Scisense PV Surgical Protocol

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

APPLICATION BASICS

Site:	Left Ventricle - Closed Chest
Species:	Rat
Body Weight:	200 - 500 grams
Duration:	Acute

CATHETER

Size:	1.9F
Type:	Pressure Volume or VSL Pressure Volume
Catalog #:	FTH-1912B-8018 or FTH-1918B-E218

SYSTEM

ADV550 / 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 rat.

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 rat cardiac surgery should include a sterile surgical instrument pack and sterile supplies (i.e. drapes, 4 x 4" gauze squares, Q-tips, disposable high-temp fine tip cautery, 5 ml syringes, saline rinse, tray, gloves, mask, head bonnet 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 rat, and determine whether animals have had enough acclimatization time (usually 3 days post arrival). Check rat's respiratory rate (65-110 breaths/min), heart rate (305-500 beats/min) and temperature (38.1-38.5°C).

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Rat Left Ventricle Acute PV Measurement (Closed Chest) Cont.

Pre-Surgical Preparations and General Anesthesia Cont.

Shave the animal while on the warming pad using a #40 blade attached to Oster small animal clippers (Harvard Apparatus). 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 rats 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 rat 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 rat'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 16-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 2.5 mL, with 85 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 rat on 2% Isoflurane by using rodent ventilator operated in pressure-controlled mode with a maximal airway pressure of 30 cm H_2O, 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 * $M_b^{1.01}$

Rat Weight (g)	RR (min^{-1})	V_t
250	77	1.53
300	73	1.84

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 ADV550/ ADVantage system to the data acquisition software, ensuring all channels are calibrated. See Manual and Quick Start Guide for more details. 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.

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Rat Left Ventricle 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 of the animal, make a straight incision in the direction towards the transversal pectoral muscles. Make the incision as straight as possible while lifting the skin with thumb forceps. Keep the scissor tips up. Using blunt scissors or medium hemostats, dissect any underlying glandular tissue from skin around the entire circumference of the wound. Take care to avoid major bleeding in the area. Minor bleeding can be stopped by Q-tip or gauze squares. Keep area moist with warm saline or PBS. Following this step the skin should be completely separated from underlying tissues all the way around the incision. Using medium scissors, cut as straight as possible through the fascia overlying the glandular tissue to expose underlying glands. Gently separate glands via blunt dissection to expose underlying muscular layer.

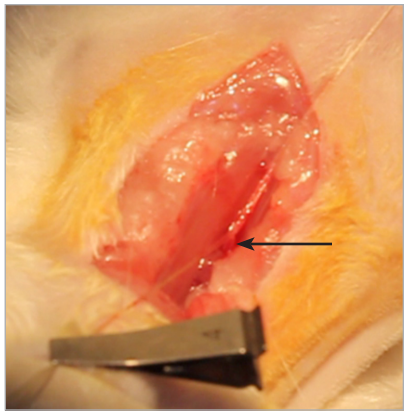

Fig. 1: Isolated RCA with sutures knotted around artery.

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 vasculature. Keep the tips of the instruments up and all tissues moist and warm. During subsequent methodical dissection and retraction of adjacent tissue, RCA can be detected next to vago-sympatic trunk (a thin white sheath lying next to the RCA).

Continue blunt dissection to expose RCA to about 25 mm in length. Dissect alongside the RCA distally towards the head to expose RCA's bifurcations. Ensure that section of the RCA is completely separated from all adjacent tissues to limit unexpected bleeding during the retraction and/or clamping procedures. RCA must be fully separated from vascular fascia and the vegus nerve.

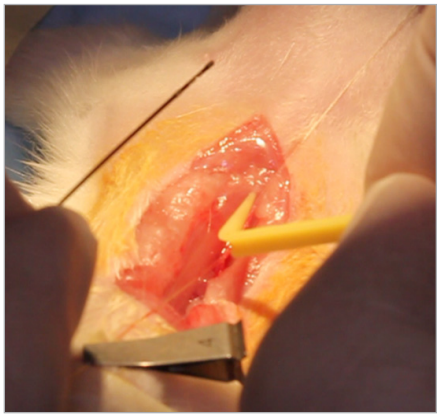

Fig. 2: Vascular inducer (yellow) is used to open the RCA in preparation for Catheter insertion.

