**Preparation for Quantification of Aortic Atherosclerotic Lesions**

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Edited by: Mark Kelly LAT, Timothy P. Fitzgibbons MD PhD

## Summary:

Atherosclerosis - Aortic Root Sectioning and Analysis

Reagents and Materials:

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| Reagent/Material | Vendor | Stock Number |
| Minutien Pins | Fine Science Tools  | 26002-20 |
| S65104 | Fisher Scientific | 09-002-20 |
| Oil Red O | Sigma Aldrich | O0625-25G |
| ImageJ | NIH | https://imagej.nih.gov/ij/ |
| Methanol | VWR | BDH1135-1LP |
| 0.45um Syringe filter | VWR | 28145-479 |

Protocol:

***WARNING HAZARDOUS CONDITION WARNED AGAINST. This comment describes a hazardous condition to which the technician may be exposed in the performance of this protocol. It also contains directions on how to avoid or minimize the danger. Warnings are always and only used for personnel safety, and precedes the first step that will expose the technician to the hazard.***

Expected procedure duration:

~30 minutes

Equipment Prep:

Prepare oil red o stock solution:
0.5 g oil red o

100 mL isopropanol

Dissolve the dye in 100% isopropanol (you may need to use a 37C water bath to get it to dissolve).

Oil red o working solution (prepare fresh each time):

Dilute 30 ml of the stock stain with 20 ml ddH20, let stand for 10 minutes, and syringe filter (0.45 microns). Cover to prevent evaporation.

78 % methanol, 1.5 ml Eppendorf tube

Procedure:

Oil red o staining of aortas

1. Place the ﬁxed aortas in 1.5 ml Eppendorf tubes, one aorta per tube.
2. Add 1ml of 78 % methanol to each tube and place on a tilted roller with slow movement for 5 min. Replace the methanol solution and repeat this step twice.
3. Discard the methanol and add 1 ml of fresh Oil red o working solution and return the tube/s to the tilted roller for an additional 50–60 min at room temp.
4. Place the aorta in a new tube and wash twice with 1 ml of 78 % methanol for 5 min each on the tilted roller.
5. Discard the methanol and ﬁll the tube with 1 ml of PBS.
6. When aortas preparation is complete, move the stained aorta using ﬁne forceps to the wax surface of the dissecting tray.
7. Under a stereomicroscope, carefully as not to damage the aorta, remove any small piece of stained remnant fat or tissue that might be attached to the outer surface of the aorta.
8. Cut the aorta longitudinally: using Micro-dissecting spring scissors introduce the tips into the lumen of the artery and cut the outer curvature of the arch from the ascending arch to the left subclavian artery, continuing to cut along the length of the aorta.
9. Pin the aorta ﬂat with lumen side up on the dissecting tray with steel minutien pins.
10. Insert the pins along the aorta at a slight outward angle to minimize the shadow that they will cast on the illuminated aorta.
11. With a digital camera attached to a stereomicroscope and connected to a computer, capture a bright-ﬁeld image.
12. Use selected software (image-J) for image analysis and quantification of plaques to convert the selected areas into relative quantitative data.