

Processing Cochlea For Paraffin Sectioning And SEM

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We have successfully sectioned decalcified paraffin embedded "inner ears" from mice, rats and humans. We have also prepared guinea pig cochlea for SEM. So, briefly:

Paraffin:

- 1) Immediately after dissection, place the cochlea in 10% neutral buffered formalin (NSF) and let fix with gentle agitation 24 hours per 2 mm thickness at room temperature.
- 2) Decalcify by immersing in 5% formic acid at least 4X specimen volume.
- 3) Gently agitate at room temperature and change at least once per 24 hours. Check for completeness of decalcification by decanting 4 mLs of "spent" formic acid into a clean, clear test tube. Add 1 mL of 5% ammonium oxalate in distilled water. Let stand undisturbed for 15 to 20 minutes. White precipitate of calcium oxalate indicates that the sample is not completely decalcified. Change formic acid at least once per 24 hours until the spent formic tests clear.
- 4) When clear, rinse in gently running tap water for approximately one hour.
- 5) Dehydrate with serial alcohols, 70% - 95% - 100% X 3.
- 6) Clear in methyl salicylate (Oil of wintergreen) X 3.
- 7) Infiltrate with 3 changes of paraffin (We use a 50:50 mixture of Fisher Tissue Prep and Stephens Type 9) at 56 to 60°C at 15 to 18 psi vacuum.
- 8) Embed in Stephen Type 9 (not a mixture).

SEM:

- 1) After dissection, fix the cochlea by injecting 2% osmium tetroxide in 0.2

M cacodylate buffer (pH 7.0 to 7.2). Make the buffer as in Bancroft and Stevens, and then add the osmium immediately prior to use.

2) Dehydrate in serial alcohols: 40%, 5 minutes; 70%, 10 minutes; 90% ,10 minutes; 100% 3 X 10 minutes then to Critical point dryer "bomb". Store in anhydrous acetone if cochlea cannot be dried immediately. Plus, the use of acetone as a transitional fluid between the alcohol and the CO₂ makes the detection of the endpoint of critical drying easier. When the odor of the acetone is completely gone, the specimen is dry and ready to coat.

We used gold sputter coating.

Note: I found the anhydrous acetone an imperative step given the extremely high humidity in Arkansas.

Cacodylate buffer

Preparation of stock solutions:

Stock A: 0.2 M sodium cacodylate (MW 214).

4.28 g sodium cacodylate into 100 mL distilled water

Stock B: 0.2 M HCl (MW 35.45)

1.7mL HCl into 100 mL distilled water

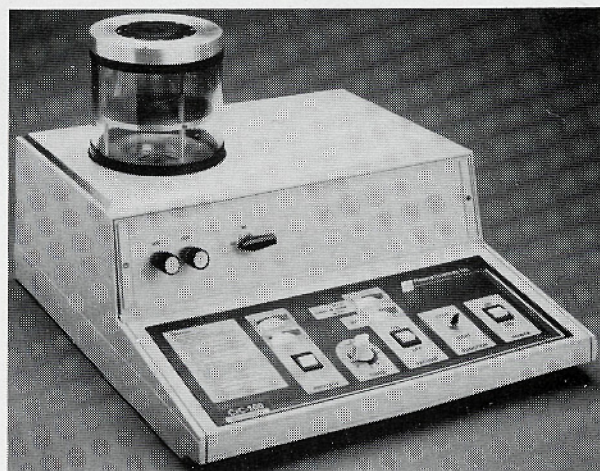
Composition of Buffer:

25 mL Stock A + x mL Stock B made up to 100 mL with distilled water

pH	x mL Stock B	pH	x mL Stock B
5.0	23.5	6.4	9.2
5.2	22.5	6.6	6.7
5.4	21.5	6.8	4.7
5.6	19.6	7.0	3.2
5.8	17.4	7.2	2.1
6.0	14.8	7.4	1.4
6.2	11.9		

References:

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