PATHOLOGY CORE

Special Stain Protocol: Trichrome

Purpose:
The purpose is to detect collagenous connective tissue fibers and differentiate between collagen and smooth muscle.

Principle:
The collagen fibers after treatment with phosphotungstic and phosphomolybdic acid will interact with the aniline blue dye to stain blue, the cytoplasm and muscle fibers interact with biebrich scarlet-acid fuchsin to stain red/pink, and solution A & B interact with the nuclei to stain brown/black.

Positive Control Tissue:
Skin tissue

Tissue Fixative:
10% Formalin fixed tissue

Reagents Required:
Picric Acid

- Vendor – Sigma Aldrich
- Catalog number – P6744-1GA

Hematoxylin (Solution A)

- Vendor – Sigma Aldrich
- Catalog number – H3136-100G

Ethanol (Solution A)

- Vendor – Epredia
- Catalog number – 6201

Ferric chloride (Solution B)

- Vendor – Sigma Aldrich
- Catalog number – 157740-1KG

Hydrochloric Acid (Solution B)

- Vendor - Fisher Chemical
- Catalog number – A144-212

Phosphomolybdic Acid

- Vendor - Sigma Aldrich
- Catalog number – A237-100

Phosphotungstic Acid

- Vendor – Sigma Aldrich
- Catalog number – 74690-500G

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Aniline Blue
- Vendor – Fisher Chemical
- Lot number – A967-25

Biebrich Scarlet
- Vendor – J.T. Baker
- Catalog number – C242-03

Acid Fuchsin
- Vendor – Fisher Chemical
- Catalog number – F97-25

Acetic Acid
- Vendor – Fisher Chemical
- Catalog number – A38-500

Solution Preparation:
Bouin's Fixative: 1500mL of Picric Acid + 500mL of Formalin + 100mL of Acetic Acid

29% Ferric Chloride: 1000mL of distilled water + 290 grams of Ferric Chloride

Solution A: 1000mL of 95% ethanol + 10 grams of hematoxylin

Solution B: 40mL of 29% ferric chloride + 950mL of distilled water + 10mL of hydrochloric acid

Phosphomolybdic/Phosphotungstic Acid Solution: 400mL of distilled water + 10 grams of phosphomolybdic acid + 10 grams of phosphotungstic acid

Aniline Blue Solution: 500mL of distilled water + 12.5 grams of aniline blue + 10mL of acetic acid

1% Acetic Acid Solution: 1000mL of distilled water + 10mL of acetic acid
(Solutions can be stored for 6 months)

Protocol:
1. Deparaffinize slides (xylene→distilled water)
2. Immerse slides in Bouin's solution and place in oven for 1 hour
3. Let slides cool under hood for 30 minutes
4. Wash slides with cold tap water for 2 minutes or until yellow color disappears
5. Rinse slides with distilled water
6. Immerse slides in equal parts of solution A and solution B for 10 minutes
7. Wash slides in running cold tap water for 10 minutes

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8. Rinse slides with distilled water
9. Immerse slides in biebrich scarlet-acid fuchsin for 2 minutes
10. Wash slides with distilled water
11. Immerse slides in phosphomolybdic/phosphotungstic acid solution for 25 minutes
    Note: DO NOT wash slides after this step
12. Immerse slides in aniline blue solution for 35 minutes
13. Wash slides with distilled water
14. Immerse slides in 1% acetic acid solution for 5 minutes
15. Rinse slides with distilled water
16. Dehydrate slides (5 dips in 70% ethanol, 5 dips in 80% ethanol, 5 dips in 95% ethanol, 5 dips in 100% ethanol, 5 mins in xylene)
17. Coverslip

Interpretation:
Collagen- Blue

Muscle Fibers- Red

Cytoplasm- Pink

Nuclei- Dark Brown to Black

References:

- Theory and Practice of Histotechnology