

Beaumont

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Histology
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Histology Special Stain - Mucicarmine - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of sulfated or carboxylated acid mucopolysaccharides in tissue sections. It will also stain the capsule of *Cryptococcus neoformans*.

II. PRINCIPLE:

Carmine is chelated to aluminum salts (aluminum hydroxide and aluminum chloride). This gives the dye a positive charge, so will bind to tissue polyanions. Due to the chelated dyes large molecular size, it can only penetrate and bind to acidic substances of low density, such as mucins.

III. SPECIMEN COLLECTION AND HANDLING:

- A. Fixation
 - 1. Any well-fixed tissue.
- B. Processing
 - 1. Standard processing.
- C. Section Thickness
 - 1. Routine specimens-5µm.
- D. Slide Drying
 - 1. 60 minutes at 60°C.
- E. Type of slide

1. Plain

IV. REAGENTS:

A. Hematoxylin

Use hematoxylin from routine H&E set-up. Gill's or Mayer preferred.

B. Stock Mucicarmine Solution

Carmine	1.0 gm
Aluminum hydroxide	1.0 gm
Anhydrous aluminum chloride, granular	0.5 gm
50% ethanol	100.0 mL

1. Mix together carmine and aluminum hydroxide in a 500 mL flask.
2. Add the ethanol and dissolve together.
3. Slowly and gradually add aluminum chloride to the flask, swirling after each addition.
4. Place flask in a boiling water bath.
5. Bring the inside solution to a boil.
6. Boil for exactly 2½ minutes.
7. Remove from water bath and cool in cold water, filter.
8. Store in refrigerator (3°C).
9. Stable for up to a year.

10. CAUTIONS:

- a. **Slowly add aluminum chloride to solution, constantly swirling, or there could be a major eruption.**
- b. **Do NOT breathe the resulting hydrochloric acid fumes.**
- c. **Use a Pyrex or similar flask to boil the carmine solution. Weak glass will break when it is placed in cold water.**

C. Working Mucicarmine Solution

Stock mucicarmine solution	5.0 mL
Distilled water	45.0 mL

Just before use mix together. Discard after use.

V. EQUIPMENT:

- A. Balance
- B. Boiling water bath
- C. Magnetic stirrer
- D. Hot plate

VI. SUPPLIES:

- A. Erlenmeyer flasks
- B. Graduated cylinders
- C. Funnel
- D. Filter paper
- E. Coplin jars

VII. QUALITY CONTROL:

- A. Use a section of intestine with goblet cells as a positive control
- B. Metanil Yellow may be used as a counter stain. Minimal amount of mucin may be masked with metanil yellow or with excess Weigert hematoxylin.
- C. An iron hematoxylin MUST be used if metanil yellow is used as the counterstain
- D. To demonstrate minimal amounts of mucin, sections may be cut at 6-7 μm thickness

VIII. SPECIAL SAFETY PRECAUTIONS:

- A. Hematoxylin has no listed hazards.
- B. Ferric chloride
 - 1. Is a corrosive.
 - 2. May cause skin and eye burns.
- C. Hydrochloric acid
 - 1. Is an acid.
 - 2. Add slowly, drop by drop, to the solution.
 - 3. May cause severe skin and eye burns.
- D. Carmine - not a hazardous substance or mixture.
- E. Aluminum hydroxide - not a hazardous substance or mixture.
- F. Aluminum chloride
 - 1. Is a corrosive.
 - 2. Keep away from water.
 - 3. May cause severe skin and eye burns.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		

2	Stain slides in hematoxylin.	10 minutes	
3	Wash in running tap water	5 minutes	
4	Place slides in WORKING mucicarmine solution.	30-60 minutes	
5	Rinse in distilled water, 2-3 changes.	5-10 seconds each	
6	Dehydrate through graded alcohols.		
7	Clear in two changes of xylene.		
8	Coverslip.		

X. RESULTS:

- A. Epithelial acid, mucopolysaccharides (mucins) - **pink to red**
(*intestinal goblet cells, bronchus mucous glands*)
- B. Cryptococcus neoformans - **pink to red**
- C. Nuclei - **blue**

XI. REFERENCES:

- A. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.
- B. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.
- C. Carson FL: Histotechnology: A Self-Instructional Text. ASCP Press. 1990.

Approval Signatures

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Applicability

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