

Trichrome Stain Kit, McLetchie, Aniline Blue Technical Memo

KIT INCLUDES:

	<u>250ml kit</u>	<u>500ml kit</u>
Solution A: Biebrich Scarlet-Acid Fuchsin Stain Solution	250ml	500ml
Solution B: Iodine, Weigert & Lugol	250ml	500ml
Solution C: Phosphotungstic Acid 2% Alcoholic	250ml	500ml
Solution D: Aniline Blue Stain Solution	250ml	500ml

****2 Complimentary Positive Control Slides****

Kit Bottles are **not available** to be purchased individually.

Component bottles may be available under separate catalog numbers.

****The positive control slides are submitted on a section of kidney to demonstrate the differentiation of this technique as opposed to traditional methods.****

APPLICATION:

Trichrome staining is useful in the demonstration of collagen and muscle. Control slides traditionally used for this stain are arteries, kidney, liver and uterus. This procedural modification removes the need to mordant tissue in Bouin Fluid. It is time effective with the staining technique being completed within 15 minutes. **The nuclei stain a dark red as opposed to blue-black in the traditional methods. Clear differentiation should be apparent with this technique.**

METHOD:

1. Deparaffinize and hydrate slides through graded ethyl alcohols to distilled water.
2. Place slides in Solution A, Biebrich Scarlet-Acid Fuchsin Stain Solution for 5 minutes.
3. Rinse slides with several changes of distilled water. (Don't use tap water, see procedure note #2.) Color should be bright red.
4. Place slides in Solution B, Iodine, Weigert & Lugol for 2 minutes.
5. Rinse slides with several changes of distilled water. Color turns orange-red.
6. Place slides in Solution C, Phosphotungstic Acid, 2% Alcoholic, for 15- 30 seconds. Gently agitate slides once. Remove within 30 seconds or it will decolorize the slides completely and the staining will have to be repeated starting with step #2. (See procedure note #3.)
7. Immediately rinse slides with several changes of distilled water to deactivate phosphotungstic acid.

8. Place Slides in Solution D, Aniline Blue Stain Solution for 1-3 minutes.
9. Rinse slides in several changes of distilled water.
10. Dehydrate and clear slides through graded ethyl alcohols, clear in xylene or substitute and coverslip with a compatible mounting medium.

RESULTS:

Cytoplasm, keratin and muscle fibers:	Magenta to Red
Collagen:	Blue
Nuclei:	Dark Red

PROCEDURE NOTES:

1. Recommendations include: Do not reuse solutions and do not use dropper method.
2. Biebrich Scarlet is not miscible in tap water. It turns gummy.
3. The Iodine Solution replaces the Bouin Fluid as the mordant. The placement of the Iodine Solution is important, as it allows the Biebrich Scarlet-Acid Fuchsin to remain red in color.
4. Establish your labs decolorization timing by running slides one at a time. Some labs have reported timing at this step as short as 5-10 seconds. Once the decolorization goes too far, you will have to rinse the Phosphotungstic Acid out of the tissue and start over with the Biebrich Scarlet step.
5. If you leave your slides in the Phosphotungstic Acid solution for any longer than 30 seconds it **will draw out** too much Biebrich Scarlet-Acid Fuchsin color and make the muscle and cytoplasm appear very pale in color.
6. The nuclear detail within this method is a dark red with very crisp definition. No glacial acetic acid rinse is needed.
7. This procedure is particularly excellent for bone marrow and renal biopsies as no overstaining occurs.
8. Timings should be adjusted at steps #6 and #8 for small core biopsies.

REFERENCES:

1. **Dr. Norman GB McLetchie** from LRG Healthcare, Laconia, NH procedure manual.
2. **“Histotechnology: A Self-Instructional Text”**, Carson, F., 2nd Ed., 1997, pp. 134-136.
3. Modifications and revisions have been made by Newcomer Supply, Inc.

COMPLIMENTARY POSITIVE CONTROL SLIDES:

Enclosed with this kit are two complimentary positive control slides to be used for the initial verification of staining techniques and reagents. We suggest running one slide along with your in-house positive control slide for the first run. Retain the second slide for further troubleshooting, if needed.

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