, in combination with General Diagnostics Automated Simplastin, was used by over 30 laboratories and the CV for all samples tested was less than 5%. Review of all reagent/analyser combinations used by 20 or more laboratories showed that most had a CV of less than 5%.

Analysis of manual prothrombin time data is not available as manual methodology is almost extinct as a means of routine coagulation testing, and as already mentioned, direct comparison with the NEQAS results may not be appropriate. None the less these results suggest that there may be greater precision with automated methods as used in North America. No doubt, there are a number of possible explanations for this. One which occurs to me is that there may be interbatch variation in the thromboplastin used by NEQAS participants, at least in relation to its performance in an automated system.

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