Atheroma Quantification

Version: 1
Modified from: Villablanca AC, *et.al*. J Cardiovasc Transl Res. 2009 Sep;2(3):289-99.
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Summary: Increased lipid accumulation in the artery wall and atherosclerotic lesion development are two hallmarks of atherosclerotic cardiovascular disease. Atheroma quantification and characterization assesses neutral lipid accumulation in the vessel wall, percentage of lesions per aortic segment (quantitative) and lesion morphology, complexity, and severity (qualitative).

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
DPBS	Invitrogen	14190
formaldehyde	Fisher	F79
Tissue-Tek OCT	Fisher	14-373-65
Oil-red-O	Sigma	O0625
Gills hematoxylin	Sigma	

Protocol:

WARNING:
Formalin is, toxic, flammable and considered a carcinogen

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.

1. Mice are anesthetized with an intraperitoneal injection with 50 mg pentobarbital/kg weight.
2. Immediately after sacrifice, aortas are perfused with phosphate-buffered saline to remove blood after venting and nicking the heart.

3. After careful removal of periadventitial fat, aortas are excised and fixed (4% followed by 10% paraformaldehyde overnight), and cut into three segments*
    a. Proximal segment including all of the aortic arch
    b. Midsegment consisting of the thoracic and suprarenal aorta
    c. Distal segment consisting of the infrarenal aorta proximal to the iliac bifurcation.

    * Note: aortas can also be analysed for a desired segment (for example: coronary sinus only, or proximal segment between aortic arch and the aortic valve only)
4. Aortic segments are placed side-by-side in cryomolds with optimum cutting temperature (OCT) compound frozen on dry ice in a cryomold, and then stored at -80 °C.
5. Tissues are later sectioned with a microtome cryostat (HM 500 M) to 10 μm thick taken at -30°C, discarding every other section. (This allows sampling of all three segments of the aorta, permitting direct comparisons of each.)
    a. Four sections per slide with a total of 9 slides
    b. 36 sections per segment and 108 cryosections per aorta
    c. Total of 360 μm from each segment and 1,000 μm from each aorta.

Note: Atheroma quantification can be completed on frozen tissue sections provided by the investigator

6. Quantitative morphometric analyses of aortic lesions is performed by staining cryosections with oil-red-O and Gill's hematoxylin
    a. oil-red-O: define and characterize neutral lipid in the vessel wall
    b. Gill's hematoxylin: provides histological assessment of lesions in artery segments
7. Sections are analyzed for lesion location and area by direct imaging (Olympus BX-40 microscope with a DP11 camera with smart media chip).
8. Atheromas are identified by a single observer blinded to experimental parameters, and mean lesion area ($\mu m^2 \pm SE$) determined for each atheroma by morphometric analysis with a computer-assisted imaging system (Image Pro Plus, version 4.1).
    a. The quantification data is highly reproducible having an intraobserver error of ~6%.
9. Atheroma distribution is expressed as percentage of lesions per segment.
10. Qualitative morphologic assessment of lesion complexity is performed with light microscopy for the following parameters: absence or presence of foam cells, wall thickness, vessel wall architecture integrity, nuclear proliferation, and luminal extension.
11. Lesion morphology, complexity, and severity are used to classify the stage of lesions per the classification of Stary.
12. Lesion complexity can also be performed (e.g., for foam cells and macrophages)

Note: the above method is for quantitative microscopy. It is also possible to provide en face analysis of aortic atheroma by Sudan IV staining.

Reagent Preparation:

Reagent 1: 4% & 10% paraformaldehyde

Formaldehyde (Fisher) is diluted to 4% or 10% in DPBS (Invitrogen)