Correspondence

Immunohistochemical detection of Ki67 antigen in formalin fixed, paraffin wax embedded sections

We read with interest the recent paper by Torp *et al*[1] reporting the detection of the Ki67 antigen in formalin fixed, paraffin wax embedded glioblastoma tissue sections using a monoclonal antibody directed against Ki67, purchased from Dako, Glostrup, Denmark. The authors did not recommend the use of this antibody on paraffin wax sections. We have also tested the antibody on sections from routine blocks from human gingiva, tonsils and lymph nodes using different time schedules for microwave pretreatment and application of the primary antibody. Two crucial procedures for detecting Ki67 antigen successfully were identified. Firstly, slides should be pretreated in a microwave oven in citrate buffer (pH 6.0) for over 20 minutes (4 × 5 minutes).[2] Secondly, Ki67 antibody (diluted 1 in 100–200) should be incubated overnight at 4°C to produce strong staining.

Torp *et al* pretreated sections by microwaving for only 10 minutes (2 × 5 minutes) and incubated the Ki67 antibody for only one hour. Under these conditions, we could not achieve satisfactory staining on sections of the three tissues described above. Bankfalvi *et al*[3] have reported that almost identical results were found with the Ki67 and MIB1 antibodies. In their method, microwave heating was carried out for 35 minutes (7 × 5 minutes) and the Ki67 monoclonal antibody was applied overnight.

However, it should be noted that microwave treatment has certain drawbacks compared with autoclave treatment. Human gingival tissue sections were sometimes disrupted and detached from slides during microwave heating, presumably because of the violent boiling procedure, whereas tonsil and lymph node sections were resistant to this procedure even after one hour in our laboratory. Furthermore, another disadvantage of the Ki67 antibody was background staining on epithelium and various cell types except polymorphs, although these were easily distinguishable from genuinely reactive structures. Thus, Ki67 monoclonal antibody is as useful as MIB1 even in formalin fixed, paraffin wax sections, if the tissue sections to be examined can withstand more than 20 minutes in a microwave oven.

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1 Torp SH, Johannesen E, Lindboe CF. Comparison of different Ki67 antibodies in human glioblastomas. *J Clin Pathol: Mol Pathol* 1995; **48:**M191–3.
2 Munakata S, Hendricks JB. Effect of fixation and microwave oven heating time on retrieval of the Ki-67 antigen from paraffin-embedded tissue. *J Histochem Cytochem* 1993;**41:**1241–6.
3 Bankfalvi A, Navabi H, Bier B, Bocker W, Jasani B, Schmid W. Wet autoclave pretreatment for antigen retrieval in diagnostic immunohistochemistry. *J Pathol* 1994;**174:**223–8.

Drs Torp, Johannesen and Lindboe comment:

We recently reported our experience with the prototypic Ki67 monoclonal antibody and a new Ki67 antiserum on frozen and paraffin wax section of human glioblastomas.[1] It was not the intention of this study to optimise the staining procedures but to use the standard procedures in our laboratory—that is, microwave oven heating 2 × 5 minutes (600 W), citrate buffer (pH 6.0) and incubation of the antibody for one hour at room temperature. Under these conditions, the prototypic Ki67 antibody produced variable immunostaining whilst the Ki67 antiserum yielded nice immunostaining on paraffin wax sections. Thus, we concluded that the latter can be recommended for use on paraffin wax sections.

We realise that time of fixation, microwave irradiation and antibody incubation as well as the pH of the buffer may all influence staining quality.[2–4] Comparison of data is more difficult when different immunostaining techniques are used. Therefore, we are of the opinion that on paraffin wax sections, only those antibodies that give satisfactory immunostaining under standard conditions should be recommended.

1 Torp SH, Johannesen E, Lindboe CF. Comparison of different Ki67 antibodies in human glioblastomas. *J Clin Pathol: Mol Pathol* 1995; **48:**M191–3.
2 Munakata S, Hendricks JB. Effect of fixation time and microwave oven heating time on retrieval of the Ki-67 antigen from paraffin-embedded tissue. *J Histochem Cytochem* 1993;**41:**1241–6.
3 Cuevas EC, Bateman AC, Wilkins BS, Johnson PA, Williams JH, Lee AHS, *et al.* Microwave antigen retrieval in immunocytochemistry: a study of 80 antibodies. *J Clin Pathol* 1994; **47:**448–52.
4 Imam SA, Young L, Chaiwun B, Taylor CR. Comparison of two antigen retrieval solutions in unmasking epitopes in formalin-fixed tissue for immunostaining (abstract). *Anticancer Res* 1995;**15:**1812.

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Adhesion Molecules and the Lung. Ward PA, Fantone JC (eds). Volume 89 in the series **Lung biology in Health and Disease.** Lenfant C (executive editor). (Pp 416; $165.00.) Marcel Dekker. 1996. ISBN 0 8217 9517 2.

This is the 89th volume in a continuing series on lung biology in health and disease and addresses the exponentially increasing information on adhesion molecules. As with all texts, we probably have our own views on how they should be presented and developed. Ideally, the book should start with a basic chapter on the whole process of cell recruitment, outlining the rolling of cells, loose adhesion, firm adhesion, and then cell migration. These processes involve different adhesion molecules. With this background, it might have been more appropriate to have started with selecting, proceeded to a chapter on the beta 2 integrins and then the other cell adhesion molecules.

There is some repetition throughout the chapters, as would be expected in such a complicated field, but this certainly does not detract from the strength of the book, which is very comprehensive, well referenced and generally easy to follow. The introduction is good and it is strong from the basic science point of view. It includes a chapter on signal transduction, the lesser known role of adhesion molecules in lung morphogenesis, the adhesion processes between the epithelial cells, and the underlying matrix. There are also chapters on bacterial adherence, a process which is a prerequisite for invasive infection, and the role of adhesion molecules in malignant metastases. From the clinical point of view, there are sections on experimental lung injury, wound healing, transplantation, and pneumonia.

One great omission from this book is the genetics of adhesion deficiency. The whole field of adhesion molecules was established following the observation of defective cell migration, shown to be related to deficiency of the adhesion molecules. There have been great advances in the understanding of the basic genetic defect and it would have been appropriate to have had a chapter on this. In addition, there is little on the immune response, which clearly requires very specific cell adhesion and what evidence is included in the text is found in sections such as those on lung transplantation. Again, it might have been better to have pulled out several chapters on cell specific adhesion processes such as those that involve the lymphocyte or the neutrophil. However, once again, this probably reflects personal preference rather than true criticisms of the book.

In summary, this is an important addition to the lung biology in health and disease series. It contains a lot of information that overlaps other fields of medicine and will appeal to both basic scientists and clinical scientists alike.

R A STOCKLEY

Gel Electrophoresis: Proteins. Dunn MJ. Series: **Introduction to Biotechniques.** (Pp 176; £17.95.) Bios Scientific Publishers in association with the Biochemical Society. 1996. ISBN 1 8 72748 21 X.

This soft back edition sets out to offer a practical introduction to undergraduates, postgraduate scientists and technicians in the field of electrophoretic protein separation methods. Overall, I found this book to be clearly written, well laid out, amply illustrated, and well referenced. I found the concept of summarising transcription, translation and protein structure in two and a half pages of text quite commendable, even if erring a little on the basic side. The book concentrates primarily on polyacrylamide gel electrophoresis (PAGE) and gives little space to any other forms of protein electrophoresis.