

DAUGHERTY LAB
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Protocols\Aortic Pathologies -
Quantification

**Quantification of Abdominal
Aortic Aneurysms**

Quantification of Abdominal Aortic Aneurysms

DEFINITION and PRINCIPLE:

Suprarenal region of the abdominal aorta is the portion from the last intercostal artery to the right renal artery (**Figure 1**). In the Angiotensin II infused mouse model, abdominal aortic aneurysms are located in this region. Maximal outer width of the suprarenal aorta is a major parameter to define abdominal aortic aneurysms. Fifty % or more increase of this parameter, compared to control (saline infusion), is defined as an abdominal aortic aneurysm.

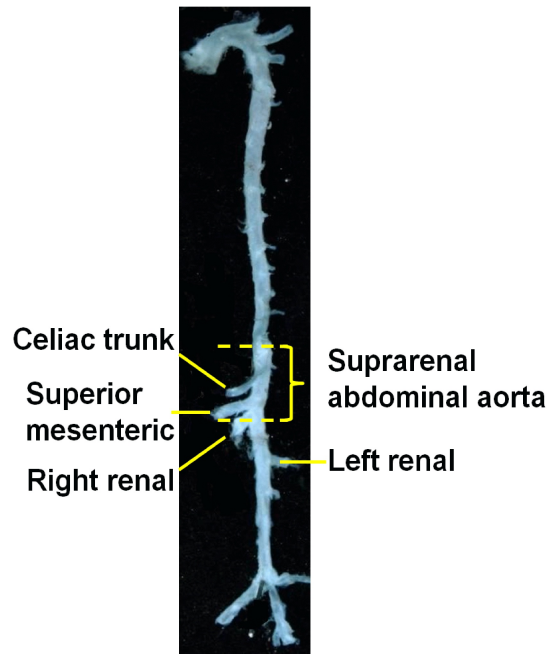


Figure 1. Definition of suprarenal aorta.

HARVEST, FIX and CLEAN:

1. Cut open the mouse ventrally, perfuse with saline and then dissect the aorta.
2. Place dissected aortas in 4% paraformaldehyde or 10% neutrally buffered formalin for 24 - 48 hours. Once fixed, aortas can be kept in saline at room temperature (or 2-8 °C).
3. Before cleaning, allow aortas to soak in saline for a few hours. Clean aortas by removing adventitial tissues. Be careful to not tear or nick the aorta and important branches. Use saline to keep the tissue moist during cleaning.

PIN:

1. Pin aorta on black wax with pins (Fine Science Tools item # 26002-20).
2. Apply saline to keep aorta moist.
3. Label mouse number on both the lid and the box of the black wax. Imaging and analysis should be done within 3 days after pinning.



IMAGE:

1. Take pictures of aorta with a Nikon digital camera. A mm ruler must be included in the images to calibrate measurements as shown in Figure 2. The abdominal region and main branches should be included.
2. Save images as TIFF.



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. Open Image Pro program 5.0 or 7.0, and open the image to be labeled.
2. 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”.
3. Burn the label to the image by pressing the double arrows ().
4. Save pictures.
5. Label the remaining images.

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. Place the line on the maximal outer width of suprarenal aortic region (**Figure 2**).
4. Select the arrow () button to adjust the line position.

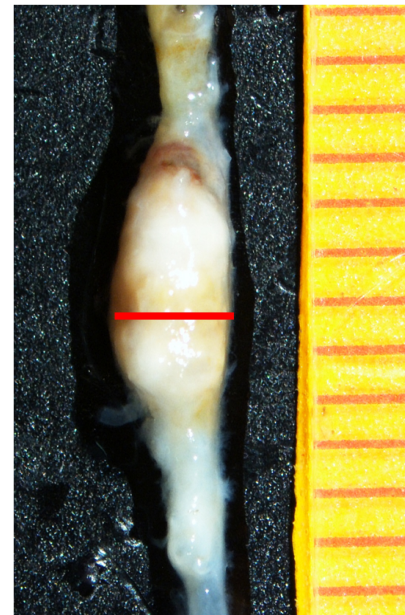


Figure 2. Example to show how maximal outer width of suprarenal aorta is measured.

EXPORTING DATA

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

2. Export into a 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.

VERIFICATION and UNPIN:

1. Quantification is verified by a second observer who is blind to study groups.
2. After verification, aortas should be unpinned, put into properly labeled tubes with saline containing 0.02% sodium azide and stored at room temperature.

Appendix - Classification of Abdominal Aortic Aneurysms

Type	ex vivo maximal width of the suprarenal aorta	number of dilations
I	1.5 - 2 times of a normal suprarenal aorta	1
II	≥ 2 times of a normal suprarenal aorta	1
III	≥ 1.5 times of a normal suprarenal aorta	≥ 2
IV = rupture		

REFERENCES:

1. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J Clin Invest.* 2000;105:1605-1612.
2. Wang YX, Cassis LA, Daugherty A. Angiotensin II-induced aortic aneurysms (Book Chapter). *A Handbook of Mouse Models for Cardiovascular Disease.* Editor: Q Xu; Publisher: John Wiley & Sons. 2006; 125-136.

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