**Sudan IV Stain, Herxheimer Alcoholic - Technical Memo**

**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

**Technical:** 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:**

<table>
<thead>
<tr>
<th>Fat</th>
<th>Orange/red</th>
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</thead>
<tbody>
<tr>
<td>Nuclei</td>
<td>Blue</td>
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</tbody>
</table>

**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:**


4. Modifications developed by Newcomer Supply Laboratory.