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Part 1400
February 2016

Sudan IV Stain, Herxheimer Alcoholic - Technical Memo

SOLUTIONS:	**125 ml**
Sudan IV Stain, Herxheimer Alcoholic	Part 1400A

Additionally Needed:

Alcohol, Ethyl Denatured, 70%	Part 10844			
Hematoxylin Stain, Mayer Modified	Part 1202			
Lithium Carbonate, Saturated Aqueous	Part 12215	or	Scott Tap Water Substitute	Part 1380
Mount-Quick Aqueous Mounting Medium	Part 6271A			

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

APPLICATION:

Newcomer Supply Sudan IV Stain, Herxheimer Alcoholic procedure is used for identification of fat/lipid in frozen sections. Herxheimer method refers to an acetone/alcohol solvent mixture; the acetone component of this solution may dissolve out small amounts of lipid.

Sudan dyes are a group of fat/lipid soluble solvent dyes, also known as lysochromes. These solvent dyes readily stain fat/lipid due to the fact that the dyes are more soluble in lipid than in the solvents from which they are applied.

METHOD:

Fixation: Fresh tissue or formalin fixed unprocessed tissue
- *a. See Procedure Note #1.*

Technique: Frozen sections cut at 8 microns on adhesive slides
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. Fix frozen section slides in Formalin 10%, Phosphate Buffered for 1 minute.
   - *a. See Procedure Note #2.*
2. Rinse sections carefully in two changes of distilled water.
3. Rinse in Alcohol, Ethyl Denatured, 70% (10844).
4. Stain in Sudan IV Stain, Herxheimer Alcoholic for 10 minutes.
   - *a. Keep tightly capped to avoid evaporation.*
5. Differentiate quickly in Alcohol, Ethyl Denatured, 70% to remove excess stain.
6. Wash thoroughly in distilled water.
7. Counterstain with Hematoxylin Stain, Mayer Modified (1202) for 2-3 minutes, depending on preference of nuclear stain intensity.
8. Wash gently in several changes of tap water.
9. Blue slides in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 10 dips.
   - *a. See Procedure Note #3.*
10. Wash gently in several changes of tap water.
11. Blot excess water from slide; coverslip with Mount-Quick Aqueous Mounting Medium.
    - *a. See Procedure Note #4.*

RESULTS:

Fat	Orange/red
Nuclei	Blue

PROCEDURE NOTES:

1. To freeze formalin fixed unprocessed tissue post-fixation: Place specimen in tissue cassette, wash in running water for 5 minutes. Remove tissue from cassette and blot well removing all excess water from tissue. Freeze tissue (fresh or formalin fixed) according to laboratory protocol.
2. Frozen formalin fixed tissue does not require an additional formalin fixation step.
3. The use of a bluing agent in this procedure is an optional step.
4. Use minimal pressure when applying coverslip or fat/lipid staining may be disturbed. To remove trapped air bubbles under coverslip; soak slide in warm water until coverslip can easily be removed. Blot excess water from slide and remount with new coverslip and Mount-Quick Aqueous Mounting Medium.

REFERENCES:

1. Culling, C. F. A. *Handbook of Histopathological and Histochemical Techniques: (including Museum Techniques)*. 3rd ed. London: Butterworth, 1974. 359-362.
2. Kiernan, J. A. *Histological and Histochemical Methods: Theory and Practice*. 3rd ed. London, Ontario: Arnold, 2003. 251-254.
3. Lillie, R. D., and Harold Fullmer. *Histopathologic Technic and Practical Histochemistry*. 4th ed. New York: McGraw-Hill, 1976. 565-568.
4. Modifications developed by Newcomer Supply Laboratory.
