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Masson's Trichrome Staining Protocol for Collagen Fibers

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Description: This method is used for the detection of collagen fibers in tissues such as skin, heart, etc. on formalin-fixed, paraffin-embedded sections, and may be used for frozen sections as well. The collagen fibers will be stained blue and the nuclei will be stained black and the background is stained red.

Fixation: 10% formalin or Bouin's solution

Section: paraffin sections at 5 μ m.

Solutions and Reagents:

Weigert's Iron Hematoxylin Solution:

Stock Solution A:

Hematoxylin ----- 1 g
95% Alcohol ----- 100 ml

Stock Solution B:

29% Ferric chloride in water ----- 4 ml
Distilled water ----- 95 ml
Hydrochloric acid, concentrated ---- 1ml

Weigert's Iron Hematoxylin Working Solution:

Mix equal parts of stock solution A and B. This working solution is stable for 3 months (no good after 4 months)

Biebrich Scarlet-Acid Fuchsin Solution:

Biebrich scarlet, 1% aqueous ----- 90 ml

Acid fuchsin, 1% aqueous -----10 ml
 Acetic acid, glacial ----- 1 ml

Phosphomolybdic-Phosphotungstic Acid Solution:

5% Phosphomolybdic acid ----- 25 ml
 5% Phosphotungstic acid ----- 25 ml

Aniline Blue Solution:

Aniline blue ----- 2.5 g
 Acetic acid, glacial ----- 2 ml
 Distilled water ----- 100 ml

1% Acetic Acid Solution:

Acetic acid, glacial ----- 1 ml
 Distilled water ----- 99 ml

Procedure:

1. Deparaffinize and rehydrate through 100% alcohol, 95% alcohol 70% alcohol.
2. Wash in distilled water.
3. Stain in Weigert's iron hematoxylin working solution for 10 minutes.
4. Rinse in running warm tap water for 10 minutes.
5. Wash in distilled water.
6. Stain in Biebrich scarlet-acid fuchsin solution for 15 minutes. Solution can be saved for future use.
7. Wash in distilled water.
8. Differentiate in phosphomolybdic-phosphotungstic acid solution for 15 minutes or until collagen is not red.
9. Transfer sections directly (without rinse) to aniline blue solution and stain for 5-10 minutes. Rinse briefly in distilled water and differentiate in 1% acetic acid solution for 2-5 minutes.
10. Wash in distilled water.
11. Dehydrate very quickly through 95% ethyl alcohol, absolute ethyl alcohol (these step will wipe off Biebrich scarlet-acid fuchsin staining) and clear in xylene.
12. Mount with resinous mounting medium.

Results:

Collagen ----- blue
 Nuclei ----- black
 Muscle, cytoplasm, keratin ----- red
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Positive Controls:

Skin, lung, stomach, intestine.