

combined with the effect of breathing mountant fumes gives one a special feeling all over. I have not noticed fading in slides prepared this way.

You should be able to make up other stain solutions to high pH in a similar fashion.

C. John Runions, Cornell University

Stacking Collodion Layers When Making Films

4% Collodion (Parlodion, nitrocellulose in amyl acetate) is good for making films, but Collodion solutions also have a wonderful additional property in that you can stack the layers.

Gently place one drop on the water surface, as the drop spreads it reaches maximum spread and then "bounces" back a little. Right when it bounces back add one more drop to the middle.

The second drop spreads on top of the first, giving a thicker film. Use the color index (just like sectioning) to determine approximately how thick the film is.

Keep adding drops of the Collodion until you get a color/thickness you like. I usually use 2 drops.

When casting collodion on water, it is important that you use a large enough container so that the solution can spread to its full extent, and not be limited by running into the sides of the container. When it hits the side(s) of the container, the film will pile-up in that area, resulting in an uneven film thickness.

Any circular dish seems to work, but I prefer using 10" x 12" Pyrex baking dishes purchased from local home stores (*i.e.*, K-mart, Walmart, etc.). They are very cost effective (\$3 to \$9 US), and have nice thick walls which stand up nicely to general lab abuse.

Richard E. Edelmann, Miami University

Processing of Skin Tissue for Light and Electron Microscopy Embedding in Epon 912

(italics are for a rapid procedure):

Skin is a problem tissue which we deal with routinely. Here is our procedure:

1) Place newly received tissue in 1/2 Karnovskys fixative overnight (2 hours to overnight).

2) Take Epon 812 mixture out of refrigerator (let it sit under the hood for at least 2 hours before opening the container). This mixture is a combination of DDSA, NMA, and EMBED 812 (48% of 812, 31% of DDSA, 21% of NMA).

3) Rinse biopsy in 0.1 M sodium cacodylate buffer x 2, 15 minutes each rinse (5 to 10 minutes).

4) Post-fix in 1% osmium tetroxide, 1.5 hours (mix 1:1, 2% OsO₄ with 0.2 M sodium cacodylate buffer) (20 minutes).

5) Rinse in distilled H₂O x 2, 15 minutes each rinse (3 to 5 minutes).

6) En-bloc stain with 1% uranyl acetate for 1.5 hours (20 minutes).

7) Dehydrate through an ascending EtOH series:

35% x 2 (15 minutes each) (5 minutes x 2)

70% x 2 (15 minutes each) (5 minutes x 2)

95% x 2 (15 minutes each) (5 minutes x 2)

100% x 2 (30 minutes each) (10 minutes x 2).

8) Note! Each resin/catalyst/propylene oxide mixture is made fresh, and the pure resin/catalyst is made fresh. Add the catalyst to the Epon 812 mixture (0.2 mL of DMP30 per 10 mL resin). Stir slowly for 10 minutes.

9) Clear biopsy in Propylene oxide x2, 15 minutes each rinse (10 minutes x 2).

10) Infiltrate by placing biopsy into:

3:1 mixture of Propylene oxide: Epon for 3 to 4 hours (30 minutes)

2:1 mixture for 12-16 hours (overnight) with caps off (30 minutes)

1:1 mixture for 12-16 hours (overnight) with caps off (60 minutes).

11) Place biopsy into an embedding mold with fresh 100% Epon for 9 hours, then place mold into 60°C oven for curing (24 to 48 hours).

(Epon 812 can be substituted with either EMBED 812 (EMS), PolyBed 812 (Polysciences), Medcast or Eponate 12 (Ted Pella). All have the ingredients DDSA, NMA, and OMP 30.)

Bob Underwood, University of Washington

Hints on Surface Decalcification of Paraffin Embedded Tissues:

To surface decalcify paraffin embedded tissues containing calcium deposits, the trimmed block is immersed face down in a few milliliters of acid decalcifying fluid contained in a shallow dish for a minute or so. Rinse the acid away with water, and/or neutralize the acid with saturated lithium carbonate or 5% sodium bicarbonate, rinse, cool block and resume sectioning.

Neutralizing the acid will help prevent acid corrosion of delicate metal parts on microtomes and microtome blades.

Any commercially or in-house prepared decaicifying solution containing hydrochloric, nitric or formic acids may be used. Strong acid decalcifiers (HCl and nitric) will decalcify rapidly, and can be diluted with water 1:1 if needed. Use safety precautions to prevent exposure to acid fumes or skin and eye contact.

Surface decalcification is not an uncommon practice on breast or other soft tissues with tiny calcium deposits. However, if residual calcium in an underdecalcified bone sample is the problem, then decalcification end point testing is advisable to insure the bone is completely decalcified.

Gayle Cailis, Montana State University

Electron Microscope Technician

A full-time position is available in our Active Transmission Electron Microscopy program with emphasis in renal and neoplastic pathology.

Responsibilities include: process, section and stain specimens for diagnosis and research; prepare micrographs for review using an automated print processor; and assist in developmental /research work.

You'll need working technical knowledge in electron microscopy, including specimen preparation, operation and maintenance of equipment. Must have proven ability to establish and maintain cooperative working relationships with faculty, housestaff, supervisors, peers and other customers.

Prefer a minimum of two years experience in clinical laboratory environment Electron Microscopy (MSA) or Histotechnologist (ASCP) certification and experience in digital imaging.

Please mail or fax resume to: **UCLA Healthcare, Attn: Maurice McGlothorn, 924 Westwood Blvd., Ste. #200, Los Angeles, CA 90095.**

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