OLIVE VIEW-UCLA MEDICAL CENTER
DEPARTMENT OF PATHOLOGY
POLICY & PROCEDURE

NUMBER: 11906
VERSION: 1

SUBJECT/TITLE: SS 013 - OIL RED O STAINING PROCEDURE

POLICY: The Oil Red O staining procedure shall be performed at the request of the pathologist.

PURPOSE: To demonstrate neutral lipids in frozen tissue sections.

DEPARTMENTS: PATHOLOGY & LABORATORY SERVICES

DEFINITIONS:

PROCEDURE:

FIXATIVE

10% neutral buffered formalin. No alcoholic fixatives should be used.

EQUIPMENT

Cryostat Coplin jars Filter paper
Erlenmeyer flasks Graduated Cylinders
Portable Scale w/ Weigh Boats
Portable Stirring Plate w/ Stir Bars

SLIDE PREPARATIONS

Cut frozen sections at 10 microns. Paraffin sections **cannot** be utilized as dehydrating and clearing agents dissolve the fat.

QUALITY CONTROL

Most tissue contains some fat, but a touch prep made with fatty tissue can also be used.

REAGENTS

I. Stock Solutions

- Oil Red O Stock Solution
  Oil Red O……..2.5 gm
  99% Isopropanol Alcohol…….500 ml

Mix Well

- 60% Isopropanol Alcohol
  Isopropanol Alcohol…….60 ml
  Distilled Water……..40 ml

II. Working Solutions

- Oil Red O Stock Solution……24 ml
- Distilled Water…….16 ml

Mix well and allow to stand for 10 minutes and afterwards filter the solution.

III. Pre-made Solutions

- Lithium Carbonate
- Gill Hematoxylin

PROCEDURE

1. Cut frozen sections at 10 microns. Fix in 37-40% formaldehyde (conc.) for 1 minute.
2. Wash well in tap water. Blot off excess water.
3. Stain sections in oil red O for 10 minutes.
4. Wash sections in tap water. Check under the microscope.
5. Differentiate briefly in 60% Isopropanol if fat is smeared on top of section.
6. Wash sections in tap water.
7. Counterstain sections in Gill hematoxylin for 1 minute.
8. Wash sections well in tap water.
9. Blue in Lithium Carbonate.
10. Wash sections well in tap water.
11. Mount section with an aqueous mounting medium.

Note: The fat in the section is relatively liquid and mobile, so care should be taken that no pressure is placed on the coverglass or the fat may be displaced. If air bubbles are present in the section, remove the coverglass by soaking the slide in warm water.

RESULTS:

Fat……. Intense Red
Nuclei ……..……..Blue

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<tr><td colspan="2">References: Freida L. Carson, PhD, HT (ASCP), Histotechnology, A Self-Instructional Text, ASCP Press, ASCP , Chicago, 1997</td></tr>
<tr><td>Approved by: Holli Mason (Laboratory Director)</td><td>Date: 05/22/2020</td></tr>
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