Washington University School of Medicine
Neuromuscular Lab
CAP: 1923316
CLIA: 26D0652044
NY: PFI 3499

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	2 g
Dissolved in deionized water	32 ml
Congo Red powder	0.5 g
Suspended in 100 % absolute alcohol	68 ml

Combine the two solutions (Store at room temperature for months)

II. Alcohol 50 %

reagent alcohol	~50ml
deionized water	~50ml

III. Alcohol 70 %

reagent alcohol	~70ml
deionized water	~30ml

IV. Alcohol 80 %

reagent alcohol	~80ml
deionized water	~20ml

V. Alcohol 95 %

reagent alcohol	~95ml
deionized water	~ 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 H_2O.

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:

1. Thompson, Samuel W. *SELECTED HISTOCHEMICAL AND HISTOPATHOLOGICAL METHODS,* Charles C. Thomas, Springfield, IL, 1966.

2. Barka, T. and Anderson, P.J., 1962. In: *THEORY AND PRACTICE OF HISTOTECHNOLOGY,* Sheehan, D.C. and Hrapchak, B.B., 2nd Edition 1980; Battelle Memorial Institute, Columbus, OH, 1987.

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

12/28/2016