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Histology Special Stain - Phosphotungstic Acid Hematoxylin (PTAH) - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of muscle striations, fibrin, collagen, and glial fibers. Muscle striations are found in striated and cardiac muscle, and in tumors arising from such tissues. Fibrin is a specific protein formed as a result of the clotting process and is a prominent feature in renal transplant rejections and tissues that have received a physical trauma. Glial fibers are found in the brain, and in gliosarcoma, a tumor of the brain.

II. PRINCIPLE:

Tungsten is a mordant that attaches to the hematoxylin, creating a blue-colored lake. This tungsten-hematein lake will bond with certain tissue components, staining them blue. Excess phosphotungstic acid will stain fibrin and connective tissue a red-brown color. Bouin's solution is used as a post-mordant, which improves the staining.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue.
    2. Zenker's, Bouin's or DB preferred

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 slides

IV. REAGENTS:

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

B. **Bouin's Fluid**

Saturated aqueous picric acid	**750.0 mL**
Formaldehyde, 37-40%	**250.0 mL**
Acetic acid, concentrated	**50.0 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 UNDER SPECIAL SAFETY PRECAUTIONS.

C. **5% Ferric Ammonium Sulfate**

Ferric ammonium sulfate (Iron Alum)	**5.0 gm**
Distilled water	**100.0 mL**

Stir together. Store at room temperature. Stable for several months. Discard after use.

D. **5% Oxalic Acid**

Oxalic acid	**5.0 gm**
Distilled water	**100.0 mL**

Dissolve together. Stable at room temperature for months. Discard after use.

E. **Phosphotungstic Acid Hematoxylin (PTAH)**

Hematoxylin	**1.0 gm**
Absolute ethanol	**10.0 mL**
Phosphotungstic Acid	**20.0 gm**
Distilled water	**900.0 mL**

1. Dissolve together 1 gm hematoxylin in 10 mL absolute alcohol.
2. Dissolve together 20 gm phosphotungstic acid in 900 mL hot distilled water.
3. Allow phosphotungstic acid solution to cool.
4. Mix together hematoxylin solution and phosphotungstic solution.
5. The solution ripens naturally in several months and is stable for at least one year.
6. May be stored at room temperature.
7. Discard after use.
8. If the stain is needed for immediate use, add 0.177 gm potassium permanganate to the solution. When artificially ripened solution is a reddish-brown color, it is ready for

use.

V. EQUIPMENT:

A. Balance

B. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Acid clean coplin jars

D. Forceps

VII. QUALITY CONTROL:

A. To demonstrate:

1. Cross-striations and connective tissue – use striated muscle.
2. Fibrin – use tissue with fibrin
3. Glial cells – use cerebral cortex

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Picric Acid

1. Is toxic, highly reactive and an extreme fire hazard.
2. Keep picric acid moist at all times.
3. If a dry powder is seen around the lip of the jar, wash off with running water before opening.
4. Store in an explosion proof cabinet.

B. Formaldehyde

1. Is poisonous.
2. May be fatal if swallowed or cause permanent blindness.
3. Possible cancer hazard.
4. Irritant to eyes, skin, respiratory system.

C. Acetic Acid

1. Add drop by drop to solutions.
2. May cause skin and eye burns.

D. Ferric Ammonium Sulfate (Iron Alum)

1. Is an irritant.

E. Oxalic Acid
    1. Is a strong reducer.
    2. Contact with other material may cause fire.
    3. Store separate.
    4. May cause skin and eye burns.
    5. May be irritating to respiratory system.

F. Hematoxylin
    1. Is incompatible with oxidizers and alkalies.
    2. Store separate from these.

G. 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 minutes	Steps 2-4 can be skipped if tissue was 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 Biebrich Scarlett- Acid Fuchsin.	2-3 minutes	
8	Rinse in running tap water, 2-3 changes.	5-10 seconds	
9	Place in phosphotungstic acid solution, jiggling occasionally.	1-5 minutes	
10	Rinse in distilled water, quickly, 2 changes each.	1-2 seconds	
11	Stain slides in aniline blue solution	1-10 minutes	Check the staining of the aniline blue with a microscope.

			The intensity of the red and blue should be equal. Do NOT check constantly, as this seems to make the colors muddy.
12	Rinse quickly in distilled water, 2-3 changes.	5-10 seconds	
13	Dehydrate through graded alcohols, clear with xylene.		
14	Coverslip.		

X. RESULTS:

A. Striated muscle fibers, fibrin, glial cells - **blue**

B. Nuclei, mitotic figures, mitochondria, keratin - **blue**

C. Collagen, reticulin, basement membrane, cartilage - **red to red-brown**

XI. LIMITATIONS:

A. PTAH must be either chemically oxidized or naturally ripened for the stain to work.

B. Chemically ripened PTAH has a shorter shelf life than naturally ripened (sun, air).

C. Over-oxidized (old) PTAH will show very pale blue staining.

1. Discard over-oxidized solution.

XII. REFERENCES:

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

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D. Preece A: A Manual for Histologic Technicians, 3rd edition. Boston, MA, Little, Brown & Co., 1972.

Approval Signatures

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Applicability

Royal Oak