

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, Microwave-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. **29% Ferric Chloride**

Ferric chloride	29.0 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**

29% ferric chloride	4.4 ml
Distilled Water	250.0 ml

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

C. **Working Colloidal Iron Solution**

Stock colloidal iron	20.0 ml
Distilled water	10.0 ml
Acetic acid, concentrated	10.0 ml

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

D. **12% Acetic Acid**

Acetic acid	120.0 ml
Distilled water	880.0 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.
 - a. Do not boil.
 - b. Cool and filter.
 - c. Add a few crystals of thymol.
2. Stable at room temperature.
3. May be reused 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.

- C. Always rinse with tap water after counter-staining with nuclear fast red. Aluminum sulfate will remain as streaks on the slide if the water rinse is skipped, as aluminum sulfate does not dissolve in alcohol.
- D. To save on the amount of nuclear fast red used, keep some in the refrigerator. When the solution being used becomes weak, add some of the fresh solution from the refrigerator to it.
- E. PAS may be used as a counterstain instead of nuclear fast red. Proceed with steps 1-6 of the colloidal iron procedure, then begin with the periodic acid step for the PAS procedure. Acid mucins will be dark blue and neutral mucins will be magenta. Mixtures of acidic and neutral mucins will be purple. Hematoxylin is usually NOT used as a counterstain for colloidal Iron-PAS 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 12% acetic acid	1 minute	

3.	Place in plastic, uncapped jar of WORKING colloidal iron solution. Microwave on power level 5	10 seconds, swirl, 10 seconds more, swirl	
4.	Allow slides to cool	10 minutes	
5.	Rinse in 12% acetic acid	4 minutes	
6.	Place in potassium ferrocyanide-hydrochloric acid solution. Microwave, high	30 seconds, swirl	
7.	Allow slides to cool	1 minute	
8.	Rinse in running tap water	3 minutes	
9.	Counterstain in nuclear fast red	1-5 minutes	Check intensity using microscope
10.	Rinse in tap water	5-10 seconds	
11.	Dehydrate through graded alcohols.		
12.	Clear in two changes of xylene.		
13.	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:

- A. Davis, MM. and Green, RM. Automation, Acceleration and Microwaving for Special Stains, Teleconference Network of Texas, San Antonio, Texas, April 16, 1999.

Approval Signatures

Step Description	Approver	Date
Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	10/25/2022
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