REAGENT ASSEMBLY KIT
ITEM# k025

JONES' METHOD FOR KIDNEY

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of basement membranes.
PRINCIPLE:	Periodic Acid oxidizes carbohydrates forming dialdehydes which can selectively bind and reduce Silver from the Hexamine Silver Solution.
CONTROL:	Kidney
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 2-4 micrometers; 5 micrometer sections do not stain well
SOLUTIONS:	1. Periodic Acid 0.5% Aqueous Item# s1860 2. Methenamine 3% Stock Solution Item# s240A 3. Silver Nitrate 5% Aqueous Item# s1890 4. Borate Buffer pH 8.2 Working Item# s127B Methenamine Silver Working Solution pH 8.2: Methenamine 3% Stock..... 42.5 mL Silver Nitrate 5%..... 2.5 mL Borate Buffer pH 8.2..... 12.0 mL *Note:* Mix just before use and filter. This solution is stable for approximately 60–75 minutes. After this time there is a breaking down process, which produces a black precipitate and is picked up on the slides. 5. Gold Chloride 0.2% Aqueous Item# s202 6. Sodium Thiosulfate 3% Aqueous Item# s1894 *Solutions can be purchased separately from Poly Scientific.*
NOTES:	*Note:* Use chemically clean glassware. It is absolutely essential that all glassware be acid cleaned with concentrated Nitric Acid and rinsed in several changes of chloride free distilled water. Distilled water may be checked for free chloride by the addition of several drops of Silver Nitrate 5% Aqueous. If a white cloud appears upon the addition of the Silver Nitrate, discard the sample of water and replace.
REFERENCE:	Luna, Lee G. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd Ed. McGraw-Hill Book Co. New York. 1968. pp. 95-97.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Place in Periodic Acid 0.5% Aqueous for 11 minutes.
3. Rinse in distilled water.
4. Filter freshly prepared Methenamine Silver Working Solution pH 8.2 into coplin jar.
5. Place slides in Methenamine Silver Working Solution and then place coplin jar in prewarmed 70°C water bath. Start timing at this point, approximately 30-60 minutes. Check under microscope when slides show a medium brown color macroscopically.*

**Note:* Solution and slides should be allowed to come to 70°C together. While slides are in the Methenamine Silver Solution, they may be examined after they begin to show a medium brown color reaction macroscopically. Before checking under the microscope, they are first rinsed in hot 70°C chloride free distilled water, checked, returned to hot water rinse and then returned into hot staining solution. Slides should be checked every ten minutes when they have reached the dark or medium brown stage. Slides should be checked as rapidly as possible because if the section cools there is an uneven staining of the section. When the desired staining time has been reached, the slide should be checked as described above, every one to two minutes. Strict adherence to the timing is essential in order to obtain a uniform consistency in staining. A properly stained section at this point should have a dark brownish-yellow background; the reticulum fibers will be intense black, as should the basement membranes. An overstained section will be too black. Differentiation will be very difficult as the black will be so intense as to obscure many or all of the tissue elements. The section may be distained with an extremely dilute solution of Potassium Ferricyanide for one or two dips.

6. Rinse sections well in distilled water.
7. Tone in Gold Chloride 0.2% Aqueous for 1 minute.

Note: If sections are over toned, place in Sodium Metabisulfite 3% Aqueous for 1-3 minutes, check periodically.

8. Rinse in distilled water.
9. Place in Sodium Thiosulfate 3% Aqueous for 1-2 minutes.
10. Wash in running tap water for 10 minutes.
11. Rinse well in distilled water.
12. Counterstain with routine Harris Hematoxylin and Eosin Stain.
13. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 3 changes each.
14. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Basement Membrane	Black Reticulum
Fibers	Black
Nuclei	Blue
Cytoplasm, Collagen and Connective Tissue	Pink to Orange