Scisense PV Surgical Protocol

Mouse Left Ventricle Acute Pressure-Volume Measurement (Open Chest Approach)

APPLICATION BASICS

Site:	Left Ventricle - Open Chest
Species:	Mouse
Body Weight:	20- 50 grams
Duration:	Acute

CATHETER

Size:	1.2F
Type:	Pressure Volume
Catalog #:	FTH-1212B-3518, FTH-1212B-4018, FTH-1212B-4518

SYSTEM

ADV500 / ADVantage

Application

The hemodynamic properties measured by the pressure-volume system can be used to determine cardiac function. Performing an IVC occlusion as part of the pressure-volume measurement process allows for the determination of load-independent indices.

Anatomical Landmarks

Open chest approach - thorax/upper abdomen area over the xyphoid, proximity of the sternal manubrium. Cut through the diaphragm to expose the apex of the heart. To reduce bleeding avoid incisions around the sternum.

Pre-Surgical Preparations and General Anesthesia

See Research Equipment Sources (RL-90-tn) for recommended equipment suppliers. Prepare an area for scrubbing in a separate location from where the surgical operation will take place. For cardiac surgery, it is best to find low-traffic area. Ideally, clean surfaces using disinfectants with low reaction to organic materials (e.g. Phenolics -- Lysol, TBQ).

Basic surgical supplies for mouse cardiac surgery should include a sterile surgical instrument pack and sterile supplies (i.e. drapes, gauze squares, Q-tips, disposable high-temp fine tip cautery, 5 ml syringes, saline rinse, tray, gloves, mask and sterile suture packs). In addition, a glass bead sterilizer, heating water blanket or approved electrical heating/feedback control unit should be used. Heat lamps are not ideal for body temperature maintenance and can often be a source of electrical noise/interference. Delicate rodent surgical instruments should be inspected for damage before sterilizing.

Set up surgical microscope (interpupillary distance, check light bulbs, adjust to check magnifications), organize surgical table and fine-tune surgical stool to a comfortable setting where the triangular position can be reached (both feet touching the ground with both arms comfortably resting on the surgical table). Turn on glass bead sterilizer.

Prepare 0.9% saline or a similar isotonic fluid and pre-warm the solution if it will be given pre-operatively. When a decision is made to use pre-warmed sterile isotonic fluids subcutaneously it is also suggested to use a preventive analgesia.

Before inducing anesthesia be sure to record weight, age, sex, strain, colony history and health status of each mouse, and determine whether animals have had enough acclimatization time (usually 3 days post arrival). Check mouse's respiratory rate (80-240 breaths/min), heart rate (500-600 beats/min) and temperature (37.1-37.5°C).

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Mouse LV Acute PV Measurement (Open Chest) Cont.

Pre-Surgical Preparations and General Anesthesia Cont.

Shave the animal while on the warming pad using ChronMini cordless clippers. Remove any remaining hair from the surgical area using a depilatory cream (e.g. Nair). Apply surgical scrub alternating between disinfectant (i.e. iodophores, chlorhexidines) and alcohol. Please remember: Iodophores will inactivate a wide range of microbes, however literature describes their reduced activity in the presence of organic matter.

Use gauze squares for scrubbing. Scrubbing should always begin along the incision line and extend outwards, ensuring contaminants are not pulled towards the surgical site. Always scrub larger surface area then surgical field. Do not wet large area of skin or fur with alcohol to avoid hypothermia. Consider using drapes to maintain a sterile field and preserve body temperature.

Pre-anaesthetize mouse for cardiac surgery with 3-4% Isoflurane (Forane) mixed with driving gas (Oxygen) 0.5 L/min inhaled in Plexiglas induction chamber with lid. It is important not to disturb mouse during induction. Apply an ophthalmic ointment to both eyes following induction of anesthesia to prevent corneal drying.

Use pre-cut Styrofoam as a reclined platform with rubber band attached to the edges at the top to allow mouse's neck to be situated at the top with rubber band attached to his upper incisors. Use atraumatic forceps to carefully pull out the tongue. Transorally intubate using a 22-gauge polyethylene catheter with help of fiberscope by directly illuminating ventral area of the neck. IInsert catheter into the larynx past the 2 valves (vocal cords). Ventilate with tidal volume of 0.2 mL, with 128 ventilation cycles per minute. Keep the intubation catheter in alcohol between intubations for disinfection, use 50 mL syringe to clear off any residual alcohol, to avoid aspiration.

When connected to ventilator, inspect breathing pattern, color of membranes and capillary refill time. If feasible, use pulse oximetry. We have found that Isoflurane produces an excellent long-term controllable anesthesia for cardiac surgery as well as for other procedures in the chest cavity. Adequate anesthesia is accompanied by loss of muscle tone and by loss of reflexes (e.g. corneal, pinnae and pedal).

