

DAUGHERTY LAB
Saha Cardiovascular Research Center
University of Kentucky

Protocols

Ultrasound for ascending aorta and heart (Vevo 2100)

Visualization and Quantification of the Ascending Aorta and Heart Function by Ultrasonography

Materials:

1. Vevo2100 ultrasound machine, heated platform and appropriate probe (MS550D: 55MHz, white; MS400: 38MHz, red)
2. Isoflurane, vaporizing machine with attachments including charcoal filters, plastic tubing, induction chamber, and nose cone.

Equipment:

1. Turn on ultrasound machine (Switch is located behind the machine and computer switch is on left side), heated platform and gel warmer.
2. Open ultrasound program. Select user name. Change application to cardiography. Enter study name, mouse #, etc.
- 3a. For analog vaporizer: Fill isoflurane vaporizer if low. Check O₂ tank. Turn on both Isoflurane vaporizer (at 1.5) and O₂ tank with induction chamber branch open.
- 3b. For digital vaporizer: Set at 5% iso for induction, 1.0-1.5% for maintenance. See somnosuite protocol for full details.

Mouse Preparation:

1. Place mouse in isoflurane/O₂ filled induction chamber.
2. Once anesthetized, remove mouse from chamber and place one drop of sterile ophthalmic lubricant in each eye. Redirect anesthesia to nose cone and close flow to induction chamber.
3. Lay mouse dorsally on heated platform with nose in anesthesia nose cone. Apply thick, even layer of Nair depilatory cream on chest using a cotton swab. Wait a few minutes. Gently wipe off cream and hair using a kimwipe. Irrigate the area with warmed water and gently wipe dry. (Note: Hair may be removed the day before scanning.)
4. Dot gel on the 4 paw sites on platform. Tape each paw pad down to site of gel for ECG readings.
5. Apply pre-warmed ultrasonic gel to the shaved site. Attach scan head to holder. Rotate platform for optimal scanning and lower scan head until it is in contact with ultrasonic gel.

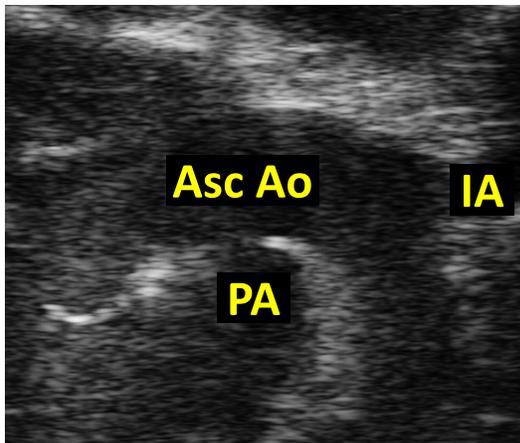
1. Aorta:

Scan of aortic root through ascending aorta:

1) Ascending aorta is observed using “modified aortic arch view (one rib lower than aortic arch view)” .



- The notch of probe is toward the head.
- Tilt down the stage to the left of mouse (See figure above).
- Put the probe on the right edge of sternum.
- Change stage and probe angle to show the aorta clearly.



An example of ascending aorta ultrasound scan:

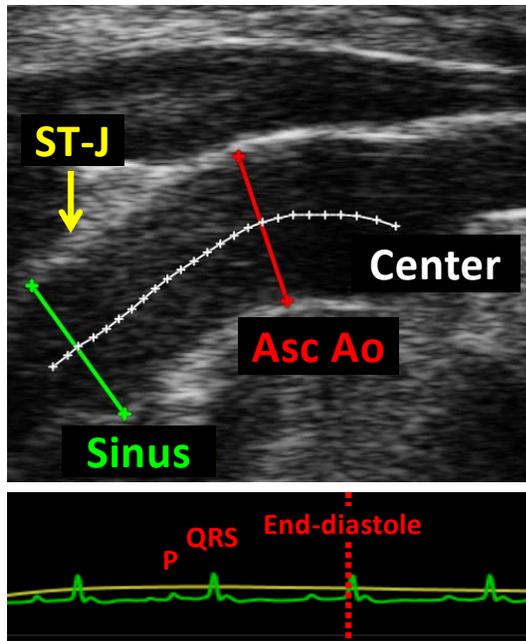
Asc Ao = Ascending Aorta

PA = Pulmonary artery

IA = Innominate (brachiocephalic) artery

2) Store one cine loop. If it is difficult to capture the proximal and distal part of the ascending aorta in one scan, images should be captured separately.

