SHORT COMMUNICATIONS


The methods most commonly used to prepare nematodes for scanning electron microscopy (SEM) are drying from a solvent with low surface tension, freeze drying or critical point drying. These techniques attempt to remove water from the tissues without distortion, leaving dry specimens suitable for exposure to the high vacuum in the microscope. Several accounts of preparing soft tissues for SEM by infiltration with an EM embedding resin to avoid tissue collapse and surface tension damage have been published (for example Alexander et al., 1973; Chambers & Hamilton, 1973; de Grisse, 1974). Chambers & Hamilton and de Grisse used a low viscosity epoxy resin (Spurr, 1969) which we have also used; de Grisse used the technique for nematodes. Green et al. (1975) found that nematodes infiltrated with Spurr’s resin were much less distorted than those prepared by other methods but did not give details of the process. Our method, which differs materially from that of de Grisse and is less complicated, is as follows:—

1. Wash heat-relaxed nematodes fixed in 2-3% formaldehyde or TAF (Courtney, Polley & Miller, 1955) in two changes of distilled water. For the first wash, two or three drops of a surfactant per 500 ml distilled water helps to remove bacteria and detritus which otherwise may obscure the cuticle surface.

2. Place the specimens in a glass staining block with a 30 mm X 10 mm cavity, containing about 0.1 ml distilled water and transfer to acetone by vapour exchange in an atmosphere saturated with acetone vapour (Stone & Green, 1971).

3. Remove half the acetone from the cavity and add an equal volume of resin mixture made up as follows: ERL 4206 (vinylcyclohexene dioxide resin) 2.5 ml; DER 736 (polypropylene glycol diglycidyl ether flexibiliser) 1.5 ml; NSA (nonenyl succinic anhydride hardener) 6.5 ml; S-1 (dimethylaminoethanol catalyst) 0.1 ml 2). Disposable syringes are convenient for dispensing the ingredients. The catalyst is added last and the mixture remains usable for about 3 days. After adding the resin mixture to the nematodes in acetone, return the staining block to an atmosphere saturated with acetone vapour for 2.5 hours.

4. Remove the block from the acetone atmosphere, top up with resin mixture if necessary, partially cover the cavity with a glass plate and leave at room temperature for a minimum of 6 hours, when most of the acetone will have evaporated.

5. Transfer the nematodes to fresh resin mixture in a staining block, place in a desiccator containing a drying agent and leave for 2 days at 5°C to infiltrate.

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2) U.K. suppliers: TAAB Laboratories, 52 Kidmore End Road, Emmer Green, Reading, Berks.