

Masson's Trichrome Stain

- 1.) Hydrate slides in deionized H₂O.
- 2.) Fix slides in Bouin's fluid for one hour at 56degrees Celsius
- 3.) Cool slides in Bouin's fluid for 5-10 minutes.
- 4.) Wash slides in running tap water until sections are colorless (approximately 5 minutes)
- 5.) Rinse in deionized water.
- 6.) Place slides in Weigert's Hematoxylin for 10 minutes. Rinse in distilled water.
- 7.) Wash in tap water for 3 minutes. Rinse in distilled water.
- 8.) Place slide in Biebrich scarlet-acid fuchsin solution for 15 minutes.
- 9.) Rinse off excess stain in distilled water.
- 10.) Place slides in phosphotungstic-phosphomolybdic acid solution for 15 minutes.
Discard solution.
- 11.) Transfer directly (no water rinse) to the 2.5% aniline blue solution for 10 minutes.
Filter after using (aniline blue can be recycled).
- 12.) Rinse off excess dye in deionized water.
- 13.) Place slides in 1% acetic acid for 1 minute. Discard solution.
- 14.) Dehydrate, clear in xylene and coverslip using a synthetic mounting medium.

RESULTS

Nuclei- black

Collagen, mucin- blue

Cytoplasm, keratin, muscle fibers- red

REAGENTS

Weigert's Solution A (stock)

Hematoxylin	10gm
95% reagent alcohol	1000mL

Weigert's Solution B (stock)

29% Ferric chloride aq.	40mL
Double distilled H ₂ O	950mL
HCl, conc.	10mL

Weigert's Iron Hematoxylin (working)

Weigert's Solution A	20cc.
Weigert's Solution B	20cc.

Phosphotungstic/Phosphomolybdic (working)

2.5% Phosphomolybdic acid, aq.	20cc.
2.5% Phosphotungstic acid, aq.	20cc.

Biebrich Scarlet- Acid Fuchsin (working)

Biebrich scarlet, 1% aq.	90mL
Acid Fuchsin, 1% aq.	10mL
Acetic acid, glacial	1mL