Echocardiography

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Ref: VisualSonics, Vevo2100 Imaging System Operator Manual

Summary:

Transthoracic echocardiography combined with Doppler ultrasound waves is used in conscious mice to provide noninvasive imaging of the heart and allows for quantification of myocardial wall and chamber dimensions and systolic and diastolic performance. Two-dimensional imaging is used to detect abnormal anatomy or movements of the left ventricle, whereas M-mode echo is used for quantification of cardiac dimensions and contractility.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Vevo 2100 Imaging System	Visual Sonics	
Rodent Surgical Monitor Platform	Scintica Indus	
Nair/Cotton swabs		
Eye ointment or gel	Puralube or others	
Lab coats/gloves/PPE		
Isoflurane USP bottles		
Isoflurane vaporizer/ delivery systems	Kent Scientific	
Compressed oxygen tanks		
Charcoal cannisters		
Anesthesia induction chamber		
70% ethanol/paper towels		
Cleaning solution	10% Nolvasan	

Protocol:

1. SET-UP

- **a.** Place mouse in induction chamber and anesthetize with 5% isoflurane. When mouse is ready, switch isoflurane flow to nose cone and set at 2.5%.
- **b.** Transfer mouse to the warmed Rodent Surgical Monitor (RSM) in a **supine** position with the nose in the cone.
- **c.** Apply electrode gel to the four paws and tape them to the ECG electrodes on the RSM.

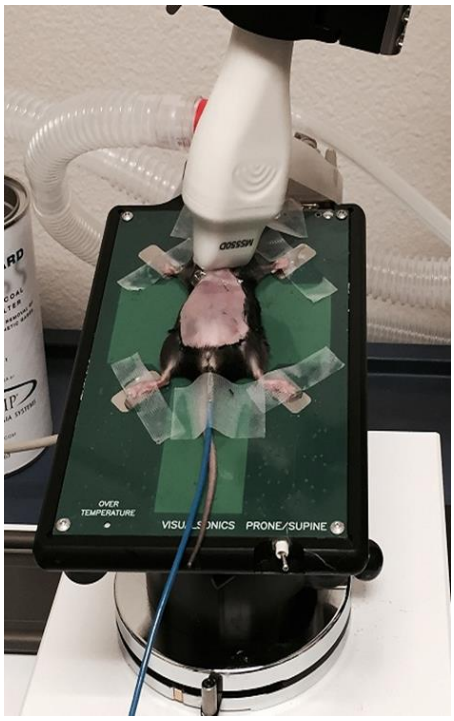
d. Apply Nair to chest area that will be used for imaging with a cotton swab and remove fur with a damp gauze or paper towel after 30 seconds.
e. Apply eye ointment or gel to both eyes with an applicator prevent drying of the sclera.
f. Position the rectal probe and monitor the temperature on the RSM (should be about 37^{o}C).
g. Reduce anesthesia to (1-1.5%) to maintain proper heart rate. Perform a toe or tail pinch to confirm sedation. If the animal shows signs of being awake, use a higher concentration of anesthetic.

IMPORTANT NOTES:

- It is important to maintain the body temperature within a narrow range (37.0 °C ± 0.5 °C), as even moderate changes in temperature and heart rate affect cardiac function in mice.
- Chest area hair removal may be performed a day ahead of procedure.

