

DAUGHERTY LAB
Saha Cardiovascular Research Center
University of Kentucky

Protocols\Aortic Pathologies - Quantification

Atherosclerosis - En Face Aorta and Analysis

Preparation and Quantification of Atherosclerotic Lesions - En Face Method

PRINCIPLE

FIX, CLEAN and CUT OPEN

1. Store each dissected aorta in 4% paraformaldehyde or 10% neutrally buffered formalin for 24 - 48 hours. Once fixed, aortas are stored in saline at room temperature or 2 - 8 °C.
2. Clean aorta by removing adventitial tissues. Be careful to not tear or nick the aorta and important branches such as innominate artery, left common carotid artery, and left subclavian artery. Before cleaning, let the aorta sit in saline for a few hours could help facilitate cleaning. Use saline to keep the tissue moist during cleaning.
3. Leave 1 mm of innominate and left common carotid artery, and cut off the entire left subclavian artery. Cut open the outer curvature through the innominate artery, then to left common carotid artery, and then to the left subclavian artery. Cut open along the inner curvature of the ascending portion to the bottom of the abdominal portion (**Figure 1**).

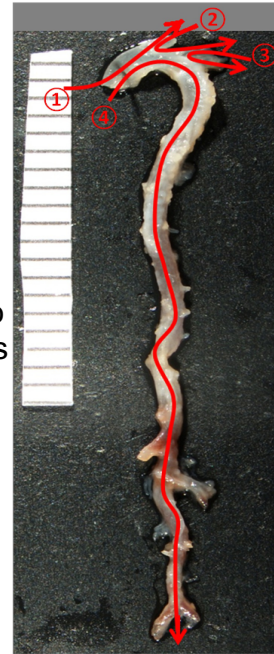


Figure 1. (1). First cut through innominate artery; (2) second cut from innominate to left common carotid artery; (3) third cut from left common carotid artery to left subclavian artery; (4) fourth cut along the inner curvature of the aorta.

PIN

1. Pin aorta flat on black wax with pins (Fine Science Tools item # 26002-20).
2. Apply saline to keep aortas from drying.
3. Label mouse numbers on the black wax box and store at 4 ° C. Imaging and analysis must be done within 3 days after pinning.

IMAGE

1. Take pictures of en face aortas with a Nikon digital camera. A mm ruler must be included in the images to calibrate measurements. Three regions of the aorta are designed for this en face method, and pictures should include these 3 regions separately (or include arch and thoracic regions in same image).

Definition of these 3 regions (**Figure 2**) are:

Arch Region - from the junction of the myocardium to 3 mm distal from the left subclavian artery.

Thoracic Region - 3 mm distal of the left subclavian artery to the last intercostal artery.

Abdominal Region - the last intercostal artery to the iliac bifurcation.

2. Save images as JPEG or TIFF.

QUANTIFICATION

General Rules:

1. Use Image Pro plus 5.0 or 7.0 software for quantification.
2. View aorta images under a microscope simultaneously while the images are used as templates for noting lesions.
3. Quantification is verified by a second observer who is blind to study groups.

NOTES: After verification, aortas should be unpinned, put into properly labeled tubes with saline containing 0.02% sodium azide and stored at room temperature.

Calibration:

1. Open Image Pro program 5.0 or 7.0, and open the 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.
4. Click "image", position line over the ruler, and change reference units to mm. Click "ok" and then "apply".

Labeling:

1. Label the image by clicking on the annotation button (🖋️) click on "Aa", then click on the picture and fill in text (study name, mouse #, date), and click "ok".
2. Burn the label to the image by pressing the double arrows (↔).
3. Save picture.
4. Label the remaining images.

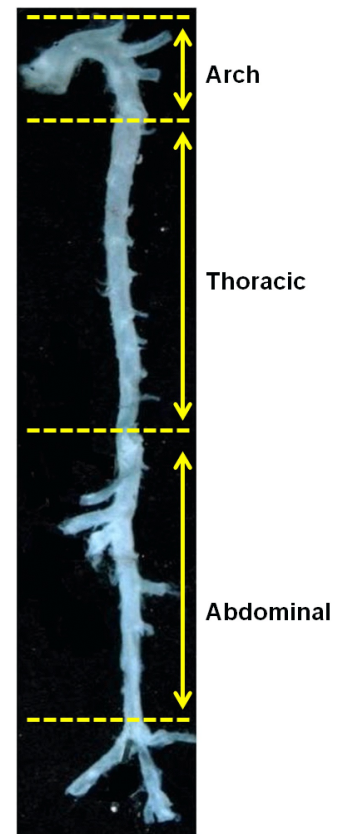


Figure 2. Definition of aortic regions.

Measuring:

1. Select the correct calibration for each image opened. “Measure” → “Calibration” → “set system” → “Apply”.
2. Click “measure” menu and select “measurements”.
3. Choose “line” (\) icon. Use the line to measure 3 mm on the ruler.
4. Select the arrow (↖) button. For example, to define the aortic arch region, drag the line so one end touches the root of the left subclavian artery. Use the line as a marker to separate the arch portion from the thoracic portion when measuring (Figure 3).
5. Select the polygon button (△) on the Measurement Toolbar and trace the intimal area of the arch region.
6. Use the polygon function to trace the lesions in arch region on the aorta image, and check the aorta through the microscope.
7. Measure from the end of the arch region (see Step 4) to the last intercostal artery (approximately 17 mm), and outline this section of the aorta for the thoracic region.
8. Look at the aorta through the microscope, and trace lesions in the thoracic aorta on the image.
9. Outline the abdominal region. From the end of thoracic region to the iliac bifurcation. Optional: Quantification may include 1 mm of both iliac arteries.
10. Trace lesions in the abdominal region and look at the aorta through the microscope.

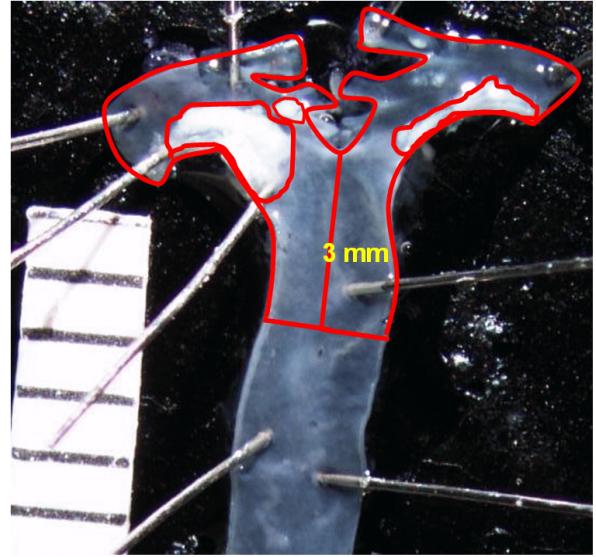


Figure 3. En face analysis of atherosclerosis lesion area and intimal area of the aortic arch region.

Exporting Data into Spreadsheet

1. Click on “input/output”. Chose 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.

NOTE: Do not close the image until the image has been printed and saved, and measurements are recorded in the spreadsheet.

Protocol Developed : Deborah A. Howatt
Updated : Jing Liu on 5/7/13
Verified : Deborah Howatt on 6/20/13

File name: K:\Protocols and forms\Aortic Pathologies - Quantification\Atherosclerosis - En Face Aorta and Analysis.wpd