Using LR White for Hard Tissue

LR White can be used for the microtomy of decalcified bone and teeth and also for microtomy or "sawing and grinding" of undecalcified tissues.

Decalcified Tissue:
These may be processed, cut and stained similarly to soft tissue (see Using LR White for Light Microscopy), except that the dehydration and infiltration times may need to be extended depending on the size of tissue. It is also recommended that bone be "de-fatted" to improve the penetration of resin into marrow cavities. This can be achieved by using chloroform after dehydration, returning to absolute alcohol to remove the chloroform before infiltrating with resin and polymerising.
Undecalcified Tissue:
Dehydration and infiltration times will vary depending on size and density of tissue. Those laboratories using Methyl or Butyl methacrylate at present can use similar dehydration times, but infiltration will probably be shortened due to the low viscosity of the resin.

Dehydration:
A graded series of alcohols should be used for dehydration of tissue, and when processing bone "de-fatting" is recommended to improve the penetration of resin into marrow cavities. This can be done using chloroform, for the same length of time that would be necessary to clear the tissue. The bone should then be taken back to absolute alcohol and given sufficient changes to remove the chloroform before infiltration with LR White (Hard Grade).

Infiltration:
Several changes of resin will be necessary and impregnation under vacuum is recommended.

Polymerisation:
The tissue can be heat or accelerator cured after embedding in strong plastic moulds, such as JB4 or Peel-a-way type, or aluminium foil dishes.
When heat curing, the moulds should first be filled with resin then the tissue added and orientated. Polymerisation will occur in 18-24 hours at 60°C ± 2°C. The surface of the block exposed to oxygen may remain slightly sticky, but this will not affect the cutting quality of the face of the block. Some ovens are not capable of controlling temperature so closely and if faced with over brittle blocks this is the first parameter to check.
When using accelerator or 'cold' curing the moulds should be placed in a bath of ice-cold water to disperse the heat produced during the exothermic polymerisation. The base of the moulds should first be smeared with accelerator using a cotton-wool bud or swab, the accelerator is then added to the resin, 1 drop per 10ml resin, and thoroughly mixed before pouring into the mould, the tissue is then placed into the mould and orientated. Polymerisation should occur in 10-20 minutes, if it occurs faster than this we recommend either more careful metering of the one drop of accelerator or a higher volume of resin per drop of accelerator. N.B. The accelerator does have some toxic risk and contact with skin and eyes should be avoided. For more information, please see the MSDS.

Cutting and mounting:
Bone marrow trephines and small pieces of cancellous bone may be cut using a motorised heavy-duty microtome or diamond wire saw, but larger pieces of cancellous bone, cortical bone and teeth offer too much resistance to the microtome knife and preparations of this material must be prepared by sawing and grinding. When using a diamond wire saw no grinding is required.

Microtomy:
Sections can be cut, using Ralph type glass knives for trephines or a tungsten carbide knife for larger pieces of cancellous bone, from 2-10µm. Blocks can be cut dry, the sections picked up and floated out on a hot plate at 60°-70°C using the following solution: to 20ml acetone add 0.5ml benzyl alcohol mix then make up to 50ml with distilled water. A section adhesive such as egg albumin can be added to this if required. Sections should be allowed to dry on the hot plate for at least 30 minutes before staining.

Sawing & grinding:
Thick slices 150-200µm can be cut using a milling machine and then ground to the required thickness, usually 20µm for staining or 70µm for microradiography; the section is inclined to fragment if grinding is continued much below 20µm.
Using the newer types of microtomes, such as the Leitz 1600 which has a diamond coated internal hole saw, sections can actually be cut at 20µm and no further grinding is necessary.

Section staining:
Sections of material embedded in LR White are stained "free floating", times of staining are usually longer than those for paraffin, sections and dehydration through alcohols should be avoided. A recommended schedule for Haematoxylin and Eosin staining is as follows:

1. Remove Ca deposits, which would otherwise interfere with the staining, from the section by treating it with Kristensen's Decalcifying Solution for about 15 minutes.
2. Wash in running tap water for a few minutes to remove the formic acid from the tissue.
3. Transfer the section to several changes of distilled water, a few minutes each and then into a 0.5% w/v solution of periodic acid in distilled water where it is left for 5 minutes.
4. Wash the section with several changes of distilled water and stain with Harris Haematoxylin for about 1 hour.
5. Transfer the section, after a short time in distilled water, into running tap water to "blue" the Haematoxylin stained tissue.
6. Check the Haematoxylin stain; if the tissue should be over stained or the surface of the resin has become stained with Haematoxylin, this may be corrected by a short differentiation in acid alcohol (0.5% HCl in 70% ethanol) and "reblueing" of the stained tissue.
7. Rinse the section in distilled water and counterstain it, using a 5% solution of Eosin Yin distilled water; leave the section in the Eosin stain for 30 minutes to 1 hour, wash briefly in running tap water and check the staining of the tissue. Nuclei and haematoxophilic elements should be bright blue, cytoplastic structures in various shades of red-pink.
8. Rinse the section in distilled water, blot dry with filter paper and either clear briefly in zylene and mount in DPX, or mount directly in LR White resin by adding a drop of accelerator to 1 ml of resin.

London Resin - acrylic resins for microscopists

This product is just one of the London Resin's range of resins specifically formulated for the needs of the microscopist. All the resins are manufactured to the same rigorous standards from one of the world's largest suppliers of histological resins.

LR White

A convenient and economical premixed resin with very wide application. Being both hydrophilic and electron beam stable it is equally suitable for light and electron microscopy, and with appropriate fixation the same specimen may be used for both techniques. Published work shows that immunocytochemical methods may be used through LR White sections without etching or any pre- treatment.

Histocryl

A conventional multi-component acrylic system offering a direct alternative for other commercial HEMA systems, but at a fraction of the cost of most.
Economical enough to allow the histologist who recognises the high-quality resin histology can bring to his work to use resin more widely and cost effectively than ever before.

LR Gold

A special acrylic resin for very specific purposes. Its infiltration and polymerisation at low temperatures down to -20°C means that unfixed tissue may be embedded in LR Gold. This enables enzyme histochemistry and immunocytochemistry of many fixation sensitive enzymes and epitomes to be performed on 1 – 2µm resin sections. Bringing the quality of resin histology to an area where only cryostat sections were previously available. LR Gold is a real step forward in histochemical technique. This resin has the ability to be cured by blue light thus making expensive ultra-violet sources unnecessary.

All these acrylic resins combine low viscosity, low toxicity and case of use, reflecting the safety-conscious standards of London Resin products.

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