

Modified Verhoeff Elastic-Masson Trichrome Stain – Technical Memo

SOLUTIONS:

	250 ml	500 ml	1 Liter	1 Gallon
Picric Acid, Saturated Alcoholic or Bouin Fluid		Part 1337A	Part 1337B	Part 1337C
Ferric Chloride 10%, Aqueous	Part 10856A	Part 10856B	Part 1020A	Part 1020B
Hematoxylin 5%, Alcoholic		Part 11623B	Part 11623C	
Iodine, Weigert & Lugol, Aqueous		Part 12092A	Part 12092B	
Biebrich Scarlet-Acid Fuchsin Stain, Elastic-Trichrome, Aqueous	Part 1016A	Part 1016B		
Phosphomolybdic-Phosphotungstic Acid, Aqueous	Part 1332A	Part 1332B	Part 1332C	
Aniline Blue Stain, Aqueous	Part 10072B	Part 10072C		
Acetic Acid 1%, Aqueous		Part 10012A		

Additionally Needed:

Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Modified Verhoeff Elastic-Masson Trichrome Stain combines elastic and trichrome staining techniques for demonstration and clear definition of elastic fibers of all sizes, connective tissue and nuclei in a single tissue section. This procedure can be useful for identification of normal tissue morphology as well as heart, liver, lung and kidney pathologic conditions.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)

Technique: Paraffin sections cut at 5 microns

Solutions: All solutions manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

STAINING PROCEDURE:

- Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - See Procedure Notes #1 and #2.
- Mordant in Picric Acid, Saturated Alcoholic (1337) for 5 minutes or Bouin Fluid (1020) at 56° C for 1 hour.
 - See Procedure Notes #3 and #4.
 - Bouin Fluid mordant; Cool at room temperature for 5-10 minutes before proceeding.
 - Skip Step #2 if tissue was originally Bouin fixed.
- Wash well in running tap water; rinse in distilled water.
- Prepare Verhoeff Working Solution:

a. Hematoxylin 5%, Alcoholic (11623)	20 ml
b. Ferric Chloride 10%, Aqueous (10856)	12 ml
c. Iodine, Weigert & Lugol, Aqueous (12092)	8 ml
- Stain slides in Verhoeff Working Solution for 15 minutes.
- Rinse in several changes of tap water.
- Prepare fresh Ferric Chloride 2%, Aqueous.

a. Ferric Chloride 10%, Aqueous	10 ml
b. Distilled Water	40 ml
- Differentiate each slide individually in fresh Ferric Chloride 2%, Aqueous with agitation; 2-10 dips.
 - Check differentiation; rinse well in tap water and check microscopically for black elastic staining with gray background. Repeat in Ferric Chloride 2%, Aqueous until desired elastic differentiation is achieved.
- Wash well in running tap water.

- Stain in Biebrich Scarlet-Acid Fuchsin Stain, Elastic-Trichrome, Aqueous (1016) for 3 minutes.
- Rinse in distilled water for 10 minutes.
- Differentiate slides in Phosphomolybdic-Phosphotungstic Acid, Aqueous (1332) for 15 minutes.
 - Check slides microscopically until collagen is colorless but muscle remains red.
- Transfer directly into Aniline Blue Stain, Aqueous (10072) for 3 minutes.
- Differentiate in Acetic Acid 1%, Aqueous (10012) for 3 minutes.
- Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Elastin	Blue-black
Muscle, keratin & cytoplasm	Red
Collagen	Blue
Nuclei	Red-brown to blue-black

PROCEDURE NOTES:

- Drain staining rack/slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during staining procedure.
- The use of Picric Acid, Saturated Alcoholic mordant will reduce overall staining time. Bouin Fluid mordant requires longer exposure but will enhance Biebrich Scarlet-Acid Fuchsin staining (Step #10).
- Dispose of Picric Acid, Saturated Alcoholic or Bouin Fluid as hazardous waste and/or according to local and state environmental regulations.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

- Dapson, Janet Crookham, and Richard Dapson. *Hazardous Materials in the Histopathology Laboratory: Regulations, Risks, Handling, and Disposal*. 4th ed. Battle Creek, MI: Anatech, 2005. 150, 265-266.
- Garvey, Winsome. "Modified Elastic Tissue-Trichrome Stain." *Stain Technology* 59.3 (1984): 213-216.
- Landas, Steve, M.T. Maher Strum and Karen Ellison. "Rapid Convenient Elastochrome Stain." *The Journal of Histotechnology* 14.3 (1991): 191-192.
- Modifications developed by Newcomer Supply Laboratory.