Silver Enhancement Protocol

Caution: Purity of water and cleanliness of glassware are crucial for optimal silver-staining results. Also, contact between metallic objects (for example, metal forceps) and the silver solution should be avoided.

Reagents: Prepare the following reagents fresh daily except for the citrate buffer.

Citrate buffer: Dissolve 23.5 g trisodium citrate dihydrate and 25.5 g citric acid monohydrate in 850 ml distilled water. Check for microbial contamination before each use if not making fresh each time.

Solution A: Dissolve 100 mg silver acetate (Fluka Chemical Co., catalog # 85140) in 50 ml distilled water and keep under cover. To speed dissolving the fluffy powder, stir vigorously. The reagent may be used for an entire day if protected from light.

Solution B: Dissolve 250 mg hydroquinone in 50 ml citrate buffer that has been freshly adjusted to pH 3.8 using citric acid.

Procedure:

Caution: Fixation of the tissue in 1-2% glutaraldehyde is crucial since the low pH (3.8) developer used in this protocol may dissociate the previously labeled reagents from the tissue.

1. Place the slide for 5 min in solution B diluted to one-half strength with distilled water.

2. Transfer the slide to the developer (equal volumes of solution A and solution B, mixed just before use). Develop under cover at room temperature for 4 - 18 min. The degree of staining may be monitored periodically under a bright-field microscope, adjusted to low light intensity. If a more intense staining is desired, place the slide back in the developer. For certain studies, addition of crude gum arabic to a final concentration of 15% to the developer should slow the rate of silver accumulation and thereby make it more controllable. For prolonged incubation, keep under a dark box.

3. After a quick rinse in distilled water, place the slide in a commercial photographic fixative for 2 min.

4. Rinse with tap water for at least 5 min.

5. Counterstain with hematoxylin and eosin (paraffin and cryostat sections) or methylene blue (semi-thin resin sections), dehydrate, clear, and mount.