At this stage, 5-0 sutures can be placed around the RCA to be used for retraction and/or clamping and hemostasis. Use micro-forceps to place sutures around the RCA. Place the first suture as close to the sternum as possible and then place a hemostat at the end to create tension towards the tail (Fig. 1). Place another suture around the RCA and double-knot tie this suture while creating tension with a clamp. Retract it towards the head (Fig. 1). At this point, the RCA has been retracted proximally and distally. The RCA's blood flow has been temporarily stopped. Note: Avoid excessive pressure on the vasculature and try to maintain normal vessel geometry. While creating tension on the sternal-suture, make a cut with micro-dissecting scissors in the middle of the free RCA segment. Keep in mind, a longer isolated section of the RCA will significantly improve chances for successful Catheter introduction. Next, loosely place a third 5-0 suture around the RCA and slide it towards the sternum. This suture will be tied off when the Catheter passes the first suture on the way into the aorta and heart.

Following a successful RCA arteriotomy, use a vascular introducer to assist in opening and lifting vascular incision, while exploring the size of this opening (Fig 2). Note: Especially for a novice surgeon, who might take more time to successfully introduce the Catheter, the introducer might allow more time for the insertion in the collapsed RCA, limiting blood loss on subsequent attempted catheterizations.

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Rat Left Ventricle Acute PV Measurement (Closed Chest) Cont.

Surgical Approach Cont.

When completely satisfied with the RCA opening, carefully proceed to insert the tetrapolar pressure-volume micro-manometer Catheter (Fig 3). Be careful not to damage the Catheter with the forceps tips and hold the Catheter in the same plane as the blood vessel during whole introduction (Please see "Optimizing Catheter Life Span" on page 42 for best practices). Use the introducer's beveled tip to lift and level the Catheter to the same plane as the sternal RCA opening for a faster and smoother introduction into the first portion of RCA (Fig 4). Make sure there is not excessive resistance present on introduction (vasoconstriction, vessel lumen distortion), which might cause excess bleeding out of the arteriotomy site upon repositioning. Position the Catheter and tie off the first suture around the Catheter past the second set of rings. Ideally, there should be little bleeding. With the Catheter in the RCA, get a feel for the degree of resistance while gently rotating the Catheter in the RCA. Then tie off the third 5-0 suture around the Catheter to prevent it slipping out. Slide the Catheter slowly towards the heart. Position the Catheter using the live volume feedback function of the ADV550. Additional data provided by phase and magnitude will help in this process as well and is covered in several of our technical notes. All signals should measure sinusoid wave signal. 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. This position will coincide with physiological data coming from the volume channel..

Record load-dependent values during steady state for at least 10 min for each animal before attempting IVC occlusion.

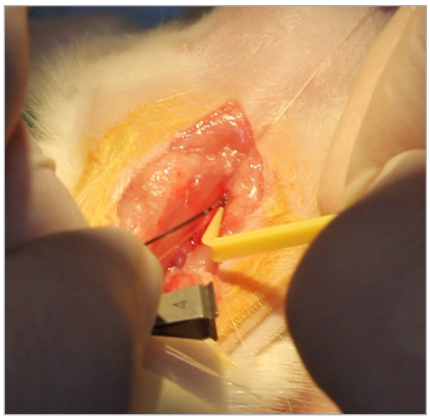

Fig. 3: Carefully remove the introducer and insert the Catheter.

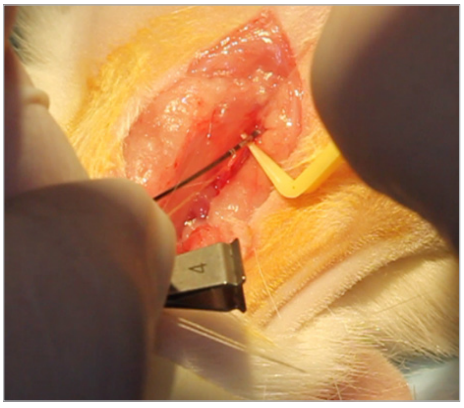

Fig. 4: Use the introducer to help Catheter insertion.

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 volume drop to better control and compare the data sets. IVC occlusion is done by pulling upward on 5-0 suture. 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).

ACKNOWLEDGMENTS

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REFERENCE

Konecny, F., Zou, J., et al. Post-myocardial infarct p27 fusion protein intravenous delivery averts adverse remodelling and improves heart function and survival in rodents. Cardiovasc Res 94, 492-500 (2012)

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