Regulate post-induction anesthesia to 2% with animal placed on a warming pad (38°C) in a supine position, with the upper and lower extremities attached to the table with surgical tape. Maintain mouse on 2% Isoflurane by using rodent ventilator operated in volume-controlled mode with a maximal stroke volume from 30-350 µL, and a positive-end expiratory pressure of 1–3 cm H_2O. Prior to surgery calculate the ventilator set up. Formula is based on animal mass (M_b):

- Respiration rate (RR, min^{-1}) = 53.5 * $M_b^{-0.26}$
- Tidal volume (V_t, ml) = 6.2 * $M_b^{1.01}$

Mouse Weight (g)	RR (min^{-1})	V_t (µl)
20	148	119
35	128	209
50	117	301

It is recommended that a "circle re-breathing circuit" with the vaporizer positioned outside of this system is used for anaesthetic delivery. Control successful ventilation by running blood gas analysis to confirm normal gas exchange.

Prior to surgery, soak the tip of the PV Catheter in 0.9% saline for ~ 20 minutes. Connect the ADV500/ ADVantage system to the data acquisition software, ensuring all channels are calibrated. See Manual and Quick Start Guide for more details. For the Admittance method, constants for "Heart Type" (sigma-epsilon ratio), blood resistivity and stroke volume reference must be input prior to data collection. For Conductance mode all volume calibration will be performed post-acquisition. After soaking, adjust the pressure balance to zero for atmospheric pressure.

Other methods of anesthesia may be used. Be sure to consider cardiovascular impact of anesthetic choice. Please adhere to your institutions guidelines for anesthesia and pain management. See Rodent Anesthesia Guidelines (RL-67-tn) for more considerations.

Mouse LV Acute PV Measurement (Open Chest) Cont.

Surgical Approach

Secure animal in supine position on the heating pad. Make skin incision in the lower thorax/ upper abdomen area over the xyphoid (Fig 1). Separate the skin from the chest wall by blunt lateral dissections. Open the abdominal wall in the proximity of the sternal manubrium (Fig 2). Use 45 cm 5-0 softsilk on 3/8 circle 19 mm cutting needle to penetrate xiphoid and to pull and attach 5-0 suture proximally (Fig 3). Cut through the diaphragm (Fig 4) to expose the heart apex (Fig 5). Try to avoid any incisions around sternum to limit bleeding. Try not to artificially retract rib cage. Gently maneuver the apex, using Q-tips into the diaphragm opening. Using microinstruments, bluntly open pericardium (Fig 6).

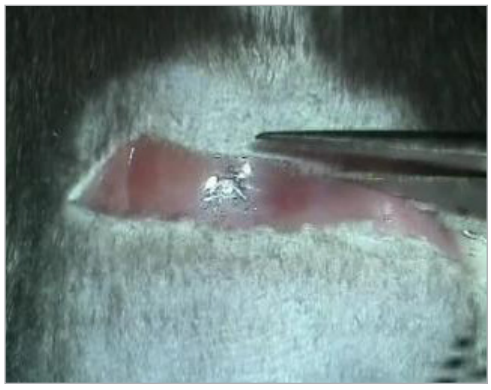

Fig. 1: Initial incision in the upper abdomen

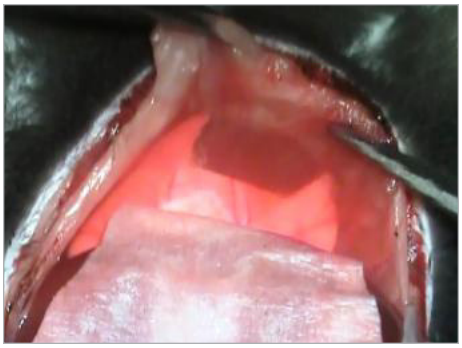

Fig. 2: Opening the abdomen wall

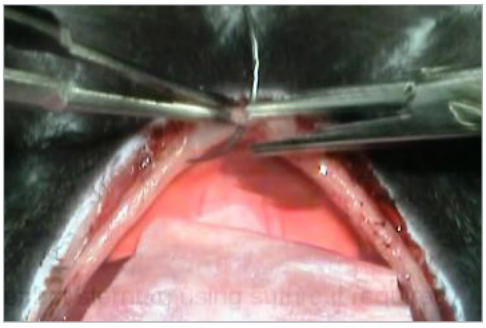

Fig. 3: Use a suture to hold the xiphoid in place

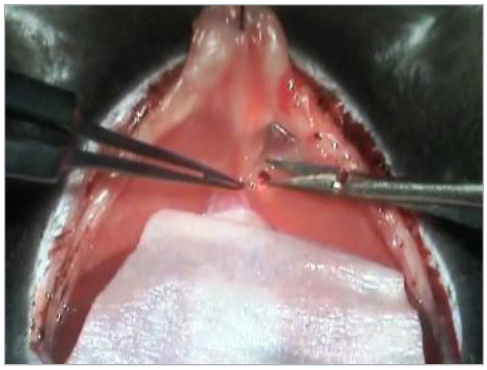

Fig. 4: Cut through the diaphragm

Use the 27 gauge needle for the LV apical stab (Fig 7). After successful stab, blood is found in the needle conus. As needle is withdrawn from the LV myocardium with your hand or with forceps covered by PE tubing, insert the 1.2F Catheter through the stab wound (Fig 8) until the distal electrode of the catheter is fully surrounded by LV muscle (Fig 9). This is critical step where all electrodes have to be fully submerged in the ventricle's cavity. Position the Catheter to control for phase angle (ɵ) and admittance magnitude (Y) and collect pressure-volume (PV) signal in form of graphical tracing known as PV loop.

