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Histology Special Stain - Oil Red O (ORO) - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of neutral lipids in tissue sections. Fat may occur under normal or abnormal pathology. Muscle has lipids in them normally. The amounts may vary, depending upon diseases and the overall well-being of the patient. Fatty emboli and degenerating myelin can be demonstrated. Tumors arising from fat cells (liposarcomas) can also be differentiated from other types of tumors.

II. PRINCIPLE:

The reaction is a process of adsorption. The dyes are more soluble in the lipid of the tissue than in the solvent in which they are dissolved. The lipids should be in a liquid or semi-liquid state. A solid or crystalline lipid will not be stained. The oil red O reagent is prepared by combining oil-soluble dye in isopropanol. The isopropanol is preferred over ethanol, as the isopropanol removes less of the lipid material from the tissue. The tissue must be a frozen section, as routine processing will extract the lipid from the tissue. Formalin fixes the tissue, stabilizing the protein around the lipid droplets, and thereby stabilizing the lipid. Hematoxylin is used to stain the nuclei.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Fresh or fixed tissue that has been frozen.
    2. 10% neutral buffered formalin preferred if fixed.

B. Processing

1. Frozen tissue or standard, overnight processing.

C. Section Thickness
    1. Cut frozen sections at 10µm.

D. Slide Drying
    1. N/A

E. Type of Slide
    1. Plus slides

IV. REAGENTS:

A. **Stock Oil Red O**

Oil red O	**0.5 gm**
99% Isopropanol	**100.0 mL**

Dissolve together. Stable at room temperature for months; discard after use.

B. **Working Oil Red O Solution**

Stock Oil red O	**30.0 mL**
Distilled water	**20.0 mL**

Mix together just before use. Let stand 10 – 15 minutes. Filter. Stable for 1-2 hours

C. **Hematoxylin**
Use hematoxylin from routine H&E set-up. Gill or Mayer preferable.

D. **Dilute Ammonia Water**
Use dilute ammonia water from routine H&E set up.

V. EQUIPMENT:

A. Balance

B. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Coplin jars

D. Funnel

E. Filter paper

VII. QUALITY CONTROL:

Frozen section of adrenal

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Oil Red O
    1. Is an irritant.

B. Isopropyl Alcohol
    1. Is a flammable liquid and vapor.

C. Hematoxylin
    1. Is incompatible with oxidizers and alkalies.
    2. Store separately from these.

D. Ammonium Hydroxide
    1. May cause severe skin and eye burns.
    2. Vapors irritating to eyes and respiratory tract. Harmful if swallowed or inhaled.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Place slides in 10% neutral buffered formalin.	5 minutes	
2	Wash slides in running tap water.	5 minutes	
3	Stain slides in WORKING oil red O.	15 minutes	At room temperature. Oil Red O Working solution should be made just before use and used only one day.
4	Rinse in warm running tap water.	1 minute	
5	Rinse in distilled water.	1 minute	
6	Counterstain slides in Mayer or Gill Hematoxylin.	3 minutes	
7	Rinse slides in running tap water.	30 seconds	
8	Dip slides in dilute ammonia water.	2-3 seconds	
9	Wash in running tap water.	5 minutes	
10	Mount with aqueous mounting media. Ring coverslip with clear fingernail polish.		Any commercial aqueous mounting media may be used, as may Apathy gum syrup or glycerin jelly. Do NOT press on the coverslip. This may dislodge the lipid.

X. LIMITATIONS:

A. The following may influence the validity of test results:

1. If the tissue has been fixed previous to sectioning, the tissue should be washed in running water for at least 30 minutes before sectioning. This will remove excess formalin from the tissue. Fixed tissue should be sectioned at a slightly warmer temperature (-10 to 15 degrees C). If the tissue has been fixed previous to sectioning, there is no need to place the slide into formalin. Start at Step 2.
2. Fix tissue, either before sectioning or before staining, in 10% neutral buffered formalin.
3. Avoid fixatives containing alcohol (extracts lipid), metals or chromates (corrodes cryostat).

XI. RESULTS:

A. Lipids (Triglycerides and Neutral fats) - **red**

B. Nuclei - **blue**

XII. REFERENCES:

A. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.

B. Carson FL: Histotechnology: A Self-Instructional Text. Chicago, IL, ASCP Press, 1990.

C. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

D. Vacca L: Laboratory Manual of Histochemistry. New York, NY, Raven Press, 1985.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak