**Oil Red O Histochemistry for *frozen sections only***

**Reagents:**

* Oil Red O (Sigma Cat. # O-0625)
* Isopropanol (Fisher Scientific Cat. # A451-4)
* Mayer’s Hematoxylin (Sigma Cat. # MHS80)
* 10% Neutral Buffered Formalin (Fisher Scientific Cat. # SF94)
* Vectamount Aqueous Mounting Medium (Vector Labs Cat. # H-5501)

**Preparation of Reagents:**

* Stock Oil Red O solution (*make this at least a day or two prior to use*)
	+ Saturated Oil Red O in 99% Isopropanol (300mg of Oil Red O in /100mL isopropanol)
* Working Oil Red O solution
	+ 30 mL of stock solution mixed with 20 mL of Distilled Water
	+ Mix and let stand for 10 minutes.
	+ Filter prior to use (*takes about an hour to filter properly)*
		- Use Whatman Paper (Cat# 1002-185)
* 60% isopropanol, diluted with MilliQ water

**Staining Method:**

1. Air dry frozen sections on slides for *30 minutes minimum*
2. Fix in 10% neutral buffered formalin for *10 minutes*
3. Dip in 60% isopropanol *1 time quickly*
4. Stain in working Oil Red O solution for *15 minutes*
5. Dip in 60% isopropanol *1 time quickly*
6. Dip in DI water *1 time quickly*
7. Counterstain with Mayer’s Hematoxylin for *3 minutes*
8. DI water *10 dips*
9. Coverslip with aqueous mounting gel
	1. IMPORTANT: Do not press on coverslips to remove air bubbles -> Lipids may move!
	2. ALSO: Do not let air dry. Coverslip *immediately* after DI water.

 Images: frozen sections of mouse liver stained with H&E (left panel) or Oil Red O (right panel)



A: Oil Red O of SKF15 HF liver at 100x magnification

B: H&E of SKF15 HF liver at 100x magnification (same location)

C: Overlay of Figures A and B