

E-300 BENHOLD'S METHOD FOR AMYLOID

Fixation: 10% Buffered Neutral Formalin

Sections: Cut Paraffin, 6 microns (12 microns for thicker sections, minute amyloid deposits with Congo Red, Sirius Red, Crystal Violet Stains)

Staining:

1. Deparaffinize and hydrate to distilled water.
2. Stain 10 minutes in **Mayer's Acid Hemalum** (E-300-3). Wash in three changes of distilled water.
3. To 40ml of stock **Alkaline Alcohol** (E-300-4) add 0.4ml **Sodium Hydroxide, 1%** (E-300-2) just before use. Transfer sections to alkaline alcohol for 20 minutes.
4. Stain 20 minutes in freshly alkalized Congo Red Solution: (to 40ml **Alcoholic Congo Red** (E-300-1) and add 0.4ml **Sodium Hydroxide, 1%** (E-300-2). Filter and use within 15 minutes.
5. Dehydrate quickly in three changes of absolute alcohol. Clear through two changes of Xylene. (C-120)
6. Mount.

E-300 AFIP CONGO RED (BENHOLD'S) MICROWAVE FOR AMYLOID STANDARD AND MICROWAVE PROCEDURE

Staining:

1. Deparaffinize and hydrate to distilled water.
2. Stain in filtered **Congo Red, 1% Aqueous** (E-300-5) for 1 hour.
2A. **If using amicrowave stain in **Congo Red, 1% Aqueous** (E-300-5) for 25 seconds. The hot stain jar is removed form the oven and allowed to stand at room temperature for 60 seconds.
3. Rinse in distilled water.
4. Differentiate rapidly in **Alkaline Alcohol** (E-300-4).
5. Wash in running tap water for 5 minutes.
6. Counterstain in **Mayer's Acid Hemalum** (E-300-3) for 5 minutes.
6A. ** If using a microwave oven-stain in microwave for 30 seconds.
7. Wash in running tap water for 15 minutes.
8. Dehydrate and clear through 95% ethyl, absolute alcohol changes each, 2 minutes each.
9. Mount using resinous medium.

Results:

Amyloids	Pink red
Nuclei	Blue
Elastic Tissue	Light Red

References:

Clark, G. (ed) Staining Procedures, 3rd ed. Williams & Wilkins, Baltimore, c. 1973,p 49
Puchtler, H & Sweat.: F.J. Histochem, 10:355f (1962)