DAUGHERTY LAB
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University of Kentucky

Protocols\Aortic Pathologies - Quantification

Atherosclerosis - Aortic Root Sectioning and Analysis

SECTIONING OF MOUSE AORTIC ROOT EMBEDDED IN OCT

Materials
1.   Dissecting scissors, forceps, Scalpel blade, and Parafilm
2.   Tissue molds 15 x 15 mm (Fisher Scientific Cat # 41-741) and (optional) microcentrifuge tube with screw top
3.   OCT compound (Fisher Scientific Cat # 14-373-65)
4.   Probe-on-Plus microscope slides (Fisher Scientific Cat # 22-230-900)

Before Start
OCT will become rubbery and difficult to cut if exposed to air for long periods. If you plan to cut the tissues within a few weeks, you may place aortic root tissues in tissue molds covered with OCT compound, wrapped tightly in parafilm, and store at -20 or -80 °C. Otherwise, please store aortic roots covered by OCT compound in microcentrifuge tube with screw top at -20 or -80 °C. Label tissue molds or microcentrifuge tubes with the study name, mouse #, and date or code as appropriate.

Dissecting Aortic Root
1.   Dissect the heart and aorta from mouse.

2.   Separate the heart from the aorta by holding the heart with the forceps and cutting with either scissors or a scalpel blade, as close to the heart as possible without causing damage.

3.   Use a scalpel blade (the red line shown in the Figure) to cut away approximately 70% (from the apex to 3 mm away from the base) of the ventricles.

4.   Place the upper portion of the sectioned heart that contains the aortic root in a microcentrifuge tube or a mold and cover with OCT.

5.   If the aortic root is placed in a tissue mold prepared for cutting, it needs to be positioned to be perpendicular to the bottom surface of the tissue mold.
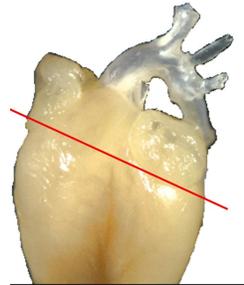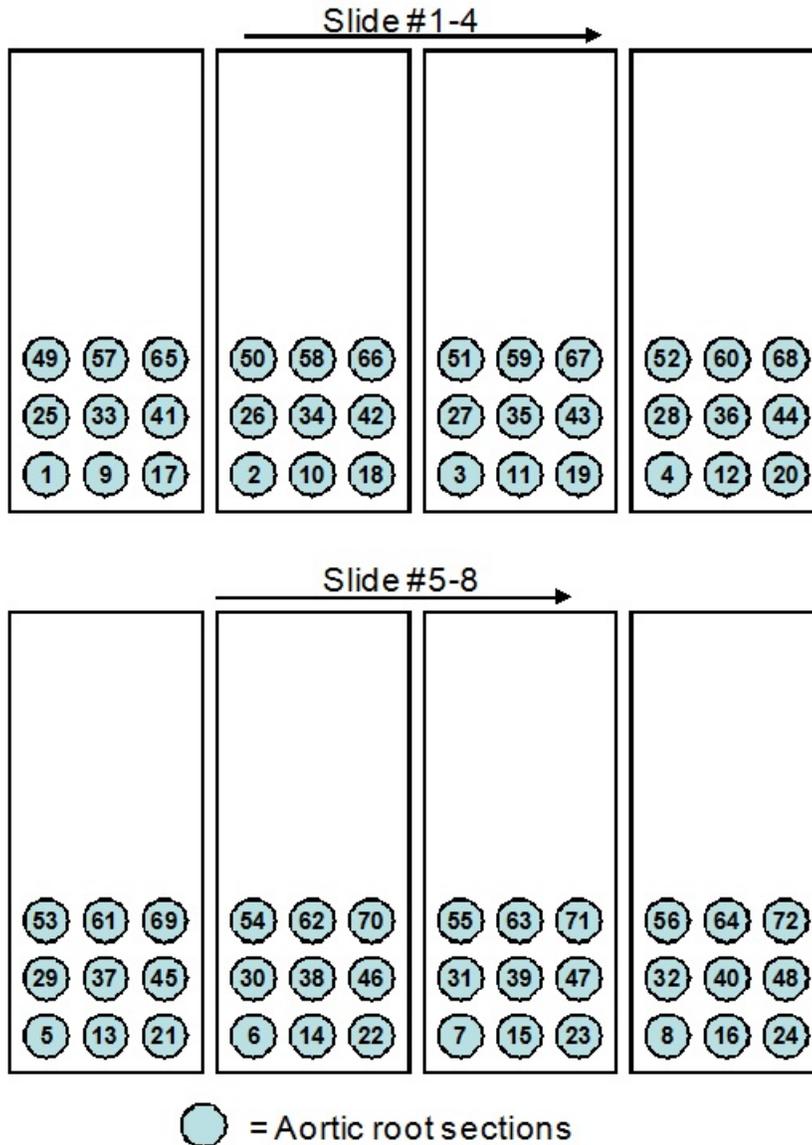
Cutting Sections of the Aortic Root
Note: The methodology requires to cut sequential sections in the correct orientation and without tearing, curling or sticking incorrectly to the slide. Therefore, do not cut aortic roots collected from your experiments until you are sure you can reliably cut sections sequentially.

1.   The frozen tissue blocks are mounted on the cryostat chuck with the ventricular tissue facing outward. If the tissue was placed correctly into the mold and level on the chuck, the aortic root will be perpendicular to the knife blade.

2.   The ventricular tissue is sectioned and discarded until the aortic sinus is reached. This is identified by checking under the microscope until the appearance of the 3 aortic valves.

