

VERHOEFF'S METHOD FOR ELASTIC TISSUE

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of elastic tissue.
PRINCIPLE:	The tissue is overstained with a soluble lake of Hematoxylin-Ferric Chloride-Iodine. Both Ferric Chloride and Iodine serve as mordants, but they also have an oxidizing function that assists in converting Hematoxylin to hematein. Differentiation is accomplished by using excess mordant, or Ferric Chloride, to break the tissue-mordant-dye complex. Sodium Thiosulfate is used to remove excess Iodine. Van Gieson's is our preferred counterstain but others may be used.
CONTROL:	Artery, aorta <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs011.</i>
SPECIMEN PREPARATION:	10% Neutral Buffered Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	<ol style="list-style-type: none"> Hematoxylin 5% Alcoholic Item# s212B Ferric Chloride 10% Aqueous Item# s180B Lugol's Iodine Working Solution Item# s234A <i>Working Solution: Mix in order, just before use and filter.</i> Hematoxylin 5% Alcoholic..... 20 mL Ferric Chloride 10% Aqueous..... 8 mL Lugol's Iodine Working Solution..... 8 mL Ferric Chloride 2% Aqueous Item# s180 Sodium Thiosulfate 5% Aqueous Item# s1895 Van Gieson's Solution Item# s289 <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
NOTES:	
REFERENCE:	Bancroft, J. D. & Stevens, A. <u>Theory and Practice of Histological Techniques</u> . 4th Ed. Churchill Livingstone. New York. 1996. p. 132.

STAINING PROCEDURE:

- Deparaffinize and hydrate to water.
- Stain in Verhoeff's Working Solution, 15-20 minutes.
- Wash in distilled water, 10 dips.
- Differentiate in Ferric Chloride 2% Aqueous, a few dips at a time, until tissue begins to appear gray macroscopically.
- Rinse in tap water to remove excess Chloride. Check under microscope for correct differentiation. Elastic Fibers should be clear, sharp black, and surrounding elements colorless. The slides should be slightly under differentiated to offset the Van Gieson's reaction which follows. If fine fibers are not visible, the slide has been over differentiated. Repeat process from step 3.
- Treat in Sodium Thiosulfate 5% Aqueous for 1 minute.
- Wash well in water.
- Counterstain in Van Gieson's Solution for 3 minutes.
- Blot slide dry before placing in Xylene to clear.
- Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Elastic Fibers Black
 Collagen..... Red
 Muscle, Cornified Epithelium Yellow
 Nuclei..... Blue to Black

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