Status **Active** PolicyStat ID **12600137**

Beaumont

Origination	11/8/2022	Document Contact	Sharon Scalise: Supv, Laboratory
Last Approved	11/8/2022	Area	Laboratory-Histology
Effective	11/8/2022	Applicability	Royal Oak
Last Revised	11/8/2022		
Next Review	11/7/2024		

Histology Special Stain - Verhoeff-Van Gieson (VVG) - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of elastic fibers, a specialized connective tissue found in tissues that undergo stretching. Changes to elastic fibers in blood vessels are of great pathologic importance. Arteries have a continuous elastic band (internal elastic lamina), and some arteries have a second band (external elastic lamina). Veins have less elastic than arteries. Elastic fibers may fray, break, duplicate, or be missing in arteriosclerosis or giant cells arteritis. This technique may be used to demonstrate if blood vessels are present, or whether the blood vessels have been invaded by tumors.

II. PRINCIPLE:

Verhoef hematoxylin uses ferric chloride and iodine to oxidize hematoxylin to hematein, and to mordant the tissue. The tissue is over stained with a hematoxylin-ferric chloride-iodine complex. The section is then differentiated with excess mordant, ferric chloride. The dye in the tissue is attracted to the greater amount of ferric chloride in the differentiating solution than is in the tissue and will be removed from the tissue. The elastic, having the strongest affinity for the hematoxylin complex, will retain it the longest. Sodium thiosulfate is used to remove excess iodine that may stain the tissue. Van Gieson is the counterstain, staining collagen red and all other tissue components yellow.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation

1. Any well-fied tissue.

B. Processing

1. Standard, overnight processing.

C. Section Thickness

1. Routine specimens 5µ.

D. Slide Drying

1. 60 minutes at 60°C.

E. Type of Slide

1. Plain slides.

IV. REAGENTS:

A. **5% Alcoholic Hematoxylin**

Hematoxylin	**25.0 gm**
Absolute ethanol	**500.0 mL**

Dissolve together with the aid of gentle heat and stirring. DO NOT LEAVE UNATTENDED. Filter.
Store in dark bottle at room temperature; stable for several months.
CAUTION: HEATING ABSOLUTE ALCOHOL may cause a fire. Heat on the lowest heat; do NOT leave unattended.

B. **10% Ferric Chloride**

Ferric chloride	**10.0 gm**
Distilled water	**100.0 mL**

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

C. **Weigert Iodine Solution**

Potassium iodide	**1.0 gm**
Iodine	**2.0 gm**
Distilled water	**100.0 mL**

Dissolve potassium iodide in 10 mL distilled water. Then add iodine, and dissolve completely. Add the remaining 90 mL
distilled water. Store at room temperature; stable for several months.

D. **Verhoeff Elastic Staining Solution**

5% alcoholic hematoxylin	**22.0 mL**
10% ferric chloride	**8.0 mL**
Weigert iodine solution	**8.0 mL**

JUST BEFORE USE, mix together, in order. Good for one day only.

E. **2% Ferric Chloride**

Ferric chloride	**2.0 gm**
Distilled water	**100.0 mL**

Dissolve together. Stable at room temperature for months; discard after use.

F. **5% Sodium Thiosulfate**

Sodium thiosulfate	**25.0 mL**
Distilled water	**500.0 mL**

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

G. **1% Aqueous Acid Fuchsin**

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

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

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

I. **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. Magnetic stirrer
C. Hot plate

VI. SUPPLIES:

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

VII. QUALITY CONTROL:

None needed, as most tissue has elastin in a vein or artery. If required, use a cross section of artery or skin.

VIII. SPECIAL SAFETY PRECAUTIONS:

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

B. Ferric Chloride
    1. Is a corrosive.
    2. May cause skin and eye burns.

C. Potassium Iodide

1. Is an irritant.

D. Iodine
    1. Is a corrosive and an oxidizer.
    2. It may be irritating to the eyes and respiratory tract.
    3. May cause skin and eye burns.

E. Sodium Thiosulfate
    1. Is an irritant.

F. Acid Fuchsin
    1. Is an irritant

G. Picric Acid
    1. Is highly reactive (4 NFPA), 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 slides through graded alcohol to distilled water.		
2	Stain slides in Verhoeff elastic staining solution.	15 minutes	
3	Rinse in distilled water, 2-3 changes each.	10 seconds	
4	Differentiate sections with 2 % ferric chloride.	2-10 minutes	Differentiate the slides individually using a microscope. Background should be pale gray to lavender, and elastin is black.
5	Rinse in distilled water, 2-3 changes.	10 seconds	
6	Place slides in 5% sodium thiosulfate.	1 minute	
7	Wash slides in running tap water.	5 minutes	
8	Counterstain slides in Van Gieson.	1 minute	
9	Dehydrate through graded		

	alcohols, clear with xylene.		
10	Coverslip.		

X. LIMITATIONS:

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

1. Time in Van Gieson must be short, otherwise, it will further differentiate the hematoxylin and decolorize the elastin.
2. Verhoeff elastic staining solution quickly over-oxidizes once it is made. It is good for one day only.

XI. RESULTS:

A. Elastin fibers - **blue-black/blue**

B. Nuclei - **blue to black**

C. Collagen - **red**

D. Muscle, cytoplasm, red blood cells, other tissue components - **yellow**

XII. REFERENCES:

A. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. 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. William Beaumont Hospital's, Royal Oak, MI. Modification of Verhorff Elastic Tissue Stain. Mallory FB: Pathological Technique, Hafner Publishing Co., 1961.

Approval Signatures

Step Description	Approver	Date
Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	11/8/2022
Policy and Forms Steering Committee (if needed)	Gail Juleff: Project Mgr Policy	11/8/2022
Policy and Forms Steering Committee (if needed)	Sharon Scalise: Supv, Laboratory	11/3/2022

Amy Knaus: Dir, Lab Operations C	11/3/2022
Jennifer Lehmann: Mgr Laboratory	11/3/2022
Sharon Scalise: Supv, Laboratory	11/2/2022

Applicability

Royal Oak