

Jones' method for reticulum and basement membranes

Fixation. 10% neutral buffered formalin, Bouin's, or Zenker's.

Embedding. Cut paraffin sections at 1 to 4 μm ; 5 μm sections do not stain so well.

Solutions

.5% aqueous periodic acid freshly prepared

3% aqueous methenamine (hexamethylenetetramine)

5% aqueous silver nitrate

Borate buffer, pH 8.2

.2 M boric Acid (6.5 mL)

.25 M sodium borate (3.5 mL)

Working methenamine silver solution. Prepare fresh just before use.

3% methenamine (42.5 mL)

5% silver nitrate (2.5 mL)

Mix and add 12 mL of Borate buffer, pH 8.2

Mix again and filter into a chemically clean Coplin jar. When used at 70°C, the working silver solution is stable for about 60-75 minutes; solution stability may be prolonged somewhat by staining at 65°C. Solution instability is indicated by the deposition of a black precipitate on the slides.

Gold chloride solution, 0.2%

1% gold chloride stock solution (10mL)

DI H₂O (40mL)

The working solution may be filtered back and reused for approximately 100 slides.

3% aqueous sodium thiosulfate

Counterstain: nuclear fast red (*Kernechtrot*) or Harris's hematoxylin

Notes

1. The second to fifth solutions mentioned above should be prepared in acid-cleaned glassware.
2. Glassware for the technique itself should also be acid-cleaned and rinsed well in DI H₂O.
3. Additional suggestions*
 - a. The Coplin jars that are to be used for the actual silver impregnation should be rinsed in DI H₂O, upended on gauze pads, and allowed to drain dry in the paraffin oven. This preheating assists in preventing cracking of the jar when it is placed in the 70°C water bath.
 - b. Prevent condensation and silver dilution by removing the water-bath lid during the silver incubation period. Use ground-glass lids to cover the Coplin jars during the incubation period.
 - c. Prepare an extra quantity of working silver solution and use half for the regular incubation at 70°C. The other half can be placed in the paraffin oven (56°C to 58°C). If the regular silver solution begins to precipitate but the reaction is not yet fully completed, the slides may be transferred to the

prepared 56°C preheated solution and the incubation then continued at 70°C with the fresh silver solution.

- d. Although the method traditionally calls for silver incubation to take place at 70°C, the use of a 65°C water bath can work just as well with a somewhat lessened possibility of precipitate production. Staining time in the silver may take a little longer, however, to compensate for the decreased temperature.
- e. The technique seems to work better on slides that have been alcohol cleaned.

Technique

1. Hydrate sections to distilled water.
2. Oxidize in periodic acid solution for 11 minutes. Discard solution after use.
3. Rinse thoroughly in distilled DI H₂O.
4. Place slides in the Coplin jar containing the freshly prepared and filtered working methenamine silver solution. Place this Coplin jar in a 70°C water bath. Place another Coplin jar containing distilled DI H₂O in the 70°C water bath. This water will be used for later rinses. Check slides macroscopically at 20 minutes for any precipitate formation. The total staining time is approximately 60 to 75 minutes when sections are incubated at 70°C. Generally, tissue fixed in formalin or paraformaldehyde will require slightly longer incubation times than does Bouin's fluid-fixed material. Tissue that has been allowed to remain in any fixative for more than a few days will tend to take longer to stain.
5. *Microscope check*: Numerous microscopic checks tend to favor precipitate formation and should not be necessary once experience has determined proper time guidelines.

When sections show a medium brown color or stain, remove from the silver solution, rinse in the 70°C distilled DI H₂O, and check microscopically for a dark yellow-brown background and black reticular fibers and basement membranes. Renal tubular basement membranes will blacken prior to the glomerular capillary basement membranes, but for a satisfactory stain, the latter basement membranes should also appear brown-black.

An understained section should be rinsed in the 70°C distilled, DI H₂O, returned to the 70°C silver solution, and checked every 5 to 10 minutes until proper intensity is reached. An overstained section may be "destained" by 1 or 2 dips in a very dilute potassium ferricyanide solution.

Do not allow slides to cool significantly during the microscopic check; otherwise uneven staining will occur.

6. Rinse sections well in distilled water
7. Tone in gold chloride solution for 1 minutes. Sections should turn gray-black. Overtone produces red tones. If overtone, treat sections with 3% sodium metabisulfite for 1 to 3 minutes.
8. Rinse in several changes of distilled water.
9. Treat with sodium thiosulfate for 1 to 2 minutes. Discard solution.
10. Wash in running tap water for 10 minutes.
11. Rinse in distilled water

12. Counterstain as desired. Nuclear fast red for 3 to 5 minutes (filter back), followed by several changes in distilled water may be used. Or sections may be counterstained with Harris's hematoxylin for 1 to 3 minutes (filter back) followed by a brief rinse in tap water, differentiation in acid alcohol, a rinse in tap water, bluing in ammonia water, and several washes of tap water.
13. Dehydrate, clear in xylene, and coverslip, using a synthetic mounting medium.

Results

Reticular fibers and basement membranes – black

Other tissue elements – colored according to counterstain employed.