T.E. M. Post Fixation Processing

Protocol.

Following secondary fixation (Osmication), the Osmium soln. is drawn off (under extraction) using a Pasteur pipette into a 2% soln. of Ascorbic Acid which serves to neutralise the Osmium soln. toxicity.

Tissues are dehydrated through a series of graded alcohols ie. 50%, 70%, 90%, 95% and pure Ethanol. The time in each step is dependant on the sample size, but as a general rule, 2X 15min in each grade is sufficient. **Note.** *(samples can be held in 70% alc. overnight if the need arises*). The pure ethanol is drawn off and replaced by Propylene Oxide (2 X 20 min.).(Work in Fume hood). Propylene Oxide acts a transition solvent between the final alcohol stage and the follow up stage of impregnation of the tissue with an Epon based resin.*(Agar Low Viscosity Resin).*

The resin is prepared to an exact formulation from a commercial kit and due care must be taken with the measuring and mixing process otherwise blocks of an inconsistent hardness will result.
Tissues are initially placed in a 50 : 50 mixture of resin and Propylene Oxide for 4 hrs. This mixture is replaced by a 75 : 25 mixture of resin and Propylene Oxide overnight on a rotator apparatus. The following morning, tissues are transferred to pure resin (preferably freshly prepared) in clean new bottles and replaced on the rotator for 5 – 6 hrs. All stages within this protocol should be carried out in a fume hood, and note all labelling should be done in pencil on adhesive labels. *(Please refer to the accompanying notes in the Bristol protocol)*
Tissues are then transferred to either pyramid head " Beem " capsules or flat embedding moulds, clearly labelled and placed in a 65degree C oven for 48hrs and allowed to polymerise.

After polymerisation, blocks are trimmed to expose tissue surface, sections of 1 micron thickness are cut onto glass slides and stained with 1% Toluidine Blue and viewed using a light microscope. These sections are known as " scout sections" as they are primarily used to ascertain tissue structure and components.
Relevant regions of interest are selected for subsequent trimming and Ultrathin sections of the magnitude of 80nm – 100nm are cut and lifted onto 3mm copper grids. These grids are stained for 30 min in 1.5% aqueous Uranyl Acetate and 10 min in Lead Citrate. We use an automated contrasting apparatus. (Leica EMAC 20).
Sections are allowed to dry and then viewed in the TEM.