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Part 10072
Revised June 2015

Trichrome Stain, Masson, Aniline Blue - Technical Memo

SOLUTION:	**250 ml**		**500 ml**	
Aniline Blue Stain, Aqueous	Part 10072B		Part 10072C	
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			
Bouin Fluid	Part 1020			
Hematoxylin Stain Set, Weigert Iron	Part 1409			
Biebrich Scarlet-Acid Fuchsin Stain, Aqueous	Part 10161			
Phosphomolybdic-Phosphotungstic Acid, Aqueous	Part 1332			
Acetic Acid 0.5%, Aqueous	Part 100121			
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, Aniline Blue procedure, with included microwave modification, is used to differentially demonstrate connective tissue elements, including collagen and muscle fibers.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns
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. 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 #1 and #2.*
2. Mordant in 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 #2 if tissue was originally Bouin fixed.*

    Microwave Modification: *See Procedure Note #3.*
    b. *Place slides in a plastic Coplin jar containing Bouin Fluid and microwave for 5 minutes at 60°C.*
3. Wash well in running tap water; rinse in distilled water.
4. 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*
5. Stain slides in fresh Weigert Iron Hematoxylin for 10 minutes.
6. Wash in running tap water for 10 minutes; rinse in distilled water.
    a. *See Procedure Note #4.*
7. Place slides in Biebrich Scarlet-Acid Fuchsin Stain, Aqueous for 2 minutes.
8. Rinse in distilled water.
9. Place slides in Phosphomolybdic-Phosphotungstic Acid, Aqueous for 10 to15 minutes.
10. Transfer slides directly into Aniline Blue Stain, Aqueous for 5 minutes.
11. Rinse in distilled water.
12. Place slides in Acetic Acid 0.5%, Aqueous for 3 to 5 minutes.
13. 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	Blue
Muscle fibers, cytoplasm and keratin	Red
Nuclei	Blue/black

PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. 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.
4. If Weigert Iron Hematoxylin is not completely washed from tissue section, nuclear and cytoplasmic staining may be compromised.
5. 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.

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Part 10072
Revised June 2015

Trichrome Stain, McLetchie, Aniline Blue - Technical Memo

SOLUTION:	**250 ml**		**500 ml**	
Aniline Blue Stain, Aqueous	Part 10072B		Part 10072C	
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			
Biebrich Scarlet-Acid Fuchsin Stain, Aqueous	Part 10161			
Iodine, Weigert & Lugol, Aqueous	Part 12092			
Phosphotungstic Acid 2%, Alcoholic	Part 13342			

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

APPLICATION:

Newcomer Supply Trichrome Stain, McLetchie, Aniline Blue procedure is useful for the demonstration of collagen and muscle fibers, has excellent staining results with bone marrow and renal biopsies and provides time effective trichrome results. This modified protocol differs from a standard trichrome procedure by not using a Bouin Fluid mordant or a hematoxylin nuclear stain.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns
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. 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 #1 and #2.*
2. Place slides in Biebrich Scarlet-Acid Fuchsin Stain, Aqueous for 5 minutes.
3. Rinse slides in several changes of distilled water.
4. Place slides in Iodine, Weigert & Lugol, Aqueous for 2 minutes.
5. Rinse slides in several changes of distilled water.
6. Differentiate slides one at a time in Phosphotungstic Acid 2%, Alcoholic for 15-30 seconds. Gently agitate slides once.
    a. *To deter over-differentiation do not exceed the 30 second timing in Phosphotungstic Acid 2%, Alcoholic.*
    b. *If sections are over-differentiated, wash slides well in distilled water and repeat Steps #2 through #6.*
7. Rinse slides immediately in several changes of distilled water.
8. Place slides in Aniline Blue Stain, Aqueous for 1-3 minutes.
9. Rinse slides in several changes of distilled water.
10. 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	Blue
Muscle fibers, cytoplasm and keratin	Magenta to red
Nuclei	Dark red

PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. The nuclear detail within this method is dark red with crisp definition.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida, *Histotechnology: A Self-Instructional Text*. 2nd ed. Chicago: ASCP Press, 1997. 134-136.
2. McLetchie, Norman G.B. "Trichrome McLetchie Modification". Laboratory Procedure: Lakes Region General Healthcare, Laconia, NH.
3. Modifications developed by Newcomer Supply Laboratory.