

AORTA STAINING

- Stain a lot of aortas at a time, possibly whole studies at a time
- Under the hood: pour off formalin into the large jar, rinse off with distilled H₂O, leave some dH₂O on the aorta to keep it wet, move over to sink
- Use the dH₂O in the sink to rinse the aorta off again, leave a little dH₂O on it to keep it wet
 - **Make sure that you keep a hold of the aorta, it can slip off
- Weigh out 70 mg of Oil Red O (.07 g on the scale)
- Use a sterile 25 ml glass pipet and bubble top to pull up 35 ml methanol (found under the sink). Put into a 50 ml tube
- Add 10 ml 1N NaOH (break down from 10N NaOH) a drop at a time. Use 2 transfer pipettes (plastic, in drawer). Drop NaOH with one and mix with the other
- Fill tube to 45 ml with NaOH
- Use Whatman filter paper (drawer) and the red funnel to filter stain. Filter into another tube
- Put pins from the aorta into a 50 ml tube (should be labeled "pins") filled with EtOH. Remove the pins using two forceps. **Be very careful not to rip the tissue.
- Lay tissue in H₂O in Petri dish. Throw paper away.
 - **Check paper to make sure there isn't anymore tissue on it
- Use a 24-well plate, label wells with permanent marker, make an extra well – 70% MetOH.
- Need a well for each aorta
- Pull tissue out of petri dish, dip in 70%, and put into correlating well.
- Cover with stain (after filtered), then go back and fill the wells to the top with stain
- Place on shaker at 1100 RPM for exactly 55 minutes.
- Aspirate stain into a used 50 ml tube
- Fill wells back up with 70% MetOH, shake for one minute
- Aspirate MetOH again
- Fill back up with 70% MetOH, shake two more minutes
- Aspirate again
- Mount the aortas:
 - Label frosted slide – project, sex, DOB, diet, date aorta was taken
 - Place aorta on slide – inside face up
 - Put mounting medium near frosted end and let it work its way down the side
 - May need to use a blue weight if the tissue is too large