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Histology Special Stain - Iron Hematoxylin - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of Protozoa in tissue.

II. PRINCIPLE:

Weigert's Iron hematoxylin uses ferric chloride to oxidize hematoxylin to hematein, and to mordant the tissue. The nuclei and protozoa, having the strongest affinity for the hematoxylin complex, will stain them black. Van Gieson is the counterstain, staining collagen red and all other tissue components yellow.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue.

B. Processing
    1. Standard, overnight processing.

C. Section Thickness
    1. Routine specimens-5µ.

D. Slide Drying
    1. 30 minutes at 60°C.

E. Type of Slide

1. Plain slides

IV. REAGENTS:

A. **1% Alcoholic Hematoxylin**

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

Dissolve together hematoxylin and warmed distilled water. Add this to the absolute alcohol. Stable at room temperature for several months.

B. **29% Ferric Chloride**

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

Dissolve together. Store at room temperature; stable for several months.

C. **Weigert's Iron Chloride**

Distilled water	**95.0 mL**
29% Ferric chloride	**4.0 mL**

Mix together, stable at room temperature for months.

D. **Weigert's Iron Hematoxylin Solution**

1% alcoholic hematoxylin	**20.0 mL**
Weigert's Iron chloride	**20.0 mL**

Mix together. Stable at room temperature for one week; may be reused.

E. **1% Aqueous Acid Fuchsin**

Acid fuchsin	**1.0 gm**
Distilled water	**100.0 mL**

Dissolve together. Store at room temperature; stable for months.

F. **Saturated Aqueous Picric Acid**

Purchased Pre-Made

G. **Van Gieson Counterstain**

1% aqueous acid fuchsin	**5.0 mL**
Saturated aqueous picric acid	**100.0 mL**

Mix together. Store at room temperature; stable for months. Reuse until weak.

V. EQUIPMENT:

A. Balance

B. 60°C oven, water bath or microwave oven

C. Hot plate

D. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Funnel
D. Coplin jars
E. Filter paper

VII. QUALITY CONTROL (QC):

Tissue with protozoa.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Hematoxylin
    1. Is incompatible with oxidizers and alkalies.
    2. Store separately.
B. Ferric Chloride
    1. Is a corrosive.
    2. May cause skin and eye burns.
C. Acid Fuchsin
    1. Is an irritant.
D. Picric Acid
    1. Is highly reactive (4NFPA), and an extreme fire hazard (4 NFPA).
    2. Keep picric acid moist at all times.
    3. If a dry powder is seen around the rim of the jar, wash off with running water before opening.
    4. Store in an explosion proof cabinet.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	Stain slides in WORKING iron hematoxylin.	10 minutes	
3	Wash sections in running tap water.	5 minutes	
4	Counterstain sections in Van Gieson.	1 minute	Time in Van Gieson must be short, otherwise, it will further differentiate the hematoxylin.
5	Differentiate in 95% ethanol, 2 changes each.	5-10 seconds	

6	Dehydrate through graded alcohols, clear with xylene.		
7	Coverslip.		

X. LIMITATIONS:

A. The following may influence the validity of test results:

1. Weigert's hematoxylin solution quickly over-oxidizes once it is made. It is good for one week only.

XI. RESULTS:

A. Nuclei - **black**

B. Protozoa - **black**

C. Collagen - **red**

D. Muscle, cytoplasm, red blood cells - **yellow**

E. Other tissue components - **yellow**

XII. REFERENCES:

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

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

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

D. Histopathology Laboratory Procedures of the Pathologic Anatomy Branch of National Cancer Institute.

Approval Signatures

Step Description	Approver	Date
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Applicability

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