

Mouse Echocardiography (iE33).v3

Preparation

1. **Turn on the On/Off bottom** left to the DVD writer. If it takes > 10 min to start it, turn off the machine from a switch at the left rear bottom and push and hold the power switch next to the DVD writer for >10 sec. Start the machine again. This will usually clean up the memory and have a fresh start.
2. **Warm the ultrasonic gel** to about 37°C in hot water in a beaker.
3. **Clean** the chest of the mice under isoflurane anesthesia using Nair.
4. Press the “**Patient Data**” button and enter the date in the “Last Name”, such as 150508 (YYMMDD), and enter a unique ID for “Patient ID” for each individual mouse, such as A1.
5. On the small touch screen on the left, select “**Loop**”. Turn the knob of “Loop Type” to “**Time**” (the other option is “Beat”). Adjust the “Time” knob to select “**2**”.
6. On the small touch screen on the right, select “**2D**” (B-mode), turn “2D Opt Gen” knob counterclockwise to adjust resolution to **140 Hz**, turn “**Focus**” knob to make focus at the level of the heart chambers, turn “**Focus range**” knob to make the focus range about the size of the heart, and turn the “**Manify**” knob to “1”.
7. **Anesthetize** the mouse under isoflurane (Induction 2.5% and 0.5 L/min O₂, maintain 2% and 0.5 L/min O₂) and put the mouse on the stage at a supine position with four limbs fixed. The stage is tilted 30 degree to the left and slightly downward toward you.
8. Apply the pre-warmed **ultrasonic gel** to the left chest.

LV short axis

9. **Position the probe** perpendicular to the long axis pointing to 8 o'clock.
10. Press the **2D key** on the small touch screen and adjust the probe to get parasternal short axis view of the heart with two papillary muscles.
11. Click the **MMode key** to start M mode and 2D mode at the same time. If you do not see the M-mode image, touch the “Upgrade Trace”. The setting of the image could be chosen by touch the “Image” on the small touch screen on the left.
12. Roll the round ball on the System Control Panel to **adjust the scan line** immediately to the left of the papillary muscle close to the posterior wall.
13. Adjust isoflurane flow to get **heart rate to 450 ± 50 bpm**.
14. Press the **Acquire key** to save the M-mode image.

Parasternal long axis

15. Turn the ultrasound probe **90 degree clockwise** and adjust the probe position to get a clear view of the left ventricle.
16. Roll the round ball on the System Control Panel to **adjust the scan line** immediately to the left of the papillary muscle in the middle of left ventricle.
17. Press the **Acquire key** to save the image.

Pulse-wave Doppler for the trans-mitral flow

18. Put the mouse in the **Trendelenburg position** (tilt the right shoulder down).
19. Change the ultrasound probe toward the mouse head and place it under the chest toward the heart (**orthogonal to the apex**). Find the left ventricle.
20. Adjust isoflurane concentration to **low the heart rate** to 300-350 bpm.

21. Touch “PW” on the touch screen on the right and place the “Sampling Region” at the position of the left ventricle chamber.
22. Touch “Upgrade” to obtain the trace.
23. Press the **Acquire key** to save the image.
24. Put the mouse **back into cage** after obtaining enough images.

Data transfer

25. Press the **Review key** and double click on the folder you want to transfer. This will open all the recordings for that mouse.
26. Press “Select All” to **select the recordings**. For the one you do not want to say, you can click on the number of the image to cancel the selection for that recording.
27. Click on “To Media as JPG/AVI” to save the recordings to a blank DVD.
28. Use a blank writable DVD disk to **save the recordings and transfer** to a computer.

Image analysis

Measurement using the caliber function of the echo machine

29. Press the **Review key** and double click on the folder you want perform measurement. This will open all the recordings for that mouse.
30. Select the file by double clicking.
31. Push the “Caliber” button to begin the measurement.
32. Place the cursor and click to **mark the beginning** of the measurement.
33. Move the cursor to a different position and click “Caliber” to **mark the end** of the measure the distance. Repeat step 32-33 to make more measurements. The machine will display the most recent 3 measurements.
34. **Record the numbers** on the Echo Data Sheet.
35. **Calculate cardiac function** offline using the Echo Calculation (Download from our website).

Offline measurement using Image J

36. **Open the video** with “Quick Time Player” and play the movie.
37. **Enlarge the image** as you like by dragging.
38. Open “Grab” and choose “Capture”>”Selection” to **copy the screen and save the image** (TIFF file).
39. **Open** the TIFF image in “Image J”.
40. **To measure distance**, click to select the line tool. Click on one point and holding the mouse and select the other point with “Shift” key pressed to make the line perfectly straight up. Click “Command” and “M” keys at the same time to calculate the distance.
41. **Measure** IVSd, LVIDd, PWd, IVSs, LVIDs, PWs. For each parameter, make three measurements.
42. Measure the scale and record the scale (in mm) for **calibration**.
43. Measure the total heart beats and record the time.

Calculate the following parameters.

HR (bpm) = total heart beats x 60/time

Systolic internal diameter, mm = IVSd

Diastolic internal diameter, mm = IVSs

EF (%) = $(LVIDd^3 - LVIDs^3) \times 100 / LVIDd^3$

FS (%) = $(LVIDd - LVIDs) \times 100 / LVIDd$

$$\text{PWT (\%)} = (\text{PW}_s - \text{PW}_d) * 100 / \text{PW}_d$$

$$\text{LV Mass (mg)} = 1.055 \times ((\text{IVS}_d + \text{LVID}_d + \text{PW}_d)^3 - \text{LVID}_d^3)$$

44. Do the same measurements for PWD images (step 29-33) except you **measure E and A peaks** and use a different scale as calibration.
45. **Calculate** E/A ratio.