Double embedding in agar/paraffin wax as an aid to orientation of mucosal biopsies

Orientation of intestinal mucosal biopsies so that histological sections can be cut perpendicular to the epithelial surface is of paramount importance to their histological examination and reporting. Many biopsies of both small and large intestine yield little or no useful diagnostic information because correct orientation has not been achieved.

Orientation of strips of mucosa in the laboratory is greatly facilitated if they are allowed to adhere, flat, to a piece of frosted glass or thin card. However, although such a procedure can be easily implemented in gastroenterological units, many intestinal biopsies, particularly rectal biopsies, are taken in diverse, non-specialised clinics and often reach the laboratory irregularly curled-up in formal saline and are a significant potential cause of wasted laboratory and clinical time and effort.

To increase the precision of orientation of all biopsies, regardless of their state on reaching the laboratory, we decided to use a method of embedding the tissue in the fixed but unprocessed state using a technique based on embedding in agar before processing into paraffin wax.

The formalin-fixed biopsy fragments are embedded in molten 1% aqueous agar at 45°C, under dissecting microscopic control. The agar solution has several remarkable useful properties; although its melting point is 98°C, gelling does not occur until the temperature falls to 42°C. Solidification is fairly slow and correct orientation of a biopsy, even if curled, is easily achieved by gentle manipulation with a mounted needle during cooling. Moreover, the agar is colourless and transparent in both liquid and gel forms so that the process of orientation can be viewed through a dissecting microscope. Even photography of the dissecting microscopic appearance is possible at this stage. The embedding in agar can be conveniently performed in standard moulds, such as those used for preparing paraffin wax blocks—for example, Tissue Tek.

The block of solidified agar, with its embedded biopsy is trimmed with a scalpel or razor blade so that it presents a flat surface perpendicular to the epithelial surface and it is then processed through alcohols and chloroform to paraffin wax in the usual way. The agar does not shrink appreciably during processing and is impregnated by wax just as if it were tissue. Paraffin sections are cut in the usual way. No technical difficulties have been experienced; the agar cuts just as if it were wax. On staining, the agar takes up a minimal amount of eosin and can just be seen as a very faint background stain but does not interfere with the histological appearances. Quantification and photomicrography are unimpeded.

This technique has been used in our laboratories now for three years. Although it is unnecessary for well presented, mounted biopsies or for tiny oesophageal and gastric biopsies it has proved invaluable for rendering small, unmounted, curled and apparently “unorientatable” biopsies reportable.

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Culture of Chlamydia

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