Status **Active** PolicyStat ID **12589645**

Beaumont

Origination	11/4/2022	Document Contact	Sharon Scalise: Supv, Laboratory
Last Approved	11/4/2022	Area	Laboratory-Histology
Effective	11/4/2022	Applicability	Royal Oak
Last Revised	11/4/2022		
Next Review	11/3/2024		

Histology Special Stain - Crystal Violet - 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 is polychromatic. Crystal violet is not a pure dye, rather a mixture of basic dyes. Amyloid is selectively stained by one of the dye components; the background is stained by other dye components. Crystal violet stains amyloid by attaching to the carboxyl ions on the amyloid. AL amyloid has fewer carboxyl ions than AA amyloid. Also, the amount of carboxyl ions on the AL amyloid varies greatly from individual to individual. Therefore, the staining of AL amyloid by crystal violet (and methyl violet) is extremely variable, AA amyloid is more likely to produce a positive result. Hydrochloric acid prevents over-staining of cytoplasmic components. Apathy is an aqueous mounting media containing sodium chloride to prevent bleeding, and thymol a preservative.

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 processing.

C. Section Thickness

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

D. Slide Drying
   1. 60 minutes at 60°C.

IV. REAGENTS:

A. **Stock Crystal Violet**

Crystal violet	**14.0 gm**
95% ethanol	**100.0 mL**

Dissolve together. Store in refrigerator (3°C). Stable for several months.

B. **Working Crystal Violet**

Stock crystal violet	**2.0 mL**
Distilled water	**60.0 mL**
Hydrochloric acid, concentrated	**0.2 mL**

Just before use, mix together stock crystal violet and distilled water. Slowly add hydrochloric acid, drop by drop, to solution.
Mix together. Stable for one day only.

C. **Modified Apathy mounting media**

Acacia (gum Arabic)	**50.0 gm**
Cane sugar	**50.0 gm**
Distilled water	**100.0 mL**
Sodium chloride	**10.0 gm**
Thymol	**0.1 gm**

Bring water to a boil. Slowly add acacia and cane sugar, stirring until both are dissolved. Restore the volume with distilled
water. Add and dissolve the sodium chloride. Add the thymol. Store in a refrigerator (3°C). Stable for months.

V. EQUIPMENT:

A. Balance
B. Magnetic Stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Coplin jars
D. Forceps

VII. QUALITY CONTROL:

Use a section containing amyloid as a positive control.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Crystal Violet
    1. May cause eye irritation.

B. Hydrochloric acid
    1. Add slowly, drop by drop, to solution.
    2. May cause severe skin and eye burns.

C. Gum Arabic (Acacia)
    1. May cause skin and eye irritation.

D. Thymol
    1. May cause skin and eye burns.
    2. May be irritating to respiratory tract.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Stain in WORKING crystal violet solution.	5 minutes	
3	Rinse in running tap water, 2-3 changes.	5-10 seconds each	
4	Mount with Apathy mounting medium.		
5	Seal edges of coverslip with clear fingernail polish.		

X. RESULTS:

A. Amyloid - **purple-violet**
B. Mucin - **purple-violet**
C. Renal hyaline - **purple-violet**
D. Other tissue components - **blue**

XI. LIMITATIONS:

A. Avoid alcohol after staining in crystal violet, as this will extract the polychromatic color from the amyloid.

XII. REFERENCES:

A. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd edition. New York, NY, Churchill Livingstone, 1990.

B. Carson FL: Histotechnology: A Self-Instructional Text. ASCP Press. 1990.

C. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd edition., Lee G. Luna, Editor. New York, NY, McGraw-Hill Book Company, 1968.

Approval Signatures

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Applicability

Royal Oak