

## GOMORI'S ONE STEP TRICHROME METHOD FOR CONNECTIVE TISSUE (GREEN) (Microwave)

<b>PURPOSE:</b>	For In Vitro Diagnostic Use: Intended for the qualitative differentiation of collagen and smooth muscle fibers.
<b>PRINCIPLE:</b>	Post fixation in Bouin's increases the intensity of the stain. The positive nuclei are then stained by an anion iron hematein lake. An acid dye is applied to the section which binds all acidophilic elements including both collagen and muscle. Collagen is more permeable than cytoplasm and thus the large phosphotungstic molecule cannot displace the acid dye from the cytoplasm. It also acts as a mordant for the Light Green to bind to collagen. Acetic Acid clears the slide of any loosely bound dye.
<b>CONTROL:</b>	Uterus, appendix  <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs025.</i>
<b>SPECIMEN PREPARATION:</b>	Formalin fixed, paraffin embedded sections cut at 6 micrometers
<b>SOLUTIONS:</b>	1. Bouin's Fixative Item# s129 2. Weigert's Iron Hematoxylin Solution Set (A & B) Item# s216B <i>Working Solution:</i> Mix equal parts of solutions A & B for use. 3. Gomori's One Step Trichrome With Light Green Item# s1815 4. Acetic Acid 0.5% Aqueous Item# s2015  <i>Solutions can be purchased separately from Poly Scientific.</i>
<b>NOTES:</b>	<i>Variations in timing may occur due to the power wattage of the microwave oven. Provided times and power levels are based on 1000 watt microwave oven.</i>
<b>REFERENCE:</b>	Lillie, R. D. <u>Histopathologic Technic and Practical Histochemistry</u> . 3rd Ed. McGraw-Hill Book Co. New York. 1965. p. 547.

### STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Place 50 mL of Bouin's Fixative in a plastic coplin jar (loosely apply cap) and microwave for 20 seconds. Stir and re-microwave for 15 seconds. Stir again and place slides in hot solution for 3-5 minutes.
3. Rinse well in running water until water runs clear.
4. Place 50 mL of Weigert's Iron Hematoxylin Working Solution in a plastic coplin jar (loosely apply cap) and microwave for 15 seconds. Stir and re-microwave for 10-15 seconds. Stir again and add slides to hot solution for 2-3 minutes. Check control. If additional time is needed place slides back in hot solution until desired intensity is reached.
5. Wash well in running water.
6. Place 50 mL of Gomori's One Step Trichrome With Light Green in a plastic coplin jar (loosely apply cap) and microwave for 15-20 seconds. Stir and re-microwave for 15 seconds. Stir again and add slides to hot solution for 3-4 minutes. Check control. If additional staining time is needed place slides back in hot solution.
7. Place in Acetic Acid 0.5% Aqueous for 2 minutes. If sections are too dark differentiate in Acetic Acid 1% Aqueous to which 0.7 grams of Phosphotungstic Acid has been added. Rinse in distilled water.
8. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
9. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

### RESULTS:

Muscle Fibers..... Red  
Collagen ..... Green  
Nuclei..... Blue to Black

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