Quantification of ascending aortic diameter:



- White line: Center line
- Yellow arrow: ST-Junction (ST-J). ST-J is located at the end of the cusp. ST-J is sometime difficult to define, because of pathologies.
- Green line: Sinus level. Sinus is located mid-cusp after valves.
- Red line: Ascending aorta. Maximal ascending aortic diameter is measured after the ST-J, before IA.

1) Select an image for analysis. Aortic diameter should be measured in either systole or diastole, consistently. Therefore, investigators need to specify during which phase of the cardiac cycle the aortic diameter is measured.

- **Systole:** Systole is defined from the R wave to the end of the T wave. In general, T wave is difficult to identify in the monitor ECG. Therefore, systole cannot be defined using monitor ECG. AoD in systole should be measured at physiologic systole: when aorta is maximally and visually dilated.
- **Diastole:** End-diastole is defined at R wave in monitor ECG. End-diastole measurements may minimize the effects of aortic stiffness.

Note: Clinical guidelines vary on which cardiac phase to measure aortic diameter. Pediatric guidelines are based on systolic measurements, whereas adult guidelines are based on diastolic measurements. In the AD lab, aortic diameter is measured in both systole and diastole. We prefer to measure in diastole in order to detect a maximal effect in size.

2) Draw a dotted line in the center of the aortic lumen. This center line will be used to correct the angle of measurement lines.

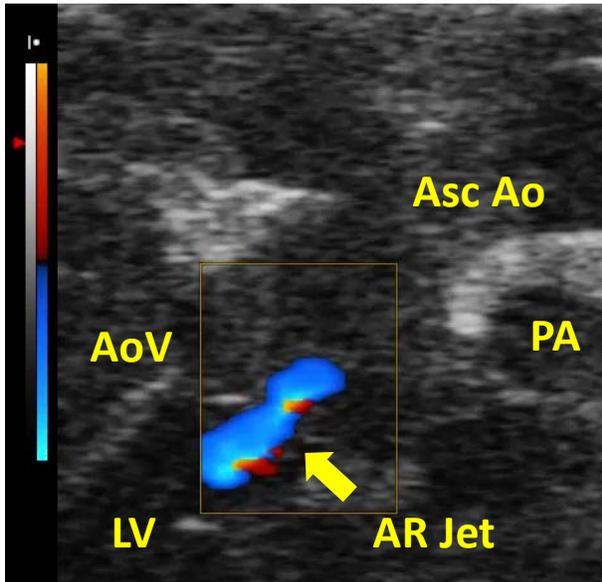
3) Draw perpendicular lines through the center line from luminal inner edge to inner edge at the sinus and maximal ascending aortic levels.

4) Measure at least 3 separate heart beats and average them.

2. Aortic valve regurgitation (AR)

Scan of aortic valve:

1) Push stage forward until aortic valve appears.



2) Place a color Doppler box on the center of aortic valve area.

