

VERHOEFF'S ELASTIC MASSON'S TRICHROME METHOD

PURPOSE:	For In Vitro Diagnostic Use: The demonstration of all connective tissue components.
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. An acid dye is applied to the section which binds all acidophilic elements including both collagen and muscle. The Phosphotungstic Phosphomolybdic Acid then displaces the acid dye from the collagen. Collagen is more permeable than cytoplasm and thus the large phosphotungstic phosphomolybdic molecule cannot displace the acid dye from the cytoplasm. It also acts as a mordant for the Aniline Blue to bind to collagen. Acetic Acid clears the slide of any loosely bound dye.
CONTROL:	Artery <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs011.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 3 micrometers
SOLUTIONS:	1. Bouin's Fixative Item# s129 2. Hematoxylin 5% Alcoholic Item# s212B 3. Ferric Chloride 10% Aqueous Item# s180B 4. Lugol's Iodine Working Solution Item# s234A 5. Phosphotungstic Phosphomolybdic Acid Item# s255 6. Biebrich Scarlett Acid Fuchsin Solution Item# s125 7. Aniline Blue Masson's Trichrome Item# s116 8. Acetic Acid 1% Aqueous Item# s100 <u>Verhoeff's Working Solution:</u> Hematoxylin 5% Alcoholic..... 20 mL Ferric Chloride 10% Aqueous..... 8 mL Lugol's Iodine Working Solution..... 8 mL <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	
REFERENCE:	Garvey, W. "Modified Elastic Tissue Masson Trichrome Stain". <i>Stain Technology</i> . Vol. 59. 3:213-216.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to water.
2. Mordant in Bouin's Fixative for 1 hour at 57°C.
3. Wash in distilled water.
4. Stain in Verhoeff's Working Solution for 15 minutes.
5. Wash in distilled water.
6. Stain in Biebrich Scarlett Acid Fuchsin Solution for 2 minutes.
7. Wash in distilled water.
8. Place in Phosphotungstic Phosphomolybdic Acid for 8 minutes.
9. Stain in Aniline Blue Masson's Trichrome for 3 minutes.
10. Rinse in distilled water.
11. Place in Acetic Acid 1% Aqueous for 1 minute.
12. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene.
13. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Red NucleiReddish-Brown to Blue-Black
 Cytoplasm, MuscleRed
 Elastic FibersBlue-Black
 Collagen.....Blue

Poly Scientific R&D Corp.

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