CONGO RED STAIN

PRINCIPLE:

Congo red stain is used for the visual detection of amyloid in muscle and nerve fresh frozen sections in patients who have amyloidosis. The dyeing of amyloid is by a mechanism similar to the direct textile dyeing of cotton. The linearity of the dye configuration permits hydrogen bonding of the azo and amine groups of the dye to similarly spaced carbohydrate hydroxyl radicals of the amyloid substance. The use of a staining solution containing high content of alcohol and free alkali releases native internal hydrogen bonding between adjacent polysaccharide chains and creates more potential sites for the binding of the dye.

QUALITY ASSURANCE:

A section of a biopsy with abundant amyloid in the muscle is used as a positive control when a new Congo Red staining solution is prepared.

SPECIMEN REQUIRED:

Snap frozen human striated muscle

METHOD:

Fixation: None. Use snap frozen tissue.

Technique: Cut 10 - 16 micron (12 µm) sections in cryostat from snap frozen biopsy. Attach one or more sections to a No.1½, 22 mm square coverslip.

Equipment:

Ceramic staining rack - Thomas Scientific #8542-E40
Columbia staining dish - Thomas Scientific #8542-C12
Columbia staining dish(jar) - Thomas Scientific #8542-E30
Forceps  Latex gloves
Filter paper (Baxter #F2217-110, Grade 363, Qualitative)

Reagents:

Absolute alcohol (100% ethanol) Quantum

FLAMMABLE Store at room temperature in a flammable cabinet

Congo Red - certified (C.I. 22120)(Sigma C-6277,
POSSIBLE TERATOGEN Store at room temperature.
Harris Modified Hematoxylin Stain, acidified (Fisher SH26-500D)
Store at room temperature
Permount (Fisher #SP15-100) FLAMMABLE, HEALTH HAZARD
Store at room temperature
Reagent alcohol, ACS - HPLC Fisher A995-4 or histochemical Fisher A962-4, **FLAMMABLE, TOXIC, TERATOGENIC**, Store at room temperature in flammable cabinet 
Sodium chloride (Sigma S-7653, store at room temp.) Xylenes - Fisher #HC700-1GAL, **FLAMMABLE** Store at room temperature in flammable cabinet.

**Solutions:**

I. Congo Red solution
   
   Sodium chloride
   Dissolved in deionized water
   Congo Red powder
   Suspended in 100% absolute alcohol
   
   Combine the two solutions (Store at room temperature for months)

II. Alcohol 50% 
    reagent alcohol deionized water 
    ~50ml ~50ml

III. Alcohol 70%
     reagent alcohol deionized water
     ~70ml ~30ml

IV. Alcohol 80%
    reagent alcohol deionized water
    ~80ml ~20ml

V. Alcohol 95%
   reagent alcohol deionized water
   ~95ml ~5ml

**Staining Procedure:**

1. Place the coverslip with section in a ceramic staining rack.

2. Immerse sections in Harris Hematoxylin for 3 – 4 dips.

3. Wash with tap water until the water is clear.

4. Filter the Congo Red solution into a Columbia staining dish (Thomas Scientific #8542-E40)

5. Place coverslips with sections in the Columbia staining dish with the filtered Congo Red solution for 3 minutes at room temperature.

6. Wash with several exchanges of deionized H2O.

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7. Dehydrate in ascending alcohol solutions (50%, 70%, 80%, 95% x 2, 100% x 2) in Columbia staining dishes - Thomas Scientific #8542-E30.

8. Clear with xylene (3 - 4 x ) also in Columbia staining dish(jar)s - Thomas Scientific #8542-E30

9. Mount coverslip onto a labeled glass slide with Permount or other suitable organic mounting medium.

**Results:**

With the light microscope, amyloid deposits are red to pink-red, nuclei are blue, other tissue elements are largely unstained. Amyloid deposits show an “apple-green” birefringence with the polarizing microscope. Nuclei of inflammatory cells, and granular basophilic debris associated with vacuoles, stain dark blue/black.

**REFERENCES:**


3. Protocol modified from the Johns Hopkins School of Medicine.

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