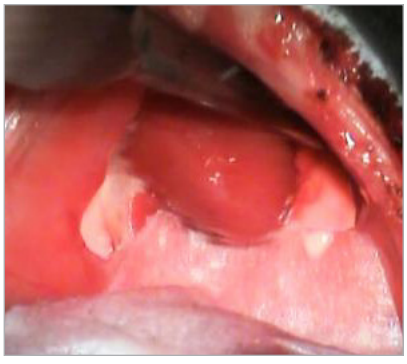

Fig. 5: Expose the heart apex.

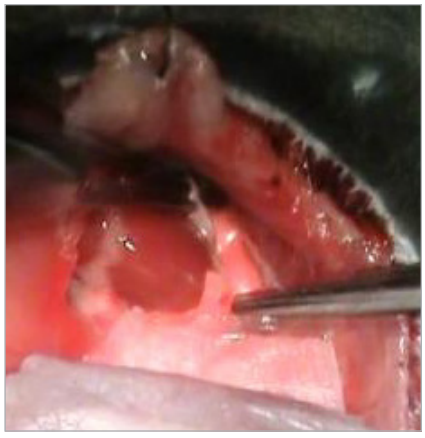

Fig. 6: Open the pericardium

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Mouse LV Acute PV Measurement (Open Chest) Cont.

Surgical Approach Cont.

Allow Catheter to stabilize in the LV for 5-10 min before marking the data file to start protocol. Catheter positional adjustment needs to be made based on acquired signals, mostly coming from phase angle (ɵ) and admittance magnitude (Y) recordings. Both signals should have a sinusoidal wave profile. If the PV Catheter lies in an off-center position the phase signal may be distorted (signals will be relatively high with a low amplitude). Reposition the Catheter until a more central position is found, where magnitude waves are at their largest and phase waves are stable and devoid of noise or spikes. Once optimal Catheter position is obtained, preform a "baseline scan" on the ADV500/ADVantage control unit - end-systolic and end-diastolic blood conductance (Gb_{ed} and Gb_{es}) values will be sampled and reported on the LCD screen. This scan is best conducted when the ventilator is turned off a few seconds prior to scanning and for the duration of the scan. Repeat the baseline scan as necessary throughout the experiment to ensure most accurate report of volume. Record load-dependent values during steady state for at least 10 min for each animal before attempting IVC occlusion.

IVC occlusion is used to derive various load-independent indices of cardiac function. Abdomen is opened and (5-0 silk) suture is placed under the vena cava, carefully separated from adventicia and thoracic aorta, above the liver at close proximity of the heart. This position will ensure an immediate drop of blood volume to better control and compare data sets. IVC occlusions can be performed by pulling on a suture placed around the vessel. Shut off the ventilation for a few seconds prior to and during occlusion to acquire data without lung motion artifacts.

At the end of the experiment, carefully remove the PV Catheter by gently pulling it back through the stab wound. Immediately, insert Catheter tip into 5 ml saline pre-filled syringe. Clean Catheter as soon as possible according to proper care guidelines to considerably prolong the Catheter's life (Catheter Cleaning & Disinfecting Guide).

See Catheter Positioning Guide for more information.

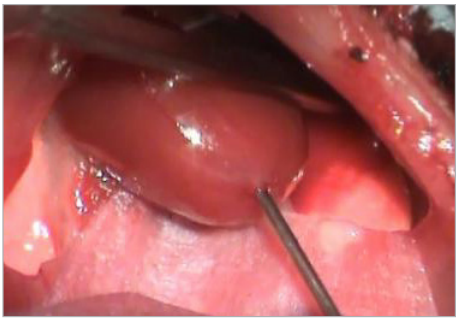

Fig. 7: Stab apex with 27G needle

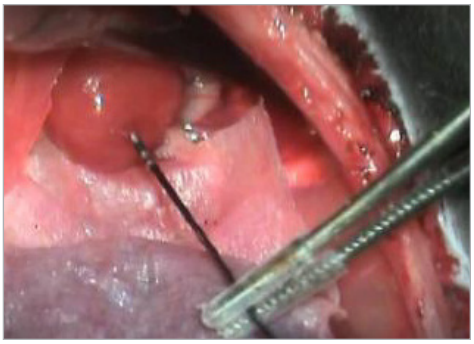

Fig. 8: Carefully insert Catheter into stab wound

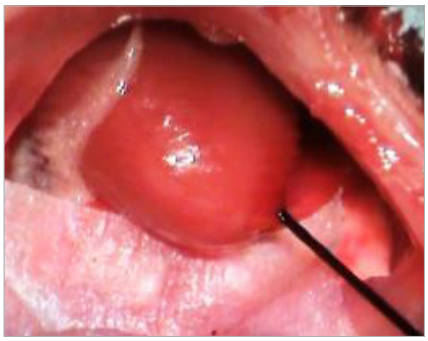

Fig. 9: Submerge all electrodes

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