3. Once all aortic valves appear, serial sections are collected at 10 µm/section, and 8 - 10 slides/aorta until the aortic wall disappears or is not intact anymore. Usually at least 9 serial sections/slide can be collected.

4. The format for placing sections on slides is shown below:

Slide #1-4

(49)	(57)	(65)	(50)	(58)	(66)	(51)	(59)	(67)	(52)	(60)	(68)
(25)	(33)	(41)	(26)	(34)	(42)	(27)	(35)	(43)	(28)	(36)	(44)
(1)	(9)	(17)	(2)	(10)	(18)	(3)	(11)	(19)	(4)	(12)	(20)

Slide #5-8

(53)	(61)	(69)	(54)	(62)	(70)	(55)	(63)	(71)	(56)	(64)	(72)
(29)	(37)	(45)	(30)	(38)	(46)	(31)	(39)	(47)	(32)	(40)	(48)
(5)	(13)	(21)	(6)	(14)	(22)	(7)	(15)	(23)	(8)	(16)	(24)

◯ = Aortic root sections

Storage
Slides should be stored in a slide box at -20 °C.

REFERENCE
Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA. Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis*. 1987;68:231-240.

IMAGE ANALYSIS OF ATHEROSCLEROTIC LESIONS
IN AORTIC ROOT SECTIONS

Aortic root sections are stained with Oil Red O (See protocol in adlab\Protocols\ Histology\Oil Red O Staining) to facilitate atherosclerotic lesion measurements.

Calibration:

1.   Open Image Pro program 5.0 or 7.0, open first image to be measured.

2.   Go to the "Measure" menu → "Calibrate" → "spacial calibration"

3.   Select "spacial calibration wizard" and follow directions, or click "new" and name your calibration.

Label:

1.   Label the image by clicking on the annotation button (✎) click on *"Aa",* then click on picture and fill in text (study name, mouse #, Sec #, date), and then click "ok".

2.   Burn the label to the image by pressing the double arrows (↙↙).

3.   Save picture.

4.   Label the remaining images.

Image Analysis:

1.   Select the correct calibration for each image opened. "Measure" →

        "Calibration" → "set system" → "Apply"

2.   Click "measure" menu and select "measurements"

3.   Select the polygon button (△) on the Measurement Toolbar and trace atherosclerotic lesions on each section (See images on Page 4). Look at the section stained with Oil Red O through the microscope and trace atherosclerotic lesions on the image.

Exporting Measurement Data into Spreadsheet

1.   Click on "input/output". Choose measurements and click "export now"

2.   Paste into spreadsheet.

3.   Save image; Input/output tab, save as "mouse #....msr". Image may be printed with overlay at this time. Do not close image until the image has been printed or saved and measurements are recorded in the spread sheet.

Quantification of Atherosclerotic Lesions
Aortic Root: Oil Red O Staining
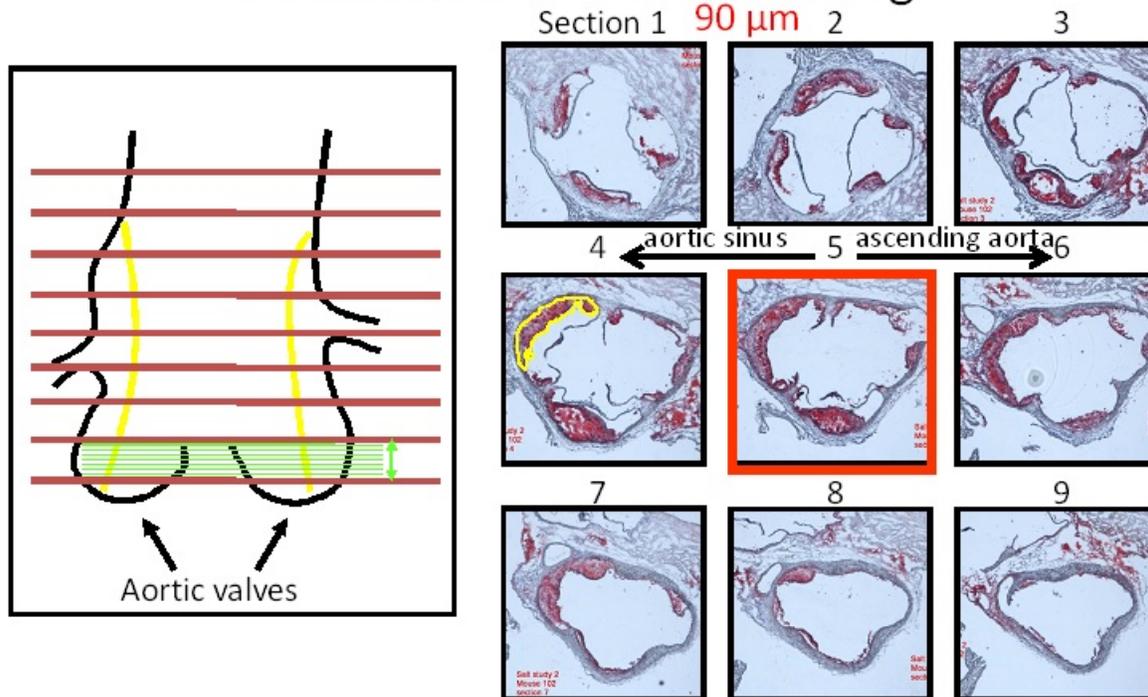
Protocol Developed : Deborah A. Howatt
Updated : Hong Lu on 5/7/13
Verified : Hong Lu on 5/7/13

File name: K:\Protocols and forms\Aortic Pathologies - Quantification\Aortic Root
Sectioning and Analysis.wpd