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UMN TMCs Masson Trichrome for Collagen and Muscle

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BLS Histology[1,2]

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Cellular Senescence Net...

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We use this protocol and it's working

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Abstract

Purpose: Trichrome stains are frequently used to differentiate between collagen and smooth muscle in tumors and to identify increases in collagenous tissue in diseases such as cirrhosis of the liver.

Principle: Trichrome procedures are so named because three dyes which may or may not include the nuclear stain, are used. Sections are first stained with an acid dye, such as Biebrich Scarlet: all acidophilic tissue elements such as cytoplasm, muscle and collagen will bind the acid dyes. The sections are then treated with phosphotungstic and/or phosphomolybdic acid. Because cytoplasm is much less permeable than collagen, phosphotungstic and phosphomolybdic acids cause Biebrich scarlet to diffuse out of the collagen but not out of the cytoplasm. Phosphotungstic and phosphomolybdic acids have numerous acidic groups that most likely act as a link between the decolorized collagen and aniline blue, the collagen dye.

Guidelines

This protocol may require prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Materials

1. Bouin's Solution:
   - Picric Acid, saturated aqueous solution: 75mL
   - Formaldehyde, 37-40%: 25mL
   - Glacial acetic acid: 5mL

2. Weigert Iron Hematoxylin:
   - Working Solution: Mix equal parts of solutions A & B

   Solution A:
   - Hematoxylin: 10gm
   - Alcohol, 95%: 1000mL

   Solution B:
   - Ferric Chloride, 29% aqueous: 20mL
   - Distilled Water: 475mL
   - Glacial Acetic Acid: 5mL

3. Biebrich Scarlet-Acid Fuchsin Solution(400mL, 200mL, 100mL, 50mL):
   - Biebrich Scarlet, 1% Aqueous: 360mL, 180mL, 90mL, 45mL
   - Acid Fuchsin, 1% Aqueous: 40mL, 20mL, 10mL, 5mL
   - Glacial Acetic Acid: 5mL, 2.5mL, 1.25mL, .625mL

4. Phosphotungstic/phosphomolybdic Acid Solution:
   - Phosphotungstic Acid: 25gm
   - Phosphomolybdic Acid: 25gm
   - Distilled Water: 1000mL

5. Aniline Blue Solution (1000mL, 500mL, 100mL, 50mL): Make fresh each time
   - Aniline Blue: 25gm, 12.5gm, 2.5gm, 1.25gm
   - Glacial Acetic Acid: 20mL, 10mL, 2mL, 1mL
   - Distilled Water: 1000mL, 500mL, 100mL, 50mL

6. Acetic Acid Solution, 1%: Make fresh each time
   - Glacial Acetic Acid: 1mL
   - Distilled Water: 99mL

Introduction

1 Purpose: Trichrome stains are frequently used to differentiate between collagen and smooth muscle in tumors and to identify increases in collagenous tissue in diseases such as cirrhosis of the liver.

2 Principle: Trichrome procedures are so named because three dyes which may or may not include the nuclear stain, are used. Sections are first stained with an acid dye, such as Biebrich Scarlet: all acidophilic tissue elements such as cytoplasm, muscle and collagen will bind the acid dyes. The sections are then treated with phosphotungstic and/or phosphomolybdic acid. Because cytoplasm is much less permeable than collagen, phosphotungstic and phosphomolybdic acids cause Biebrich scarlet to diffuse out of the collagen but not out of the cytoplasm. Phosphotungstic and phosphomolybdic acids have numerous acidic groups that most likely act as a link between the decolorized collagen and aniline blue, the collagen dye.

3 Fixation: 10% NBF or Formal-saline*

4 Sections: Paraffin Sections at 4-5 microns

5 Control: Practically every tissue has an internal control, so no other controls are needed. However, if a control is desired, uterus, small intestine, or fallopian tube will provide good material.

Procedure

6 Deparaffinize and hydrate in water.

7 Rinse well in distilled water.

8 Mordant sections in Bouin solution overnight at room temperature.

9 Wash in running water until the yellow color disappears.

10 Rinse in distilled water.

11 Stain sections in working Weigert hematoxylin for 10 minutes.

12 Wash in running water for 10 minutes.

13 Rinse in distilled water.

14 Stain sections in Biebrich Scarlet-Acid Fuchsin solution for 2 minutes. If desired, solution may be kept for one more run only.

15 Rinse in distilled water.

16 Place slides in phosphotungstic/phosphomolybdic acid solution for 15 minutes. After 8 minutes replace with fresh solution and agitate. Discard solution.

17 Stain sections in Aniline blue for 5 minutes. Make fresh each time.

18 Rinse slides in distilled water.

19 Place slides in 1% acetic acid solution for 5 minutes. Discard solution.

20 Dehydrate, clear and coverslip.

Results

21 Nuclei: Black
Cytoplasm, Keratin, Muscle Fibers: Red
Collagen and Mucous: Blue