**Masson's Trichrome Staining**

**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 5um

**Solutions and Reagents:**

Mayers or Weigert's iron hematoxylin

Biebrich Scarlet-Acid Fuchsin Solution:

      Biebrich scarlet, 1% aqueous --------- 225 ml

      Acid fuchsin, 1% aqueous --------------25 ml

      Acetic acid, glacial --------------------- 2.5 ml

Phosphomolybdic-Phosphotungstic Acid Solution:

     Phosphomolybdic acid ------------- 6.25 g

     Phosphotungstic acid -------------- 6.25 g

 Distilled Water ---------------------- 250 ml

Aniline Blue Solution:

      Aniline blue ------------------------------- 7.5 g

      Acetic acide, glacial --------------------- 5.0 ml

      Distilled water --------------------------- 250 ml

1% Acetic Acid Solution:

      Acetic acid, glacial ------------------- 2.5 ml

      Distilled water ------------------------ 247.5 ml

**Procedure:**

1. Dewax in Xylene – 2 mins
2. Dewax in Xylene – 2 mins
3. Dewax in Xylene – 2 mins
4. Wash in Absolute Alcohol – 2 mins
5. Wash in Absolute Alcohol – 2 mins
6. Wash in 90% Alcohol – 2 mins
7. Wash in 70% Alcohol – 2 mins
8. Wash in Running Water – 2 mins
9. Stain in Mayers or Weigert's iron hematoxylin working solution for 10 minutes.
10. Wash in distilled water 2 min.
11. Stain in Biebrich scarlet-acid fuchsin solution for 10-15 minutes. Solution can be saved for future use.
12. Wash in distilled water.
13. Differentiate in phosphomolybdic-phosphotungstic acid solution for 10-15 minutes or until collagen is not red.
14. Transfer sections directly (without rinse) to aniline blue solution and stain for 5-10 minutes.
15. Rinse briefly in distilled water and
16. Differentiate in 1% acetic acid solution for 2-5 minutes.
17. Wash in distilled water.
18. Dehydrate very quickly through 95% ethyl alcohol,
19. Absolute ethyl alcohol 10 -20 dips
20. Absolute ethyl alcohol 10 -20 dips
21. Absolute ethyl alcohol 10 -20 dips
22. Clear in xylene for 2 mins
23. Clear in xylene for 2 mins
24. Clear in xylene for 2 mins
25. Mount with resinous mounting medium.

**Results:**

      Collagen ---------------------------------------- blue

      Nuclei ------------------------------------------- black

      Muscle, cytoplasm, keratin ------------------- red