

Mouse Echocardiography Protocol.v1

This protocol is for measuring cardiac function in mice with modification from Gao et al. (2011, Echocardiography in mice. Current protocols in mouse biology, 71-83)

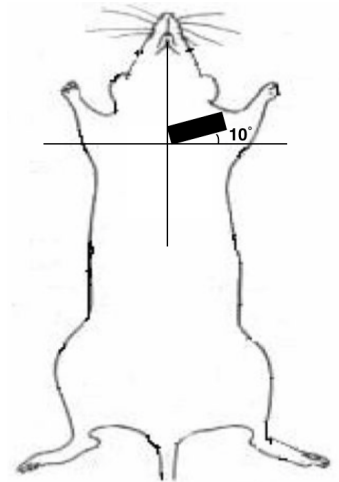
Important Note: Measuring ECG before Echocardiography will avoid influences by isoflurane. Alternatively, measure ECG and echo on different days.

Procedures:

1. **Use Nair to remove hairs** around the left chest area of the mice (use cotton swabs) the day before echo. Make sure the entire area on left side of the chest (from mid chest to arm and neck) are free of hairs.
2. **Turn on the echo machine** by pushing the power button.
3. **Turn on the heating bed** and set temperature (31°C for our system. Make sure the temperature is right) so that the heating bed temperature is 37-38°C.
4. **Enter mouse information** by pressing “Patient” and clicking on “New”; then entering “date + #” (e.g. 210429AY1) for both “MRN” and “Last Name” before measuring each mouse.
5. **Select the mode of data acquisition** by pressing “M-Mode” until screen displays both wave scans (left) and real-time images (right) with a movable cursor.
6. **Anesthetize the mouse** under isoflurane (2.5% and 0.5 L/min O₂), put the mouse on the platform at supine position, then fix the limbs onto the platform with tapes.

LV short axis view

7. **Position the probe** with a 80° angle to the head-tail axis as showed on the right. Mouse head should point away from you.
8. **Tilt the platform** to the left (right of mouse) ~30°, and then slightly tilt it toward you (~20°). **This is a critical step to visualize the heart chamber under ultrasound.** **This may take many practices.**
9. **Apply ample ultrasonic gel** to the chest area where the heart is.
10. **Raise the platform** and have the probe touch the gel, but not the skin.
11. **Look for beating signals** on right screen, by moving the platform away or closer to you. You can start from the upper portion of the chest (closer to neck) and scan slowly down toward the apex of the heart until the probe reaches the posterior wall (PW) of the heart (a clustered, white beating signal on the right screen). PW beating can serve as the starting position.
12. **Find the optimal position** where the chamber is most clearly visible by repeating previous steps and **place the cursor at the middle of the chamber** where the background should be low (dark).
***try to avoid any interferences from papillary muscles which are two white beating bands. They are usually to the bottom right of the center. Sometimes they are just not visible.
13. Press “**Acquire**” to save the view when you have four good quality waves on left screen and the beating chamber on the right is clearly visible.
14. **Repeat the above step to capture** 2-3 good images for the mouse.
15. Press “**Review**” to see the captured images and click on “**Save**” to save the images.

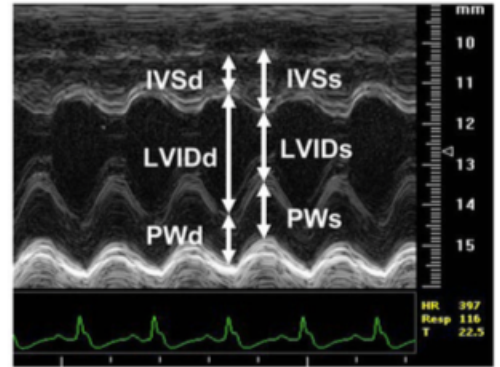


Export Images to DVD

16. **Label an empty DVD** and put it into the DVD drive.
17. **Close any opened study** in image review window. Press “**Search for Study**” and **multi-select the files** you want to export and click “**Export**”.
18. **Format the exports to “PC”** (not DICOM), choose the **highest quality** and export.
19. **Wait** until it is finished. The images are ready for viewing on computers and analysis by Image J.

Image Analysis in Image J

20. Open the echo analysis template (in Excel) and Image J software.
21. Click “**Analyze**” -> “**Set Scale...**” and type “**468**” in “Distance in pixels” in Image J (FIJI). This is the pixel distance of the 20 yellow markers on the right side of the waves.
22. Change “**Known distance**” to 20, and “**Unit of length**” to “mm”.
23. Check “**Global**” (this is important as you will need to use the same scale for all echo analysis in the same study) and click “**OK**”.
24. Measure each of 6 parameters with straight line tool (3 systolic and 3 diastolic) **by pressing “M” on keyboard** following the excel template.
25. To analyze heart rate, count the **number of heart beats** across a horizontal line and measure the according **pixel length** of that line and record both values.
26. The formulas for calculation are listed below (embedded in the excel template).



$$HR = 20280 / ((\text{pixel lengths} * 23.4) / \text{beats\#})$$

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

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

$$PWT(\%) = (PWs - PWd) * 100 / PWd$$

$$LV \text{ Mass (mg)} = 1.05 \times ((IVSd + LVIDd + PWd)^3 - LVIDd^3)$$