

Correspondence

Misunderstandings about methyl methacrylate

I read with interest the recent article by Schmid and Isaacson.¹ Unfortunately, the article propagates the myth that methyl methacrylate (MMA) embedded material does not preserve antigens for subsequent immunocytochemistry. The literature is confounded by similar accounts^{2,3} which have served to prevent development of the technique and may have been responsible in part for many departments in this country losing interest in the use of resin embedding for diagnostic purposes. Although the use of MMA has become unfashionable for high resolution studies compared with other resins such as those based on glycol methacrylate (JB-4) and polyhydroxy aromatic dimethacrylate resin (LR White), it offers the advantage of access to a wide range of antibodies for immunocytochemistry. Immunocytochemistry can be performed on tissue embedded in resins other than MMA, but the techniques required are often troublesome and idiosyncratic.

In recent years I have been one of the main advocates for the use of MMA as a suitable embedding medium for simultaneous high resolution morphology and immunocytochemistry.^{4,6} Though acrylic resins, including MMA, can be polymerised at raised temperatures, perhaps overnight as suggested by Schmid and Isaacson, our studies use polymerisation with a chemical accelerator at room temperature or less, which is quicker and more practical. To date, about 40 monoclonal and polyclonal antibodies have been successfully applied to MMA embedded tissue for the demonstration of hormonal, lymphoid, mesenchymal, neuronal and smooth muscle antigens, but the number is rapidly expanding.

The technique has proved simple, quick, and reliable in a routine laboratory and has been used on a variety of tissues, including lymph node, skin, renal and bone marrow trephine biopsy specimens for diagnostic purposes. Although MMA is being used in some pathology departments, wider recognition of the benefits to be gained will not be helped by perpetuating the fallacy that immunocytochemistry can not be done.

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- Schmid C, Isaacson PG. Bone marrow trephine biopsy in lymphoproliferative disease. *J Clin Pathol* 1992;45:745-50.
- Islam A. A new instrument for longitudinal hemisectioning of undecalcified marrow cores for simultaneous processing and examination with useful and complementary techniques. *J Clin Pathol* 1987;40:112-6.
- Frisch B, Bartl R. In: Gresham GA, ed. *Atlas of bone marrow pathology*. Dordrecht: Kluwer Academic Publishers, 1990.
- Hand NM, Morrell KJ, MacLennan KA. Immunocytochemistry on resin embedded tissue for light microscopy: a novel post-embedding procedure. *Proc Roy Microbiol Soc* 1989;24:A54-A5.
- Hand NM, Morrell KJ. Immunocytochemistry on plastic sections for light microscopy: a new technique. *Proc Roy Microbiol Soc* 1990;25:111.

- Johns L, Hand NM, Fish DCW, Miller KD. Immunocytochemistry on methyl methacrylate embedded tissue. *J Pathol* 1992; 167:suppl 154.

Processing of trephine biopsy specimens

We read with interest the article by Schmid and Isaacson. The authors discuss methods of processing trephine biopsy specimens and conclude that decalcification remains the method of choice. We strongly disagree with this view for the following reasons.

1 Methyl-methacrylate embedded trephine biopsy specimens provide excellent morphology. This permits detailed, critical assessment of bone marrow architecture and cellular differentiation at a level which we consider impossible in the best prepared decalcified sections.

2 Processing specimens in MMA can be automated and in this laboratory biopsy specimens can be reported within one working day. The method which we have been using for the past two years (about 1 000 biopsies) was developed by Mr N Hand of the Queen's Medical Centre, Nottingham. The method is also used for renal biopsy specimens and is of particular value in the assessment of cutaneous lymphoid infiltrates.

3 Contrary to the statements in this article, immunocytochemistry is not only possible on MMA sections, but can be carried out to a very high standard, which when combined with the degree of morphological detail makes this a very powerful analytical method. A wide range of antibodies can be used permitting the immunocytochemical identification of lymphoid infiltrates, normal haemopoietic cell lines, and metastatic tumours of the types normally seen in marrow. Around 25 antibodies are in routine use in this laboratory: staining techniques are similar to those used in paraffin wax sections.

4 Immunocytochemical studies on trephine biopsy specimens are of great value in identifying minor cell populations such as in early involvement by myeloma, in distinguishing regenerating blasts from large cell lymphoma, and in demonstrating details of the bone marrow microarchitecture. In cases where there is extensive lymphoid infiltration, flow cytometry is the method of choice in establishing the cell phenotype. The need for frozen section immunocytochemistry is minimal.

5 In several recent cases we have extracted DNA from MMA sections and have demonstrated lymphocyte clonality by PCR. In one case the extent of lymphocyte infiltration seen in the biopsy specimen was minimal.

6 In our opinion it is technically more demanding to prepare decalcified sections to a consistently high standard than to use MMA embedding. The standards achieved in many centres are very poor. The equipment costs of MMA embedding are modest in comparison with many standard items of laboratory equipment.

For these reasons we regard decalcifica-

tion as an obsolete method in the preparing trephines. We strongly recommend the use of MMA embedded biopsy specimens, combined with flow cytometric immunophenotyping, in the assessment of all suspected neoplastic disorders of the bone marrow. We agree with the authors that bone marrow biopsy specimens are often regarded as "Cinderella" specimens. Using the methods described above bone marrow, Pathology becomes a demanding and interesting subject, which, if properly applied, has a major role in the management of haematological malignancy.

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- Schmid C, Isaacson PG. Bone marrow trephine biopsy in lymphoproliferative disease. *J Clin Pathol* 1992;45:745-50.

Drs Schmid and Isaacson comment:

Dr Jack and his colleagues may have read our article "with interest" but clearly, they have not read it with "great care". Nowhere do we state that formalin fixation followed by decalcification and paraffin embedding is the "method of choice". Instead we have given details of several methods, including methyl-methacrylate (MMA) embedding. Indeed, MMA embedding is the method used routinely in the laboratory of one of us (CS) while paraffin wax embedding following formalin fixation with decalcification is the method used in the other author's laboratory (PGI).

We are entirely in agreement with the first two points raised by Jack *et al*, who would, if they had read our paper carefully, have gleaned this from paragraph 5 on page 1 in which we state "the use of methyl-methacrylate (MMA) provides excellent morphology (fig 1B) and sections can be processed and stained within 16-24 hours when rapid fixation is used".

Their third point, relates to performance of immunocytochemistry on MMA sections. We, too, have had some experience with this¹ and have had success with the same range of antibodies that can be applied to paraffin wax sections, with the exception of antiimmunoglobulins where staining of perinuclear space Ig is more difficult. We have encountered some minor problems with the technique relating to the requirement for processing at 4°C and changes in enzyme digestion for certain antibodies. A recent paper by Wolf *et al*² reports success with the MMA technique but describes a requirement for specially tailored fixatives and prolonged washes. The range of antibodies listed by Wolf *et al* is also restricted to those that can be applied to paraffin wax embedded material. We doubt, therefore, that Jack *et al* have had success with the wider range of antibodies that can be applied to frozen sections³ which are sometimes useful in analysing lymphoproliferative and other disorders of the marrow.

We are, of course, in full agreement with point 4, having taken great pains to point out and illustrate the value of immunocytochemistry on bone marrow trephines, and are grateful for the anecdote which forms the fifth point of the letter which, however, has no relevance to our review.