2. PROCEDURE

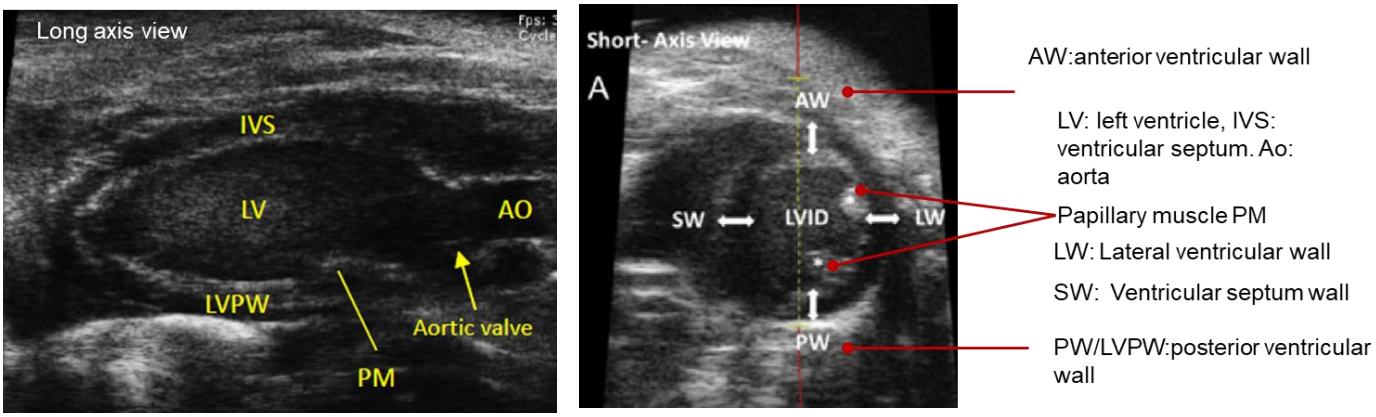
B mode (2-dimensions) & M mode imaging



a. Open the Vevo Study Browser or a Mode window open, press the key for the Mode you want to image in. For example, press B-Mode and select the views: parasternal long-axis, parasternal short axis, four chamber view, etc.
b. Apply gel on to the area to be imaged.
c. Lower the probe to the gel until it makes contact, making sure that all areas of the probe are covered with gel.
d. To view the heart two dimensionally in the parasternal long-axis mode, tilt the platorm at a **45°angle** counterclockwise and place the transducer probe at ~30° clockwise to the animal body on the left side of its sternum with the notch of transducer pointing to the animal head. Adjust gain settings (45 dB) for good visualization of endocardial and epicardial walls. A proper image in this orientation includes the left atrium and ventricle, a slight portion of the right ventricular wall and the output of the aorta, with the heart forming a gourd-like structure. The beginning ascending aorta and the apex of the heart are on the same horizontal line.
e. When a good clear image is obtained press the Cine store button to save data. Each animal should have at least 3 B-Mode, long parasternal axis images captured.
f. Then switch to the parasternal short-axis mode. Rotate the transducer probe so it **is 90°** with the mouse (use papillary muscles as point of reference). A proper image in this orientation will include the left ventricle and a slight portion of the right ventricular wall with the posterior and anterior walls of the LV, the intraventricular septal wall (see figure 2).
g. Capture 3 short parasternal axis view images to assess systolic & diastolic functions.

h. In the short axis view, press the M-mode button and place the yellow line in the middle of the LV. Change the Display window to 1000ms. Use the transducer or move the platform to ensure right placement of the yellow line in the LV. When a good clear image is obtained press the Cine store button to save the M-mode data.

Figure 2: Parasternal long & short view axis for M mode



Pulse Wave Doppler Imaging

a. While still viewing the parasternal short axis, perform tissue Doppler imaging (TDI) to measure velocity of myocardial motion.

b. Pulse-wave Doppler (PWD) is used to measure blood flow velocity within a small area at a specific depth in the myocardial tissue. To image the trans-mitral flow pattern, tilt the animal backwards in the Trendelenburg position. Tilt the probe upward, such that the probe is orthogonal with the apex of the heart.

c. Increase the isoflurane level to lower the heart rate to 300-350 bpm, which will slow the movement of the mitral valve. NOTE: For comparison studies, it is important to maintain similar heart rates between animals.

3. ANALYSIS

Image files are analyzed using the Vevo 2100 software using the cardiac measurement package. Perform the analysis using the best image by tracing a minimum of four cycles as well as averaging two wall measurements for diastole and systole. The data can be derived using the LV trace, the Parasternal long axis (PSLAX), or Parasternal Short axis (SAX) using the protocols specific to the image views and include systolic/diastolic diameter (d;s/d;d), systolic/diastolic volume (V;s/V;d),stroke volume (SV), ejection fraction (EF), fractional shortening (FS), heart rate (HR) and body temp (temp). Power Dopler images can be analyzed with the measurement tools within the software to calculate blood velocity and flow and cardiac output.