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Histology Special Stain - Gomori Trichrome - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of connective tissue and muscle and can be used to differentiate muscle from collagen or to demonstrate an increase in collagen.

II. PRINCIPLE:

The reaction is based on the acid/base (+/- charges) and the hardness/softness of the tissue and the dyes. The negative charged dyes will be attracted to the positively charged tissue components. Hard dyes will be attracted to hard tissue components, while soft dyes will be attracted to soft tissue components. Chromotrope 2R will stain cytoplasm and muscle. Fast Green FCF will stain connective tissues. Phosphotungstic acid is taken up by the collagen. Fast Green FCF is subsequently bound to the phosphotungstic acid. The acetic acid changes the charges/pH of the solution. The acetic acid rinse after the stain gives a more delicate stain but will not change the ratio. Hematoxylin stains the nuclei.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue.
    2. Use a fixative containing picric acid (Bouins; Dubosq-Brazil).

B. Processing
    1. Standard processing.

C. Section Thickness

1. Routine specimens- 5µm.
2. Cut liver biopsies at 4 µm.

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

E. Type of slide
   1. Plain slides

IV. REAGENTS:

A. **Saturated Aqueous Picric Acid**
Purchased Pre-Made

B. **Bouin's Fluid**

Saturated aqueous picric acid	**150.0 mL**
Formaldehyde, 37-40%	**50.0 mL**
Acetic acid, concentrated	**10.0 mL**

Mix together, adding the formaldehyde and acetic acid slowly, as not to splash. Store at room temperature. Stable for months.
SEE CAUTION CONCERNING PICRIC ACID IN SPECIAL SAFETY PRECAUTIONS.

C. **1% Alcoholic Hematoxylin-Solution A**

Hematoxylin	**1.0 gm**
Distilled water	**5.0 mL**
Absolute alcohol	**95.0 mL**

Dissolve hematoxylin in warmed distilled water. Add the absolute alcohol. Store at room temperature in a dark brown bottle.
Stable for months.

D. **29% Ferric Chloride**

Ferric chloride	**29.0 gm**
Distilled water	**100.0 mL**

Dissolve together. Store at room temperature. Stable for months.

E. **Iron Chloride-Solution B**

29% ferric chloride	**4.0 mL**
Distilled water	**95.0 mL**
Hydrochloric acid, concentrated	**1.0 mL**

Mix 29% ferric chloride and distilled water together. Slowly add hydrochloric acid, drop by drop, with stirring, to solution. Store at room temperature. Stable for months.

F. **Working Weigert Hematoxylin**

1% alcoholic hematoxylin (Solution A)	**20.0 mL**
Iron chloride (Solution B)	**20.0 mL**

Mix together. Store at room temperature. Stable for 2-3 days.

G. **Gomori Trichrome Solution**

Chromotrope 2R	**0.30 gm**
Fast green FCF	**0.15 gm**
Phosphotungstic acid	**0.40 gm**

Distilled water **50.00 mL**
Acetic acid, concentrated **0.50 mL**

Dissolve together. Store in refrigerator. Best when used within one (1) week.

H. **0.5% Acetic Acid**

Acetic acid **0.5 mL**
Distilled water **100.0 mL**

Stir together. Store at room temperature. Stable for several months.

V. EQUIPMENT:

A. Balance
B. Magnetic stirrer
C. 60°C oven

VI. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Acid clean coplin jars
D. Forceps
E. Funnel
F. Filter paper

VII. QUALITY CONTROL:

None needed; every tissue has its own built-in control. Look for areas of muscle and collagen, such as around blood vessels.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Picric Acid
    1. Toxic.
    2. Highly explosive.
    3. An extreme fire hazard.
    4. Keep picric acid moist at all times.
        a. If dry around the tops of the jar, wash off dry particles before opening.
    5. Store in an explosion-proof cabinet.

B. Formaldehyde
    1. Is a poison.
    2. May be fatal or cause blindness if swallowed.

3. Cannot be made non-poisonous.
4. Possible cancer hazard.
5. Irritating to eyes, skin and respiratory tract.
6. Can cause severe eye burns.

C. Acetic Acid
   1. Is an acid.
   2. Add slowly, drop by drop, to solution.
   3. May cause skin and eye burns.

D. Hematoxylin
   1. Is incompatible with oxidizers and alkalies.
   2. Store separate.

E. Ferric Chloride
   1. Is a corrosive.
   2. May cause skin and eye burns.
   3. Can be irritating to respiratory tract.

F. Hydrochloric Acid
   1. Is an acid.
   2. Add slowly, drop by drop, to the solution.
   3. May cause severe skin and eye burns.

G. Chromotrope 2R
   1. Is an irritant.

H. Fast Green FCF
   1. Is a carcinogen.
   2. Is an oxidizer.

I. Phosphotungstic Acid
   1. Is a corrosive.
   2. May cause skin and eye irritation.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place in Bouins solution in 60°C oven	35-60	Steps 2-4 can be skipped if tissue was

		minutes	fixed in a picric acid fixative (Bouins; DB).
3	Remove from oven, and place slides in a different coplin jar containing water.		
4	Wash in running water until all the yellow color is gone.	2-10 minutes	
5	Place in Weigert hematoxylin.	10 minutes	
6	Rinse in running tap water.	1 minute	
7	Stain in Gomori trichrome.	10 minutes	A decrease in staining time will lessen the green color. Aged trichrome solutions stain less red and more green.
8	Rinse in 0.5% acetic acid at room temperature.	10 seconds	This rinse does not alter the color schemes, it only makes the shades more delicate.
9	Dehydrate through graded alcohols.		
10	Clear in two changes of xylene.		
11	Coverslip.		

X. LIMITATIONS:

A. Tissue must either be fixed in a picric acid fixative (Bouins or DB) or must be post-mordanted in Bouins. Failure to do so will result in muddy colors - the collagen will be reddish-green, and the muscle will be greenish red.

B. An iron hematoxylin like Weigert's must be used. The acid dyes in the trichrome solution decrease the intensity of nuclear stains. If an alum hematoxylin is used, no nuclear staining will be seen. Nuclear staining may be poor even with the iron hematoxylin.

XI. RESULTS:

A. Collagen - **green**

B. Muscle fibers - **red**

C. Nuclei - **black**

XII. REFERENCES:

A. Carson FL: Histotechnology: A Self-Instructional Text. Chicago, IL, ASCP Press, 1990.

B. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak