Status **Active** PolicyStat ID **12430080**

Beaumont

Origination	10/25/2022
Last Approved	10/25/2022
Effective	10/25/2022
Last Revised	10/25/2022
Next Review	10/24/2024

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Area	Laboratory-Histology
Applicability	Royal Oak

Histology Special Stain-Colloidal Iron-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.

II. PRINCIPLE:

This reaction uses colloidal iron that is absorbed into tissue polyanions such as carboxylated and sulfated mucins. Acetic acid is used to maintain a low pH, and reduce non-specific background staining. Prussian blue reaction is used to visualize the absorbed iron. Nuclear fast red is the counterstain.

III. SPECIMEN COLLECTION AND HANDLING:

A. **Fixation**
   1. Any well fixed tissue.
   2. Avoid chromate fixatives.

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. **30% Ferric Chloride**

Ferric chloride	30.0 gm
Distilled water	100.0 ml

1. Dissolve together.
2. Store in dark bottle at room temperature.
3. Stable at room temperature for months.

B. **Stock Colloidal Iron**

30% ferric chloride	4.4 ml
Distilled Water	250.0 ml

1. Bring the distilled water to a boil.
2. Add 4.4 mL of 29% ferric chloride to the boiling water.
3. Continue to boil, stirring with mechanical stirrer.
4. When solution becomes a dark red, remove from heat.
5. Allow mixture to cool.
6. Store in a dark bottle at room temperature.
7. Stable for months.

C. **Working Colloidal Iron Solution**

Stock colloidal iron	10.0 ml
Distilled water	18.0 ml
Acetic acid, concentrated	12.0 ml

1. Just before use mix together in the order above.
2. Discard after use.

D. **15% Acetic Acid**

Acetic acid	22.5 ml
Distilled water	127.5 ml

1. Slowly add acetic acid to water.
2. Store at room temperature.
3. Stable for months.

E. **4% Hydrochloric Acid**

Hydrochloric acid	40.0 ml
Distilled water	960.0 ml

1. Slowly add hydrochloric acid to water, stirring constantly.
2. Store at room temperature.

3. Stable for months.

F. **2% Potassium Ferrocyanide**

Potassium ferrocyanide 20.0 gm
Distilled water 1000.0 ml

1. Dissolve together.
2. Store in dark bottle at room temperature.
3. Stable for 1 month.

G. **Potassium Ferrocyanide-Hydrochloric Acid Solution**

4% hydrochloric acid 20.0 ml
2% potassium ferrocyanide 20.0 ml

1. Just before use mix together.
2. Discard after use.

H. **Nuclear Fast Red**

Nuclear fast red (Kernechtrot) 0.1 gm
5% aluminum sulfate 100.0 ml

1. Dissolve together with the aid of gentle heat.
2. Do not boil.
3. Cool and filter.
4. Add a few crystals of thymol.
5. Stable at room temperature for several months.
6. May be re-used until weak.

V. EQUIPMENT:

A. Balance
B. Magnetic stirrer
C. Hot plate

VI. SUPPLIES:

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

VII. QUALITY CONTROL (QC):

A. Umbilical cord, especially if staining for hyaluronic acid (carboxylated acid mucopolysaccharide)
B. To demonstrate hyaluronic acid, proceed with step 1 with 2 controls and 2 unknowns. Place

one of the controls and one of the unknowns in hyaluronidase for 1 hour at room temperature. Keep the other control and unknown in distilled water. At the end of 1 hour, gently rinse off the hyaluronidase, combine all slides together, and continue with step 2. Carboxylated hyaluronic acid mucopolysaccharides will NOT stain with alcian blue. All other acid mucopolysaccharides will continue to stain.

VIII. SPECIAL SAFETY PRECAUTIONS:

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

B. **Acetic Acid**
   1. Add drop by drop to solutions.
   2. May cause skin and eye burns.

C. **Hydrochloric acid**
   1. Add drop by drop to solutions.
   2. May cause severe skin and eye burns.

D. **Potassium Ferrocyanide**
   1. No hazards listed.

E. Nuclear fast red
   1. Is a corrosive.
   2. May cause skin and eye burns.

IX. PROCEDURE:

Step	Action	Time	Notes
1.	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2.	Place in Working colloidal iron solution	2 hours	
3.	Rinse in 15% acetic acid, 3 changes	10 minutes each	
4.	Place in potassium ferrocynide-hydrochloric acid solution	20 minutes	
5.	Rinse in distilled water, 2 changes	5-10 seconds each	
6.	Rinse in running tap water	5 minutes	
7.	Counterstain in nuclear fast red	1- 5 minutes	
8.	Rinse in distilled water, 3 changes	5-10 seconds each	

9.	Dehydrate through graded alcohols		
10.	Clear in two changes of xylene		
11.	Coverslip		

X. RESULTS:

A. Acid mucopolysaccharide-**blue**
(intestinal goblet cells, umbilical cord, cartilage, cornea, dermis, mast cell granules, calcium salts, some fungi)

B. Nuclei- **pink**

XI. REFERENCES:

1. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.
2. 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