Scisense PV Surgical Protocol

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

APPLICATION BASICS

Site:	Left Ventricle - Closed 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.

Note: Preforming an IVC occlusion will require a second incision in the abdomen of the mouse.

Anatomical Landmarks

Right Carotid Artery (RCA) passes cranially along the right side of the trachea near the larynx in the close proximity to the vago-sympatic trunk. Major muscles (sternohyoid and strenomastoid) in the area have to be moved aside to allow ventral neck access.

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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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. Insert 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. 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 using saline bolus infusion. 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 (Closed Chest) Cont.

Surgical Approach

For right common carotid artery (RCA) access, secure animal in supine position on the heating pad. Using sharp scissors, starting immediately below the chin, make a straight incision in a direction towards the transversal pectoral muscles. Make the incision as straight as possible while lifting the skin with thumb forceps (Fig 1). Keep the scissor tips up. Using blunt scissors or medium hemostats, blunt dissect any underlying glandular tissue from skin around the entire circumference of the wound (Fig 2). Minor bleeding can be stopped by Q-tips or by pre-made spear shaped nitrocellulose sponges (Harvard app, QC). Keep area moist with warm sterile saline or PBS. Gently separate glands via blunt dissection to expose underlying muscular layer and use retractors to make trachea and ventral neck muscle visible (Fig 3).

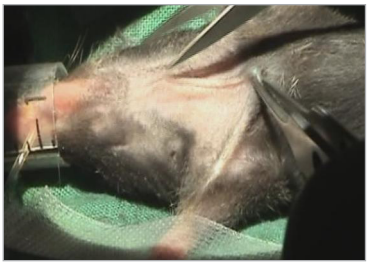

Fig. 1: Initial incision under the chin

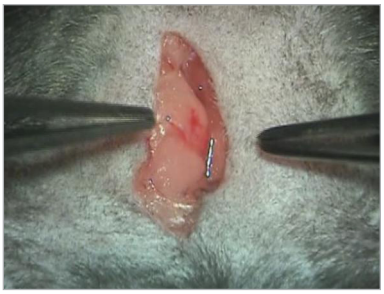

Fig. 2: Dissect glandular tissue from skin

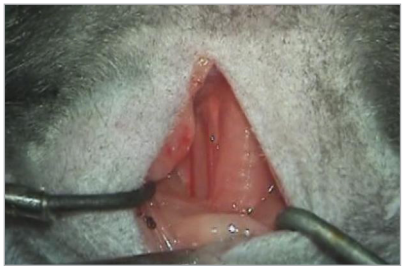

Fig. 3: Retract skin to expose site

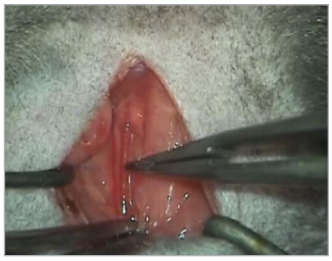

Fig. 4: Expose & dissect RCA

Bluntly dissect along the longitudinal right central and adjacent muscular group (sternocleidomastoid, thyrohyoid, sternohyoid, omohyoid) and remember to avoid pressure on these muscles to maintain the rat's ability to breath. Carefully separate the central muscle from parallel neck muscles and the diagonal thin muscular band (omohyoid) lying directly over the carotid vasculature. Retract skin and muscular tissues for visualization of the underlying carotid artery (Fig 4). Keep the tips of the instruments up and all tissues moist and warm. During subsequent methodical dissection and retraction of adjacent tissue and sheets, RCA can be detected next to vago-sympatic trunk (a thin white sheath lying next to the RCA).

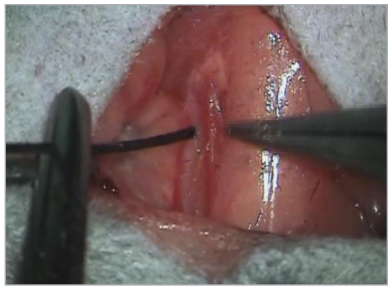

Fig. 5: Use hemostat to draw suture under the RCA

Continue blunt dissection to expose RCA to about 20 mm in length. Dissect alongside the RCA distally towards the head to expose RCA's bifurcation into branches. Ensure that section of the RCA is completely separated from all adjacent tissues to limit an unexpected bleeding during the retraction and/or clamping procedures. RCA must be fully separated from vascular fascia and the vagus nerve.

At this stage 5-0 sutures can be placed around RCA to be used for retraction and/or clamping and hemostasis. Use micro-forceps to place sutures around the RCA (Fig 5). Place the first suture to the most proximal visible end on the RCA (as close to the head as possible) and tie it off using surgical knot (Fig 6), while creating tension with a clamp and retract it towards the head. Place 2nd suture (Fig 7) and retract distally towards the tail. At this point the RCA has been retracted proximally and distally and blood flow has been temporally stopped. Avoid excessive pressure on the vasculature and try to maintain normal vessel geometry.

