**UW Medicine - Pathology**

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**GT Green**

Gomori's One-Step Trichrome Method Procedure

Hand Stain Method (Manual Backup)

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| Adopted Date: 07/15/13Review Date: Revision Date:  |

PURPOSE

To identify an increase in collagenous connective tissue fibers, or to differentiate between collagen and smooth muscle fibers.

PROCEDURE

**Fixation:**

10% buffered neutral formalin.

**Technique:**

Cut Autopsy Brain at 6 microns, other tissues at 4 microns.

**Procedure:**

1. De-paraffinize to 80% and hydrate to running water.
2. Place in pre-heated Bouin's solution in 37° oven for 30 min.
3. Wash in running H2O until yellow color is removed from tissue.
4. Stain nuclei with Weigert's hematoxylin (working solution) - 12 min.
5. Wash in running H2O - 10 min.
6. Flood with Trichrome solution from Artisan Stain kit for 20 min.
7. Place directly into 0.5% acetic acid from renal service reagents for 2 min.
8. Rinse with DH2O.
9. Dehydrate, clear and mount as usual.

**Results:**

Muscle fibers, reactive astrocytes, myelin Red

Connective tissue Green

Nuclei Blue-Black

**Solutions:**

**Bouin's Fixative**

Picric acid, saturate aqueous solution 750 ml

37-40% Formaldehyde 250 ml

Glacial acetic acid 50 ml

**Working Weigert's Hematoxylin Solution**

 **Solution A -** premade from Richard Allyn Scientific

 **Solution B -** premade from Richard Allyn Scientific

 **\*\*\*\*Working Solution:** mix equal parts of Solutions A & B.

**One-Step Trichrome Stain (Green)** - premade in Artisan GT stain kit

**0.5% ACETIC ACID**

Glacial acetic acid 0.5 ml

DH2O 99.5 ml

**Comments:**

**Principle**: In the one-step trichrome procedure, a plasma stain (chromotrope R) and a connective tissue fiber stain (fast green FCF, light green, or aniline blue) are combined in a solution of phosphotungstic acid to which glacial acetic acid has been added. Phosphotungstic acid favors the red staining of muscle and cytoplasm. The tungstate ion is specifically taken up by collagen, and the connective tissue fiber stain is subsequently bound to this complex, coloring the collagen green or blue, depending on the counter-stain used.

**Notes**:

1. Coloration of fine connective tissue fibers is affected by the dye solution pH, with maximum binding occurring around pH 1.3. The pH of Gomori's trichrome is about 2.5, which decreases affinity for anions by approximately 50%, so that by replacing the acetic acid with hydrochloric acid, a pH of approximately 1.3 can be obtained. The intensity of coloration of the fine connective tissue fibers can be varied by altering the pH.

REFERENCES

1. *Manual of Histologic and Special Staining Technics*, McGraw-Hill Book Co., 1960, pg. 66. Modified by Histopathology Laboratory, Harborview Medical Center, Seattle, WA.
2. Sheehan, D.C. and Hrapchak, B.B.: *Theory and Practice of Histotechnology*, The C.V. Mosby Co., 1980, pg. 191.

3. Newcomer Supply, Trichrome, Gomori One-Step Technical Memo, revised December 2009

4. A.F.I.P., 3rd ed., p93-4.

Written By: Director Approval:

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