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**COLLAGEN - MASSON'S TRICHROME STAIN(TRI)**

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**PURPOSE:** Used to differentiate between collagen and smooth muscle in tumors, and the increase of collagen in diseases such as cirrhosis. Routine stain for liver and kidney biopsies.

**PRINCIPLE:** As the name implies, three dyes are employed selectively staining muscle, collagen fibers, fibrin, and erythrocytes. The general rule in trichrome staining is that the less porous tissues are colored by the smallest dye molecule; whenever a dye of large molecular size is able to penetrate, it will always do so at the expense of the smaller molecule. Others suggest that the tissue is stained first with the acid dye, Biebrich Scarlet, which binds with the acidophilic tissue components. Then when treated with the phospho acids, the less permeable components retain the red, while the red is pulled out of the collagen. At the same time causing a link with the collagen to bind with the aniline blue.

**FIXATIVE:** Bouin's is preferred, 10% formalin.

**TECHNIQUE:** Cut paraffin sections 4m

**EQUIPMENT:** Rinse glassware in DI water. Coplin jars, 60°C oven or waterbath, microwave

**REAGENTS:**

**Bouin's Fixative**

Saturated picric acid	1500.0 ml
Formaldehyde	500.0 ml
Glacial acetic acid	100.0 ml

Mix well, label, date and initial.  
Stable for 2 years.

**CAUTION:** Carcinogenic, irritant.

**Biebrich Scarlet:**

Biebrich scarlet	2.7 gm
Acid fuchsin	0.3 gm
Distilled water	300.0 ml
Glacial acetic acid	3.0 ml

Mix well, label with initial and date. Solution is stable for 6 months.

**CAUTION:** Avoid contact and inhalation.

CONNECTIVE TISSUE  
MASSON'S TRICHROME

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**Weigert's Iron Hematoxylin**

**Stock Solution A:**

Hematoxylin	5.0 gm
95% alcohol	500.0 ml

Mix well, label with initial and date. Stable for 1 year.

**CAUTION:** Flammable, avoid contact and inhalation.

**Stock Solution B:**

29% ferric chloride	20.0 ml
Distilled water	475.0 ml
Hydrochloric acid	5.0 ml

Mix well, label with initial and date. Stable for 1 year.

**CAUTION:** Corrosive, avoid contact and inhalation.

**Weigert's Hematoxylin**

**Working Solution**

Solution A	25.0 ml
Solution B	25.0 ml

Mix well, solution will remain stable for 3 - 4 days.

**Phosphotungstic/**

**Phosphomolybdic Acid Solution:**

Phosphotungstic acid	25.0 gm
Phosphomolybdic acid	25.0 gm
Distilled water	1000.0 ml

Mix well, label with initial and date. Solution is stable for 6 months.

**CAUTION:** Avoid contact and inhalation.

**Aniline Blue:**

Aniline blue	2.5 gm
Distilled water	100.0 ml
Glacial acetic acid	1.0 ml

Mix well, label with initial and date. Solution is stable for 6 months.

**Caution:** Avoid contact and inhalation.

**1% Acetic Acid:**

Glacial acetic acid	10.0 ml
Distilled water	1000.0 ml

Mix well, label with initial and date. Solution is stable for 1 year.

**Caution:** Avoid contact and inhalation.

**SAFETY:** Wear gloves, goggles and lab coat. Avoid contact and inhalation of dyes and chemicals.

Bouin's contains: formaldehyde, a known carcinogen, picric acid can become explosive when dry. Toxic by skin absorption. Keep hot uncapped Bouin's under the hood.

Phosphomolybdic, phosphotungstic acid powders, and acetic acid solutions are skin and eye irritants, and strong corrosives.

**PROCEDURE:**

1. \*Mordant in Bouin's solution, microwave 1 minute, allow to stand 15 minutes.
  2. Wash in running tap water to remove the picric acid, 5 minutes.
  3. Weigert's working hematoxylin, 10 minutes.
  4. Blue in running tap water for 5 minutes, rinse in distilled water.
  5. Biebrich scarlet for 5 minutes.
  6. Rinse in distilled water.
  7. Phosphotungstic/phosphomolybdic acid for 10 minutes, discard solution.
  8. Transfer directly into Aniline blue for 5 minutes.
  9. Rinse in distilled water.
  10. 1% Acetic acid for 1 minute, discard solution, rinse in distilled water.
  11. Dehydrate, clear, and coverslip.
- \*Conventional method: Mordant in Bouin's solution, 60°C for 1 hour.

**RESULTS:**

Nuclei	black
Cytoplasm, muscle, erythrocytes	red
Collagen	blue

**NOTES:**

1. Light green may be substituted for Aniline blue..
2. 5% phosphotungstic acid for 5 minutes, must be substituted when using Light green.
3. When staining liver biopsies, the collagen is better light blue than dark blue.

