

G-481 METHENAMINE SILVER METHOD FOR ARGENTAFFIN CELLS

FIXATION: Formalin. Bouin's or other formalin-containing fixatives. Dichromates and mercury salts impair contrast between granules and background.

SECTION: Paraffin

STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Treat for 10 minutes in **Weigert's Iodine** (G-481-1) followed by bleaching for 10 minutes in **Sodium Thiosulfate, 5%** (G-481-2). Wash for 10 minutes in running water. Rinse in two changes of distilled water.
3. Place sections in chemically clean Coplin jars containing the buffered methenamine-silver nitrate solution* at room temperature and place in a 60°C paraffin oven for 3-3 ½ hours. (preheating the solution to 60°C reduces impregnation time by ½ to 1 hour.) Rinse in distilled water.

*Prepare methenamine-silver nitrate by mixing:

Silver Nitrate, 5% (G-481-3A) 5 ml

Methenamine, 3% (G-481-3B) 100 ml

For working buffered stain add 8ml of **Borate Buffer, pH 7.8** (G-481-4) to 30 ml of methenamine-silver nitrate. Use chemically clean glassware throughout.

4. Tone for 10 minutes in **Gold Chloride, 0.1%** (G-481-5). Rinse in distilled water.
5. Fix in **Sodium Thiosulfate, 5%** (G-481-2) for 2 minutes. Wash in running water for 5 minutes.
6. Counterstain in **Acetic Safranin O, 0.1%** (G-481-6) for 5 minutes.
7. Dehydrate with acetone, clear in xylene and mount.

RESULTS:

Argentaffin Cells	Black
Coarse connective tissue of the submucosa	Variable amount of blackening by 3-3 ½ hr. of impregnation
Granules of eosinophil leukocytes, nuclei, smooth muscle & surface epithelium	Show additional blackening after incubation beyond 3 - 3 ½ hr.
Granules of mast cells	Remain red after nuclei & reticulum are blackened

REFERENCES:

- Clark, G.: Staining Procedures, Williams and Wilkins Co., Baltimore, 3rd ed., c.1973, p.161.
Gomori, G., Arch. Path., 45:48, 1948.
Burtner, H.J. and Lillie, R.D., Stain Techn., 24:225-7, 1949.