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Beaumont

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Histology Special Stain-Congo Red-Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of amyloid in tissue sections.

II. PRINCIPLE:

The reaction uses hydrogen bonds to attach the linear Congo red dye molecule to the anti-parallel B-pleated sheets of amyloid. The Congo red dye is attached to other tissue components with electrochemical bonds. The use of the alcohol and salt in the staining solution suppresses the background electrochemical staining. The use of the graded alcohols after staining further weakens the electrochemical bonds, thereby removing more of the Congo red dye molecules from the background tissue components. Hematoxylin is used to stain the nuclei. A polarizing microscope is used to obtain the apple-green birefringence found with amyloid stained with Congo red. Dense connective tissue, which may also stain with Congo red, will birefringe white.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well fixed tissue.
    2. B-5 fixative or prolonged fixation in 10% NBF may reduce stain intensity.

B. Processing
    1. Standard, overnight processing.

C. Section Thickness

1. Routine specimens-7-8µm.
2. Frozen section muscle biopsies-10µm.

D. Slide Drying

1. 60 minutes at 60° C.

E. Type of slide

1. Plain

IV. REAGENTS:

A. Hematoxylin

1. Use Gill or Mayer's hematoxylin.

B. Dilute Ammonia (ammonium hydroxide)

1. Use ammonia water from H&E set-up.

C. Saturated Modified Congo Red Solution

Sodium chloride	2.0 gm
Distilled water	20.0 mL
95% Ethanol	80.0 mL
Congo red	0.2 gm

1. Dissolve together sodium chloride and distilled water.
2. Stir in 95% ethanol.
3. Add Congo red and stir for several hours.
4. Allow to set overnight before using to ensure saturation.
5. Do NOT filter.
6. Shake before using.
7. Stable at room temperature for several months; may be reused until weak.

V. EQUIPMENT:

A. Balance

B. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Coplin jars

D. Forceps

VII. QUALITY CONTROL (QC):

A. Extended time in the alcohols after the staining will reduce staining intensity.

B. Do NOT filter Congo red solution.

C. Use a polarizing microscope. Do NOT rely on red staining for determination.

D. Fluorescence microscope may also be used to view amyloid stained with Congo red. Deposits will fluoresce orange. This is a good method for finding minimal deposits.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Hematoxylin

1. Incompatible with oxidizers & alkalis
2. Store separately

B. Ammonium Hydroxide

1. May cause severe skin and eye burns
2. Vapors are irritating to eyes and respiratory tract
3. Harmful if swallowed or inhaled

C. Congo Red

1. Is a suspected carcinogen

IX. PROCEDURE:

Step	Action	Time	Notes
A.	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
B.	Stain in Mayer or Gill hematoxylin at room temperature	5 minutes	
C.	Rinse in running tap water	1 minute	
D.	Ammonia water	2-3 seconds	An alternative is tap water if pH is 9 or higher
E.	Wash in running tap water	5 minutes	
F.	Place in Congo red solution at room temperature	30 minutes	
G.	Decolorize in 2 changes of 70% ethanol	10 dips each	

H.	Decolorize in 2 changes of 95% ethanol	10 dips each	
I.	Decolorize in 2 changes of 100% ethanol	10 dips each	
J.	Clear in two changes of xylene		
K.	Coverslip		

X. LIMITATIONS:

A. If over-decolorized Amyloid will stain similarly to collagen, sections may be placed back into the Congo red solution and re-stained.

B. Large deposits that have been in the body a long time will stain less intense than the small, newly formed deposits.

C. Control slides lose their intensity of staining after about 6 months. Cut only enough slides to last about 2 months.

D. 7-8 µm sections give the best apple-green birefringence, due to a half-wavelength retardation. Sections 5-10 µm thick may be used. Sections thinner than this will exhibit more of a blue birefringence, while sections thicker than this will exhibit more yellow.

E. As the beam of polarized light is split while passing through the amyloid, the amount of light is lessened. Therefore, the power of the light source on the microscope will need to be increased.

XI. RESULTS:

A. With light microscope:
   1. Amyloid - **red/orange**
   2. Collagen - **pink**
   3. Nuclei - **blue**

B. With polarizing lenses:
   1. Amyloid- **apple-green**
   2. Collagen- **white**
   3. Background- **black**

XII. REFERENCES:

1. Arch Path. Vol 104, June 1980.
2. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.
3. Carson FL: Histotechnology: A Self-Instructional Text, Chicago, IL, ASCP Press, 1990.
4. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

5. Wolman M and Bubis JJ (1965). The cause of the green polarization color of amyloid stain with Congo red. Histochemie. 4, 351.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak