

F-396 JONES' METHOD FOR KIDNEY

Fixation: 10% Buffered Neutral Formalin (F-113) or Bouin's Solution (F-40) or Zenker's (F-155)

Sections: Paraffin, 2 microns

Procedure: Acid washed glassware must be used!!!!

1. Deparaffinize and hydrate to distilled water.
2. Oxidize in Periodic Acid 0.5% (F-396-1) 11 minutes. Wash in chloride-free water.
3. Prepare Methenamine Silver solution by mixing:
42.5 ml Methenamine 3% (F-396-2), 2.5 ml Silver Nitrate, 5% (F-396-3) and
12.0 ml Borate Buffer, pH 8.2 (F-396-4).
4. Place slides in the solution and the entire jar in a water bath at 70°C for approx. 60-75 minutes. Check under microscope when slides appear medium brown microscopically. Every 10 minutes, once the medium brown color has been established, rinse a slide in 70°C, chloride free water and check under a microscope. Rinse again in hot water and return to the hot staining solution. As the staining time approaches the end point, check the slides, as above, every 1-2 minutes. The entire procedure must be performed quickly to prevent an uneven staining of the tissues. The slides should exhibit a brownish-yellow background, intense black reticulum fibers, and black basement membranes. If the slides become oversaturated, i.e. too black, destain in a dilute Potassium Ferricyanide Solution (F-396-11) for one or two dips.
5. Rinse well in distilled water. Tone in Gold Chloride 0.2% (F-396-5), 1 minute. If sections are overtone place in Sodium Metabisulfite, 3% (F-396-12) for 1-3 minutes. Rinse well in distilled water.
6. Sodium Thiosulfate 3% (F-396-9), 1-2 minutes. Wash in running tap water, 10 minutes. Rinse well in distilled water.
7. Stain in Harris' Hematoxylin (F-396-6) containing 2-4ml of Glacial Acetic Acid per 100 ml for 5-15 minutes. Wash in water.
8. Differentiate in Acid Alcohol 1% (F-396-13) until the sections turn red.
9. Blue section in Ammonia Water, 0.3% (F-396-14). Wash thoroughly.
10. Counterstain in Eosin Y, 1%, Alcoholic Solution (F-396-7).
11. Dehydrate in 95% alcohol, absolute alcohol and clear in xylene 3 changes each. Mount.

Stain Results:

Basement membranes, reticulum fibers	Black
Nuclei	Blue
Cytoplasm, collagen, connective tissue	Pink-orange

References: Jones, D.B., Amer.J.Path. 27:99 (1951).

AFIP Manual of Histological Staining Methods, 3rd ed., Ed. L. Luna: NY: McGraw-Hill Publications, c. 1968, p. 97.