AoV: aortic valve

Asc Ao: ascending aorta

AR Jet: aortic valve regurgitation jet

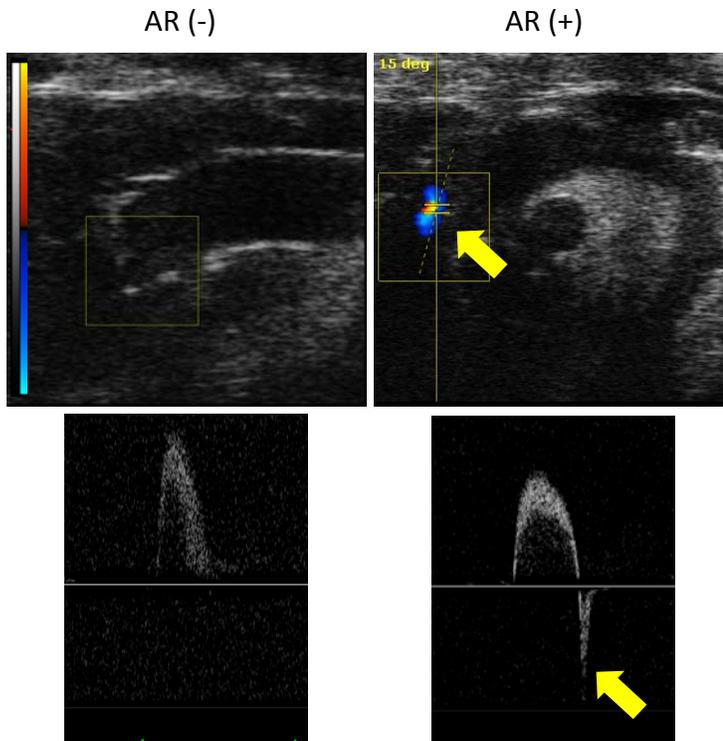
LV: left ventricle

PA: pulmonary artery

3) Push the PW (pulse wave) button and place the sample gate on the LV outflow. Correct the angle ($-30 < \text{angle} < 30$), if the angle is too oblique, the test is not as sensitive.

4) Push the update button to capture the PW image.

Examples:

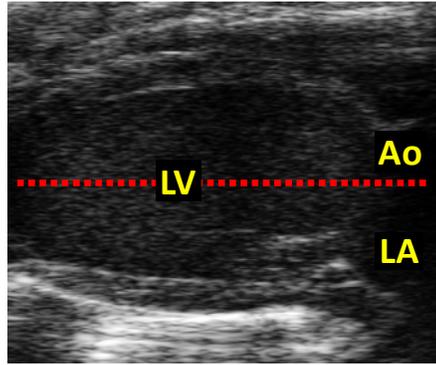
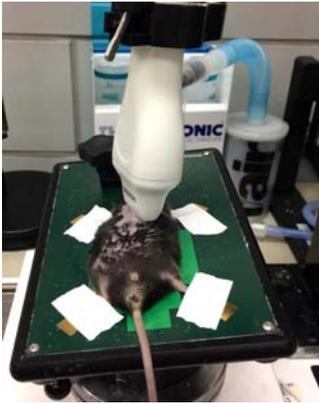


Yellow arrows indicate AR jet.

3. Heart

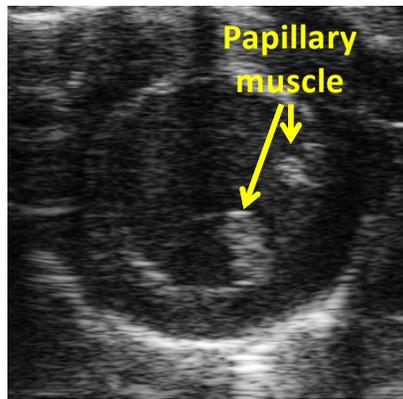
Scan of heart:

1) Heart should be observed using parasternal long axis (PSLAX) view at first.

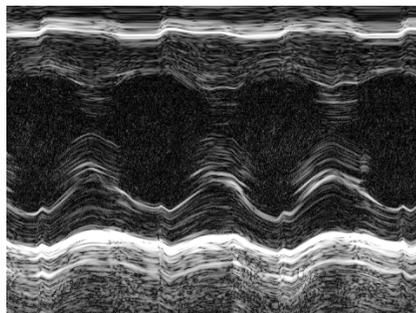
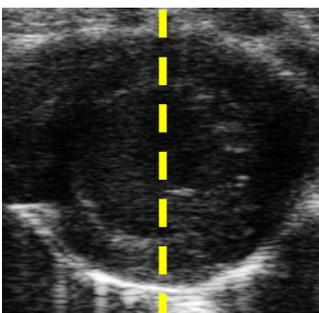


- Change stage angle by tilting mouse head up or down to correct heart angle (red dotted line exactly horizontal). Store one PSLAX image.

2) To capture short axis (SAX) view, rotate the probe 90 clockwise. Store one SAX image.



3) Place M mode line on the center of LV and push the update button for capturing M mode images.



- Keep heart rate between 400-600 bpm. Deep anesthesia would decrease cardiac systolic function.

