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# Atherosclerotic Morphometry in Pigs V.2

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

### Summary:

These assays are performed on all the pig models studied by the DiaComp to assess the extent of atherosclerotic lesions in diabetic cardiovascular disease models. Each measurement/assay is listed below with the details of how the data is collected. Details of the reagents and materials are in the assay.

### Diabetic Complication:



Cardiovascular

1 **En face abdominal aorta total area**  
**En face abdominal aorta lesion area**  
**En face abdominal aorta lesion percent**

The abdominal aorta is opened longitudinally, immersion fixed with 10% buffered paraformaldehyde, and photographed for measuring the percent surface area with raised lesions when macroscopically present (see Coronary atherosclerosis for details of measurement methods).

**Aortic atherosclerosis morphometry – intimal area**

**Aortic atherosclerosis morphometry – intimal area as % medial area**

The fixed aorta is then divided into 1 cm sized segments taken perpendicular to the direction of blood flow from the level of the renal arteries to the origin of the iliac arteries, producing 10 to 12 sections per pig. Light microscopic sections were prepared from the proximal half of each segment and stained with Verhoeff-van Gieson and hematoxylin and eosin. All slides were prepared with at least three cross sections and the image of each aortic cross section was digitized and measured in a blinded fashion with computer-assisted planimetry using the same system described for the coronary arteries. The extent of aortic atherosclerosis is defined as the intimal plaque area (Eq. 1) and the intimal plaque area as a percent medial area (Eq. 3), and the percent surface area with raised lesions. The summary statistics are reported as the mean of all values for each group of pigs for the entire abdominal aorta (i.e., from the renal arteries to the aortoiliac junction). The inter and intra observer variability is comparable to that seen for the coronary arteries and differences were resolved in the same manner.

**Right coronary artery atherosclerosis morphometry – intimal area**

**Right coronary artery atherosclerosis morphometry – intimal area as % medial area**

**Right coronary artery atherosclerosis morphometry – intimal area as % medial area**

**Circumflex coronary artery atherosclerosis morphometry – intimal area**

**Circumflex coronary artery atherosclerosis morphometry – intimal area as % medial area**

**Circumflex coronary artery atherosclerosis morphometry – intimal area as % medial area**

**Left anterior descending coronary artery atherosclerosis morphometry – intimal area**

**Left Ant. Desc. coronary artery atherosclerosis morphometry – intimal area as %**

## **medial area Left Ant. Desc. coronary artery atherosclerosis morphometry – intimal area as % medial area**

The heart and coronary arteries were fixed with 10% buffered paraformaldehyde. Cross sections of each coronary artery were taken perpendicular to the direction of blood flow at one cm intervals from their origin. The number of sections reflects the distance covered. In general there are a total of 19 to 21 total coronary artery sections per pig heart: 7 left anterior descending coronary artery, 8 right coronary artery, 4 to 6 circumflex. Previous studies performed in which sections were obtained at 0.25 and 0.5 cm yielded equivalent results to the strategy of obtaining sections at 1 cm intervals (data not shown). The coronary artery cross sections were stained with Verhoeff-van Gieson to highlight the internal and external elastic lamina. Adjacent sections were stained with hematoxylin and eosin to confirm lesion details. The image of each coronary artery cross-section was digitized using a Nikon Microphot-FXA (Japan) connected to a Macintosh computer via an Optronics TEC 470 CCD Video Camera System (Optronics Engineering, Goleta, CA) camera. The external elastic lamina (EEL), internal elastic lamina (IEL), and lumen were measured using NIH Image software. The extent of atherosclerosis was evaluated from three indices calculated from the computerized tracings: (Eq. 1) intimal plaque area, (Eq. 2) % luminal narrowing by intima, and (Eq. 3) intimal area as a percent of medial area.

**(Eq. 1)** intimal plaque area ( $\mu\text{m}^2$ ) = area within the IEL - luminal area.

**(Eq. 2)** % luminal narrowing by intima = (intimal area  $\div$  area within the IEL) X 100.

**(Eq. 3)** intimal area as % medial area = [intimal area  $\div$  (area within EEL - area within IEL)] X 100.

The summary statistics for the total amount of coronary atherosclerosis are reported as the mean and standard deviation of all values for each group of pigs for all three coronary arteries. All microscope slides contained at least three arterial cross sections that were measured by at least two blinded observers using the same microscope, computer, and software. The inter and intra observer variability is between 4 and 7%.