

## Modified Verhoeff Elastic-Masson Trichrome Stain – Technical Memo

### SOLUTIONS:

Biebrich Scarlet-Acid Fuchsin Stain, Elastic-Trichrome, Aqueous	250 ml Part 1016A	500 ml Part 1016B
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### Additionally Needed:

Picric Acid, Saturated Alcoholic	Part 1337	or Bouin Fluid	Part 1020
Ferric Chloride 10%, Aqueous	Part 10856		
Hematoxylin 5%, Alcoholic	Part 11623		
Iodine, Lugol's, Aqueous	Part 12092		
Phosphomolybdic-Phosphotungstic Acid, Aqueous	Part 1332		
Aniline Blue Stain, Aqueous	Part 10072		
Acetic Acid 1%, Aqueous	Part 10012		
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 for demonstration and definition of elastic fibers of all sizes, connective tissue and nuclei in a single tissue section. This procedure is useful in identifying 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 4 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 provided staining procedure.

### 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 Note #3.
  - 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, Lugol's, 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 Ferric Chloride 2%, Aqueous with agitation; 2-10 dips.
  - Check differentiation: rinse well in tap water, check microscopically for black elastic staining with gray background.
  - If needed, 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 for 3 minutes.

- Rinse in distilled water for 10 minutes.
- Differentiate in Phosphomolybdic-Phosphotungstic Acid, Aqueous (1332) for 15 minutes.
  - Until collagen is colorless but muscle remains red.
- Transfer 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 slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during procedure.
- The use of:
  - Picric Acid, Saturated Alcoholic will reduce staining time.
  - Bouin Fluid requires longer exposure but enhances Biebrich Scarlet-Acid Fuchsin staining (Step #10).
- 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.