**UW Medicine - Pathology**

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Oil Red-O Stain in Propylene Glycol Method for Demonstration of Simple Fat Procedure

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PURPOSE

To identify the method for performing the special stain of Oil Red-O Stain in propylene glycol method for demonstration of simple fat.

PROCEDURE

**Fixation:**

10% buffered neutral formalin.

**Sectioning:**

Frozen sections at 6 microns.

**Solutions:**

(Use Type II de-ionized water for all solution preparation.)

**Oil Red-O Solution**

Richard Allan Scientific

**85% Propylene Glycol**

Propylene glycol, absolute 42.5 ml

Distilled water 7.5 ml

Discard after use.

**Harris’s Hematoxylin**

Richard Allan Scientific

**Bluing Solution**

Richard Allan Scientific

**Procedure:**

Use control slide.

1. Cut frozen sections and attach to warm slide.
2. Fix in 10% buffered neutral formalin for at least ten minutes.
3. Wash in tap water for five minutes and drain slides.
4. Absolute propylene glycol for 5 minutes.
5. Place in Oil Red O solution, put in 60°C oven for 1 hour
6. Differentiate in 85% propylene glycol for three minutes.
7. Rinse in distilled water.
8. Stain in Harris’s hematoxylin for 45 seconds.
9. Rinse in distilled water.
10. Blue in ammonia water 20 dips.
11. Wash in water, two changes.
12. Mount with aqueous mounting medium.

**Results:**

Fat red

Nuclei blue

**Comments:**

The oil red-O is dissolved in a lipid solvent, and sections are treated with the dye-solvent solution. Since the dye is comparatively more soluble in the lipid in the tissue section than in the original solvent, the dye will move out of the solvent and color the tissue lipid. Substances stainable by this method are referred to as sudanophilic. Boundary-surface adsorption plays a prominent role in the process. Since the staining is of such a physical nature, chemically different lipids cannot be distinguished by this method.

The temperature at which a lipid melts will determine to a large extent whether the lipid will stain by the oil-soluble dye. Usually only liquid lipids take up this stain, but if the staining is carried out at 60 C, a lipid that is solid at room temperature is more likely to melt and admit the dye and consequently be stained. Fat-staining ability increased with the molecular weight of the dye, and factor parallels the solubility of the dye itself.

The actual staining process should be carried out in a closed container; otherwise, some of the dye solvent can evaporate and the dye itself can precipitate on the tissue.

If air bubbles are present after cover slipping, they should not be pressed out, since this process will displace stained lipid. Instead, re-immerse the slide in warm water until the coverslip falls off; re-wipe excess water from the slide and re-cover slip.

REFERENCES

1. Luna, Lee G.: *Manual of Histologic Staining Methods of the AFIP* , McGraw-Hill Book Co., 1968, pg. 140-141. Modified by Histopathology Laboratory, Harborview Medical Center, Seattle, WA.
2. Sheehan, D.C. and Hrapchak, B.B.: *Theory and Practice of Histotechnology*, The C.B. Mosby Co., 1980, pg. 203-204.

Written By: Director Approval:

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Histology Supervisor