

MOVAT'S PENTACHROME METHOD FOR CONNECTIVE TISSUE

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of all connective tissue components and mucins.
PRINCIPLE:	An Iron Hematoxylin is used here since it will resist removal by the acidic stain that follows. The iron resorcin lake binds to elastic fibers selectively. The counterstain binds selectively by dye competition, size and charge. An acid dye is applied to the section which binds all acidophilic elements including both collagen and muscle. The Phosphotungstic Acid then displaces the acid dye from the collagen. Collagen is more permeable than cytoplasm and thus the large phosphotungstic molecule cannot displace the acid dye from the cytoplasm.
CONTROL:	Heart, lung <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 6 micrometers
SOLUTIONS:	<ol style="list-style-type: none"> 1. Weigert's Iron Hematoxylin Solution Set (A & B) Item# s216B <i>Working Solution: Mix equal parts of solutions A & B for use.</i> 2. Alcian Blue 1% in 1% Acetic Acid Item# s111 3. Resorcin Fuchsin Working Solution Item# s265 4. Woodstain Scarlet Acid Fuchsin Set Item# s290 5. Saffron 3% Alcoholic Item# s269 6. Alkaline Alcohol (Movat's Pentachrome) Item# s2087 7. Acetic Acid 0.5% Aqueous Item# s2015 8. Phosphotungstic Acid 5% Aqueous Item# s2090 <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
NOTES:	
REFERENCE:	Movat, H. A. "Demonstration of All Connective Tissue Elements in a Single Section". <i>Arch. Path.</i> 60: pp. 289-295.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Place in Alcian Blue 1% in 1% Acetic Acid for 20 minutes.
3. Wash in running water for 3 minutes.
4. Place in Alkaline Alcohol (pH must be over 8) for 2 hours. This converts the Alcian Blue into the insoluble pigment Monastral Fast Blue.
5. Wash in running water for 10 minutes.
6. Rinse in 70% Alcohol.
7. Place in Resorcin Fuchsin Working Solution for 16 hours.
8. Wash in running water for 10 minutes.
9. Place in Weigert's Iron Hematoxylin Working Solution for 5 minutes.
10. Rinse in running water.
11. Place in Woodstain Scarlet Acid Fuchsin Solution for 5 minutes. Differentiation of nuclei will take place in this solution.
12. Rinse in Acetic Acid 0.5% Aqueous.
13. Differentiate in Phosphotungstic Acid 5% Aqueous for 10-20 minutes until the collagen is pale pink and the background substance, which is covered by the red, is bluish again.
14. Rinse in Acetic Acid 0.5% Aqueous.
15. Rinse thoroughly in 3 changes of Absolute Alcohol.
16. Place in Saffron 3% Alcoholic for 10-15 minutes.
17. Dehydrate in 3 changes of Absolute Alcohol and clear in several changes of Xylene. If the collagen is not sufficiently yellow, repeat the staining with Saffron Solution.
18. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Nuclei.....	Black
Collagen and Reticulum Fibers.....	Yellow
Fibrinoid	Intense Red
Elastic Fibers	Dark Purple to Black
Ground Substance.....	Blue to Bluish Green
Muscle	Red

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