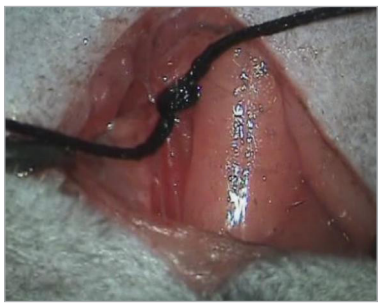

Fig. 6: Tie suture to proximal end of RCA

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Mouse LV Acute PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

Slide 3rd suture under the segment but not tie it off (Fig 7). This suture will be tied off when PV Catheter passes the second suture on the way into the aorta and heart. While creating tension on the distally placed sternal-suture, make a cut with micro-dissecting scissors closer to the head (proximally on the free RCA segment) (Fig 8). Keep in mind longer a isolated section of the RCA will significantly improve chances for successful Catheter introduction.

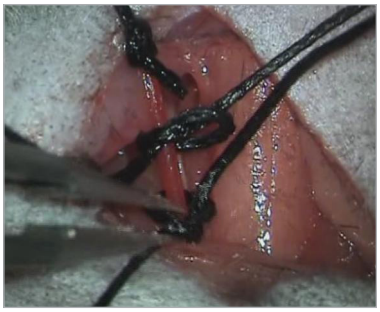

Fig. 7: Three sutures around the RCA

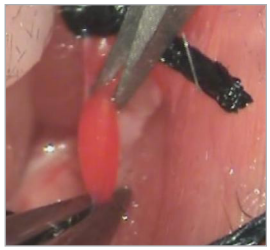

Fig. 8: Carefully cut RCA

Following a successful RCA arteriotomy use vascular introducer as described in Rat RCA catheterization (RPV-2-sp) or micro forceps (Fig 9) to open and lift the incision, while exploring the size of this opening. Especially for a novice surgeon, who might take more time to successfully introduce the Catheter, an introducer might allow more time for location of the insertion in the collapsed RCA, limiting blood loss on catheterization. When completely satisfied with RCA opening carefully proceed (Fig 10) and lift the sternal clamp and insert 1.2F tetrapolar pressure-volume Catheter into the opening passing both sets of volume electrodes. Position and tie off the first suture around the catheter passed the second set of rings (Fig 11). At the same time, please make sure there is not an excessive resistance present upon introduction (vasoconstriction, vessel lumen distortion), which might cause excess bleeding out of the arteriotomy incision on repositioning(s).

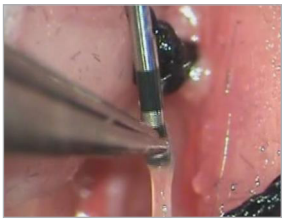

Fig. 9: Carefully insert Catheter

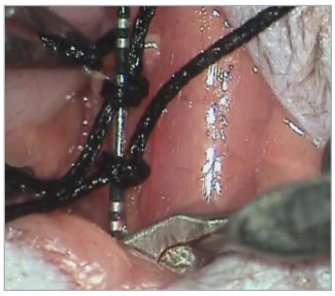

Fig. 10: Proceed with Catheter insertion

With the Catheter in the RCA, get a feel for the degree of resistance while gently rotating the Catheter in the RCA. Slide the Catheter slowly towards the heart. Then tie off the second 5-0 suture around the Catheter to prevent slip out (Fig 12). Be careful not to damage the catheter with the forceps tips, and be sure to hold the Catheter in the same plane as the blood vessel during the entire introduction process (please see technical note, How to Optimize Scisense Pressure and PV Catheter Life Span, RPV-200-tn). 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.

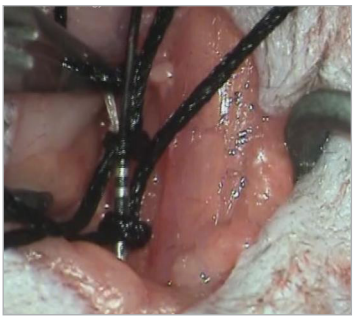

Fig. 11: Fully insert both sets of volume rings past the sutures

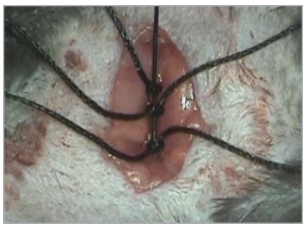

Fig. 12: Secure Catheter in place

ACKNOWLEDGMENTS

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Mouse LV Acute PV Measurement (Closed 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.

See Catheter Positioning Guide for more information.

IVC OCCLUSION

IVC occlusion is used to derive various load-independent indices of cardiac function. In order to preform an IVC occlusion, a second surgical incision must be made in the abdomen to expose the vena cava. Carefully separate the vena cava from adventicia and thoracic aorta, above the liver at close proximity to the heart. The best technique is to place a 5-0 silk suture around the vena cava located as close as possible to 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).

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