B mode

M mode



OK

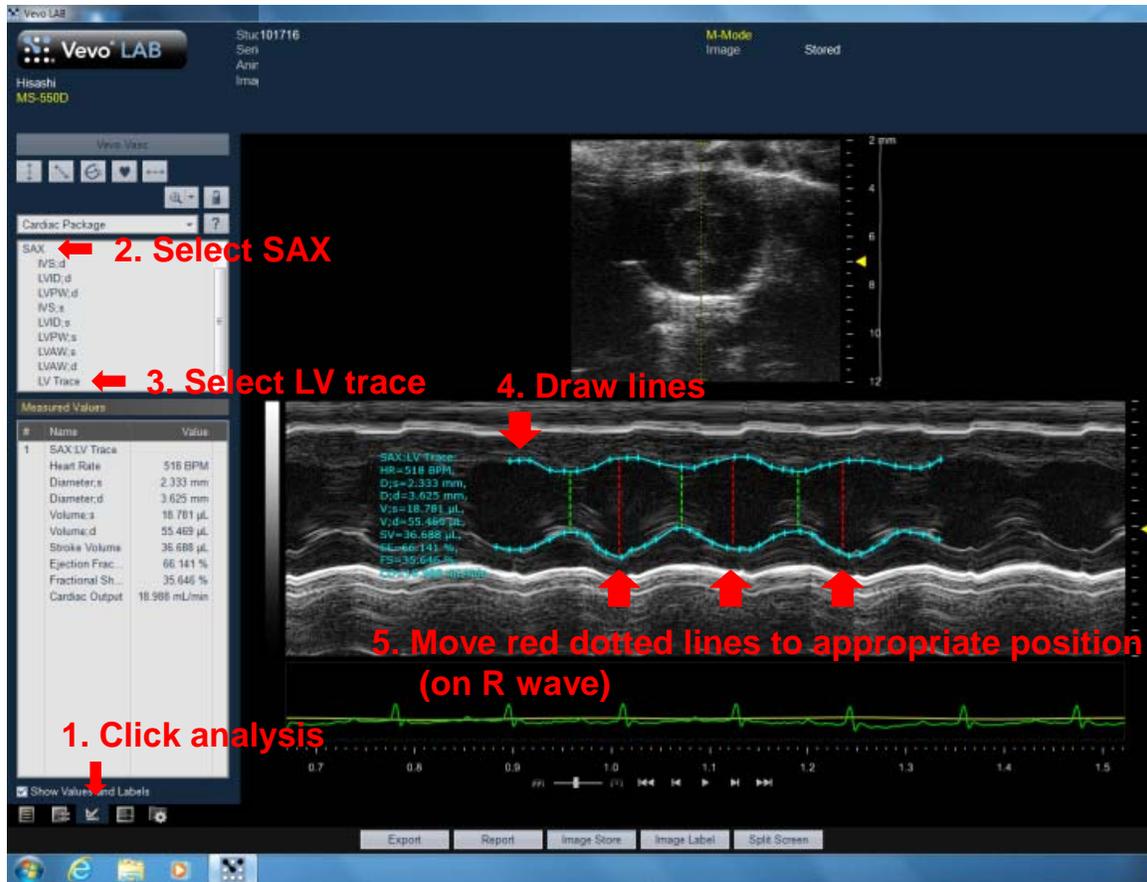


NO

- Make sure the papillary muscle attachment are not within the M-mode measurement line.

Quantification of cardiac function:

0) Open Vevo LAB application (USB key is required for opening the app).



1-3) Click analysis tab and select SAX and LV trace mode.

4) Trace anterior and posterior wall edge through 3 beats.

5) Correct green and red line to appropriate position.

Green (systole): shortest width

Red (diastole): R wave

6) FS, EF, LVDD, and LVDs are calculated automatically.

This method is only for LV dimension, FS, and EF.

If you need more data including LV volume, extra tracings are needed.

Post-scanning mouse care:

1. Ultrasonic gel is wiped off using a kimwipe. Area is irrigated with warmed water and gently wiped dry. Mouse is returned to its cage, which is resting on a heating pad. Mice are only returned to animal housing rooms when they are fully awake.

Clean up:

1. Turn off isoflurane vaporizer. Refill vaporizer if isoflurane level is low. Turn off O₂ tank. If O₂ level is low, please contact DLAR to replace tank.
2. The ultrasound machine, probe, platform and surrounding area is cleaned using a disinfectant supplied by the manufacturer (Transeptic by Parker).
3. Download all files collected during the scan. Turn off ultrasound machine.
4. Return mice to animal housing rooms and heating pad to cupboard.

Protocol Developed: Hisashi Sawada on 3/13/17

Verified: Alan Daugherty on 3/13/17

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