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Trichrome Stain, Masson, Light Green - Technical Memo

SOLUTIONS:	250 ml	500 ml	1 Liter	1 Gallon
Bouin Fluid			Part 1020A	Part 1020B
Hematoxylin Stain Set, Weigert Iron		Part 1409B	Part 1409A	
Biebrich Scarlet-Acid Fuchsin Stain, Aqueous	Part 10161B	Part 10161C		
Phosphotungstic Acid 5%, Aqueous	Part 13345A	Part 13345B		
Light Green SF Yellowish Stain 2%, Aqueous	Part 1221A	Part 1221B		
Acetic Acid 0.5%, Aqueous		Part 100121A	Part 100121B	

Additionally Needed:

Trichrome, Liver Control Slides	Part 4690	or	Trichrome, Multi-Tissue Control Slides	Part 4693
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			
Coplin Jar, Plastic	Part 5184 (for microwave modification)			

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

APPLICATION:

Newcomer Supply Trichrome Stain, Masson, Light Green procedure, with included microwave modification, is used to differentially demonstrate connective tissue elements, collagen and muscle fibers.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns
a. *See Procedure Note #1.*
Solutions: All solutions are 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:

1. Preheat Bouin Fluid to 56-60°C in oven or water bath.
   (Skip if using overnight method or microwave procedure.)
2. 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.
   a. *See Procedure Notes #2 and #3.*
3. Mordant in preheated Bouin Fluid for one hour at 56-60°C or overnight at room temperature. Cool at room temperature for 5-10 minutes.
   a. *Skip Step #3 if tissue was originally Bouin fixed.*
   Microwave Modification: *See Procedure Note #4.*
   b. *Place slides in a plastic Coplin jar containing Bouin Fluid and microwave for 5 minutes at 60°C. Allow slides to sit an additional 10 minutes in solution.*
4. Wash well in running tap water; rinse in distilled water.
5. Prepare fresh Weigert Iron Hematoxylin; combine and mix well.
   a. *Solution A: Ferric Chloride, Aqueous* *20 ml*
   b. *Solution B: Hematoxylin 1%, Alcoholic* *20 ml*
6. Stain slides in fresh Weigert Iron Hematoxylin for 10 minutes.
7. Wash in running tap water for 10 minutes; rinse in distilled water.
   a. *See Procedure Note #5.*
8. Place slides in Biebrich Scarlet-Acid Fuchsin Stain, Aqueous for 2 minutes.
9. Rinse in distilled water.
10. Place slides in Phosphotungstic Acid 5%, Aqueous for 5 minutes.
11. Transfer slides directly into Light Green SF Yellowish Stain 2%, Aqueous for 5-6 minutes, depending on stain intensity preference.
12. Rinse in distilled water.
13. Place slides in Acetic Acid 0.5%, Aqueous for 2 quick dips.
14. 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:

Collagen and mucin	Green
Muscle fibers, cytoplasm and keratin	Red
Nuclei	Blue/black

PROCEDURE NOTES:

1. Using ammonium hydroxide to soak or face tissue blocks will alter the pH of tissue sections and greatly diminish trichrome staining.
2. Drain staining rack/slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during staining procedure.
4. The suggested microwave procedure has been tested at Newcomer Supply using an “EB Sciences”, 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer’s instructions, to prevent exposure to chemical vapors.
5. If Weigert Iron Hematoxylin is not completely washed from tissue sections, nuclear and cytoplasmic staining will be compromised.
6. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Brown, Richard. *Histologic Preparations: Common Problems and Their Solutions*. Northfield, Ill.: College of American Pathologists, 2009. 95-101.
2. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text.* 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 162-165.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 191-192.
4. Vacca, Linda L. *Laboratory Manual of Histochemistry*. New York: Raven Press, 1985. 308-310.
5. Modifications developed by Newcomer Supply Laboratory.