Correspondence

Which proliferation markers for routine immunohistology?

In their paper on proliferation markers, Rose *et al*¹ mention the problem of non-specific staining of renal tubular cytoplasm. We have observed this artefact both in the normal tubular epithelium and in renal cell carcinomas. In the latter instance, disruption of normal tissue integrity can make it difficult to distinguish specific from nonspecific labelling. For standard immunohistochemistry, we use an indirect Streptavidin immunoperoxidase technique (Dako, High Wycombe, UK) and we initially attempted to eliminate the background problem in kidney by applying standard blocking procedures for endogenous peroxidase and avidin/biotin (Vector Laboratories, Peterborough, UK) (fig 1). When this was not successful, we went on to use an indirect alkaline phosphatase-based ABC protocol (Dako), which also gave high background staining in renal tubular cells, and a glucose oxidase based protocol (Vector), which was insufficiently sensitive. Our solution to the problem has been to use a digoxigenin (DIG) based protocol, details of which are given later. This method is reliable, sensitive and eliminates the background staining problems peculiar to kidney.

Our protocol is as follows. Paraffin sections are dewaxed and subjected, as appropriate, to antigen retrieval. Sections are incubated sequentially (1) for five minutes in 10% sheep serum, (2) for 60 minutes in primary antibody, (3) for 30 minutes in F(ab)₂ fragments of sheep anti-immunoglobulins conjugated to DIG (diluted 1 in 200; Boehringer Mannheim), and (4) for 30 minutes in F(ab)₂ sheep anti-DIG conjugated to horseradish peroxidase (diluted 1 in 400; Boehringer Mannheim), with washing after stages 2 to 4. The

Figure 1 High non-specific background staining in normal kidney using the standard immunoperoxidase procedure.

Figure 2 Specific labelling for CD31 in normal kidney using the DIG technique.

bound antibody is visualised using a DAB/H₂O₂ substrate reaction and sections are lightly counterstained with haematoxylin, dehydrated and mounted in DPX (fig 2).

In our quest to find a solution to the high background staining problem, we initially spoke to a number of routine and research immunohistochemistry laboratories and found that high background staining of renal tubular cytoplasm was widely accepted as unavoidable. It is likely that the background staining is due to the large number of mitochondria present in the tubular epithelial cells. Mitochondria contain high levels of endogenous biotin,² to the extent that the avidin/biotin blocking procedure may be insufficient to quench background staining. Hence, we trust that our solution to the problem will be of widespread interest to his-topathologists and researchers engaged in immunohistochemical analysis of kidney and its neoplasms.

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- 2 Varma VA, Cerjan CM, Abbott KL, Hunter SB. Non-isotopic in situ hybridization method for mitochondria in oncocytes. *J Histochem Cytochem* 1994;42:273-6.

EBNA expression in Reed-Sternberg-like cells in post-transplant lymphoproliferative disorders

The paper by Khan and Naase raises very interesting issues.¹ We have conducted an immunohistochemical study on 28 cases of post-transplant lymphoproliferative disorders (PTLD) (17 renal and 11 heart/heart and lung), looking specifically at Epstein-Barr virus (EBV) encoded nuclear antigen-1 (EBNA-1) and -2 immunoreexpression, using the antibodies IH4-1 and PE2.² We also used a pressure cooking method of antigen retrieval.

Fifteen of the 28 cases were positive for EBNA-1 or EBNA-2, or both. Only two of the 15 positive cases showed discordance of EBNA-1 and EBNA-2 staining. In other words, one case was EBNA-1 positive/EBNA-2 negative; the other was EBNA-1 negative/EBNA-2 positive. Positive staining was seen in the entire cellular spectrum encountered in PTLD: small lymphoid cells, immunoblasts and Reed-Sternberg-like cells. Strikingly, variation in staining intensity was noted within each case and between cases. This heterogeneity was also noted by Oudejans *et al*.³ They concluded that PTLD exhibited a heterogenous pattern of EBV gene expression within individual cases.

The apparent down-regulation of EBNA-1 expression has important implications, because EBNA-1 is thought to be necessary for maintaining the EBV genome in an episomal form and to be consistently expressed in all EBV infections. The results obtained by Khan and Naase and ourselves, using immunohistochemistry, indicate that EBNA-1 and -2 are not consistently expressed in all EBV associated conditions.^{1,2} This may be because of lack of sensitivity of the antibodies, which

cannot detect the small amounts of protein that may be expressed. Alternatively, this negative reaction may be a true reflection of the EBNA status. This can be verified with the use of other monoclonal antibodies, when they become available.

Finally, we would like to endorse the view of Khan and Naase that pressure cooking is a sensitive and reliable method of antigen retrieval. In our hands, this form of antigen retrieval results in better preservation of cellular morphology than microwaving, and also allows for greater dilution of antibodies.

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Notice

UK NEQAS for Blood Coagulation: Participants' Meeting

March 6 1996

Venue: Octagon Centre,
University of Sheffield, Sheffield

A one day meeting has been organised for participants and interested observers of the UK National External Quality Assessment Scheme for Blood Coagulation.

Invited speakers include: Professor R M Bertina, Leiden, The Netherlands; Professor S J Machin, London; Professor M Greaves, Aberdeen; Dr D J Goldie, Bristol; and Dr T Baglin, Cambridge. Subjects to be covered include: APC resistance and the factor V Leiden defect; haemostatic markers of hypercoagulability; acute haemostatic failure; Clinical Pathology Accreditation; and near patient testing in the community. The topics of plasma calibrants and automated INR, and external quality assessment of near patient testing will be discussed by Mr S Kitchen and Mr I Jennings of UK NEQAS for Blood Coagulation. Opportunity will be given for open discussion.

For further details, please contact: Mr T A L Woods, UK NEQAS for Blood Coagulation, Room E24, CSUH Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF. (Tel: 0114 270 0862; fax: 0114 275 8989.)