

Trichrome, Multi-Tissue Control Slides – Technical Memo

CONTROL SLIDES:	Part 4693A	Part 4693B
	10 Slide/Set	98 Slide/Set

Trichrome, Multi-Tissue Control Slides contain sections of positive staining liver, positive staining kidney and positive staining uterus.

PRODUCT DESCRIPTION:

The enclosed positive control slides are intended to be used to verify histological techniques and reagent reactivity. These slides are to be used for the qualitative purpose of determining positive or negative results, and are not intended to be used for any quantitative purpose. The first serial section within the control box is stained and provided for your reference. **Before using the unstained slides, review the enclosed stained slide with your pathologist to ensure that this tissue source is acceptable. Newcomer Supply will not accept a return with missing slides in the series. Newcomer Supply guarantees reactivity of these control slides for one year from the date of receipt. Revalidate after one year to verify continued reactivity. Store at 15-30°C in a light deprived and humidity controlled environment.**

These positive control slides were produced from human surgical or autopsy tissues under carefully controlled conditions. Reasonable measures are used to deliver quality control slides that are as consistent as possible. However, characteristics of quality control slides may be dissimilar due to variations in the reagents, stains, techniques, laboratory conditions, and tissue sources used. Newcomer Supply Laboratory uses a manual method of performing quality control procedures, specifically avoiding automation, in order to provide reactive control slides for even less aggressive methods of staining that our customers may be using.

CONTROL SLIDE VALIDATION:

With Trichrome, Gomori One-Step, Aniline Blue Stain Kit:	Part 9176B/A	Individual Stain Solution
Solution A: Bouin Fluid	250/500 ml	Part 1020
Solution B: Ferric Chloride, Acidified	125/250 ml	Part 1409
Solution C: Hematoxylin 1%, Alcoholic	125/250 ml	Part 1409
Solution D: Trichrome Stain, Gomori One-Step, Aniline Blue	250/500 ml	Part 1403
Solution E: Acetic Acid 0.5%, Aqueous	250/500 ml	Part 100121

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

APPLICATION:

Newcomer Supply Trichrome, Multi-Tissue Control Slides, use a combination of tissue sources for the positive histochemical staining of connective tissue and to differentially demonstrate collagen and muscle fibers.

METHOD:

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

Technique: Paraffin sections cut at 5 microns on Superfrost® Plus
a. See Procedure Note #1 (page 2).

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

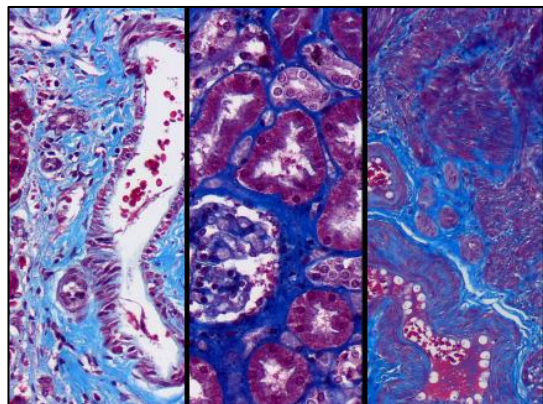
NEWCOMER SUPPLY VALIDATION PROCEDURE:

- Preheat Solution A: Bouin Fluid to 56-60°C in oven or water bath. **(Skip if using overnight method or microwave 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.
a. See Procedure Notes #2 and #3 (page 2).
 - Mordant in preheated Solution A: 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 (page 2).
- Place slides in a plastic Coplin jar containing Solution A: Bouin Fluid and microwave for 5 minutes at 60°C. Allow slides to sit an additional 10 minutes in solution.
 - Wash well in running tap water; rinse in distilled water.
 - Prepare fresh Weigert Iron Hematoxylin; combine and mix well.
 - Solution B: Ferric Chloride, Acidified 20 ml
 - Solution C: Hematoxylin 1%, Alcoholic 20 ml
 - Stain slides in fresh Weigert Iron Hematoxylin for 10 minutes.

- Wash in running tap water for 10 minutes; rinse in distilled water.
a. See Procedure Note #5 (page 2).
- Stain in Solution D: Trichrome Stain, Gomori One-Step, Aniline Blue for 20 minutes.
a. Thinner sections may require additional staining time.
- Directly differentiate in Solution E: Acetic Acid 0.5%, Aqueous for 2 minutes.
- Rinse quickly in distilled water.
- 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. 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 may be compromised.
6. Newcomer Supply Trichrome, Multi-Tissue Control Slides are validated with Trichrome Stain Kit, Gomori One-Step, Aniline Blue but can also be used as positive controls with the end users Trichrome stain procedure of preference.
7. 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. 165-166.
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.