**REFERENCE:**

- Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp 189-190, Battelle Press, Ohio
- Bancroft J, Stevens A, Theory and Practice of Histological Techniques, 2nd Ed, 1982, pp 131-135, Churchill-Livingston, NY
- Luna L, Manual of Histologic Staining Methods of the AFIP, 3rd Ed, 1968, pp 94-95, McGraw-Hill, NY
- Carson F, Histotechnology A Self-Instructional Text, 1st Ed, 1990, pp 142-143, ASCP, III

CONNECTIVE TISSUE  
MASSON'S TRICHROME

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Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology  
Laboratory, 2nd ED, 1991, Anatech

Prepared: \_\_\_\_\_ By: \_\_\_\_\_

Approved: \_\_\_\_\_ By: \_\_\_\_\_

Downloaded from WebPath: Internet Pathology Laboratory  
<http://www-medlib.med.utah.edu/WebPath/webpath.html>

## PROCEDURE CARD

### TRICHROME STAIN, MASSON'S (TRI)-COLLAGEN

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#### PROCEDURE:

1. \*Mordant in Bouin's solution, microwave 1 minute, allow to stand 15 minutes.
2. Wash in running tap water to remove the picric acid, 5 minutes.
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6. Rinse in distilled water.
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\*Conventional method: Mordant in Bouin's solution, 60°C for 1 hour.

#### RESULTS:

Nuclei	black
Cytoplasm, muscle, erythrocytes	red
Collagen	blue

**SAFETY/PPE:** Wear gloves, goggles and lab coat. Keep hot Bouin's under the fume hood.

#### Weigert's Iron Hematoxylin

##### Stock Solution A:

Hematoxylin	5.0 gm
95% alcohol	500.0 ml

Mix well, label with initial and date. Stable for 1 year.

##### CAUTION: Flammable

##### Stock Solution B:

29% ferric chloride	20.0 ml
Distilled water	475.0 ml
Hydrochloric acid	5.0 ml

Mix well, label with initial and date. Stable for 1 year.

#### Working Solution

Solution A	25.0 ml
Solution B	25.0 ml

Mix well, solution will remain stable for 3 - 4 days.

#### Bouin's Fixative

**CAUTION:** Carcinogenic, irritant, toxic.

##### Biebrich Scarlet:

Biebrich scarlet	2.7 gm
Acid fuchsin	0.3 gm
Distilled water	300.0 ml
Glacial acetic acid	3.0 ml

Mix well, label with initial and date. Solution is stable for 6 months.

##### CAUTION: Possible carcinogen

##### Phosphotungstic/

##### Phosphomolybdic Acid Solution:

Phosphotungstic acid	25.0 gm
Phosphomolybdic acid	25.0 gm
Distilled water	1000.0 ml

Mix well, label with initial and date. Solution is stable for 6 months.

PROCEDURE CARD

**TRICHROME STAIN, MASSON'S (TRI)-COLLAGEN**

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**Aniline Blue:**

Aniline blue	2.5 gm
Distilled water	100.0 ml
Glacial acetic acid	1.0 ml

**1% Acetic Acid:**

Glacial acetic acid	10.0 ml
Distilled water	1000.0 ml

Mix well, label with initial and date. Solution is stable for 6 months.

Mix well, label with initial and date. Solution is stable for 1 year.

**1% ACETIC ACID:**

Glacial acetic acid            10.0 ml  
Distilled water                1000.0 ml

Mix well, label with initial and date. Solution is stable for 1 year.

Caution: Skin and eye irritant

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**ANILINE BLUE:**

Aniline blue                    2.5 gm  
Distilled water                100.0 ml  
Glacial acetic acid            1.0 ml

Mix well, label with initial and date. Solution is stable for 6 months.

Caution: Avoid contact and inhalation.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**ANILINE BLUE**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

**BIEBRICH SCARLET:**

Biebrich scarlet                2.7 gm  
Acid fuchsin                    0.3 gm  
Distilled water                300.0 ml  
Glacial acetic acid            3.0 ml

Mix well, label with initial and date. Solution is stable for 6 months.

Caution: Avoid contact and inhalation.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**BIEBRICH SCARLET:**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

**PHOSPHOTUNGSTIC/  
PHOSPHOMOLYBDIC ACID  
SOLUTION:**

Phosphotungstic acid	25.0 gm
Phosphomolybdic acid	25.0 gm
Distilled water	1000.0 ml

Mix well, label with initial and date. Solution is stable for 6 months.

Caution: Avoid contact and inhalation.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**PHOPHOTUNGSTIC /  
PHOSPHOMOLYBDIC ACID:**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

**WEIGERT'S IRON HEMATOXYLIN:  
Stock Solution A:**

Hematoxylin	5.0 gm
95% alcohol	100.0 ml

Mix well, label with initial and date. Stable for 1 year.

CAUTION: FLAMMABLE, avoid contact and inhalation

TECH: \_\_\_\_\_

DATE: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**WEIGERT'S IRON HEMATOXYLIN:  
Stock Solution B:**

29% ferric chloride	20.0 ml
Distilled water	475.0 ml
Hydrochloric acid	5.0 ml

Mix well, label with initial and date. Stable for 1 year.

CAUTION: Corrosive, avoid contact and inhalation.

TECH: \_\_\_\_\_

DATE: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**WEIGERT'S HEMATOXYLIN**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_