Myocardial injection protocol.

Prior to beginning surgery bring the anticipated number of animals to the “surgical suite” and prepare work area with the necessary items.

Equipment

1. Notebook for documentation:
   a. Animal number.
   b. Vector or vehicle injected.
   c. Quality of injection.
   d. Animal weight.
   e. Other notation as appropriate.

2. Surgical instruments sterilized, including:
   a. Needle holder. (Fine Science Tools, cat. no. 12002-12 $95.50 Olsen-Hegar extra delicate with 1.5 mm jaw surface at serrated tip and 10 mm cutting edge. 1-800-521-2109, 650-349-1636, e-mail: info@finescience.com, http://www.finescience.com, Fine Science Tools Inc. 373-G Vintage Park Drive, Foster City, CA 94494-1139.
   b. Forceps. (FST cat. no. 11002-12 $17.25, 12 cm straight)
   c. Hemostatic forceps. (Fine Science Tools, cat. no.13011-12 $48.25, curved, 12 cm micro-mosquito)
   d. Scissors. (FST, cat. no. 14002-14 $33.50, straight, sharp/sharp)
   e. Rib spreader. (Fine Science Tools, cat. no. 17012-11 $154.25, Weitlaner-Locktite. 2x3 blunt teeth, maximum spread 4.5 cm.)
   f. Scalpel handle. (FST, cat. no 10003-12 $11.25, 12 cm, #3 scalpel handle)
   g. Non-sterile #10 scalpel blade. (FST cat. no. 10010-00 $41.75/box 100)
   h. Vascular clamp. (FST forcep-type clip applicator cat. no. 18057-14 $59.50, 14 cm: micro-serrefines cat. no. 18055-01 $44.25, 10 mm x 2 mm and pressure 60 grams)

3. Beaker (250 ml) with 70% ethyl alcohol for holding surgical instruments.
4. Beaker (30 ml) with sterile water.
5. Beaker (30 ml) with sterile saline.
6. Beaker (1000 ml) with bleach for contaminated waste.
7. Beaker (150 ml) to hold Q-tips.
8. Laryngoscope (Welch Allyn model 60200 Autoclave) with blade (Flagg-0 SS, Welch Allyn 63470). (2.5 vacuum lamp #04700 Welch Allyn, State Street Road, Skaneateles Falls, NY 13153-0220
9. Povidone iodine antiseptic germicide bottle. (The Clinipad Corporation, Rocky Hill, CT 06067, reorder #4444, 4 fl. oz.)
10. Gauze sponges (approximately five to ten). (4in x 4 in- 12 ply, Johnson & Johnson, reorder 8519)
11. Suture (4-0 silk with FS-1 cutting needle), one for each animal. (629H Ethicon)
12. Suture (6-0 ethilon with P-1 cutting needle), one for several animals. (697G Ethilon)
13. Lidocaine viscous 2%. (100 ml bottle, Roxane Laboratories Inc. Columbus, Ohio 43216, NDC 0054-3500-49)
14. Surgical blades, one for several animals. (no. 10 blade Cat. No. 371110 Bard-Parker, rib-back carbon steel, Becton Dickinson Acute Care, Franklin Lakes, New Jersey 07417/Grenoble, France BD 234)
15. Chest tube (16 gauge 1.77 in iv catheter. (B-D, Vialon, Ref 381257) and 10 cc syringe (reorder no. 309604, luer lok, Becton Dickinson, Franklin Lakes, New Jersey 07417-1884)
16. Clippers for shaving incision site.
17. Blue underpads.
   a. Cover work area with one layer of three overlapping underpads. Place one underpad above this layer, in the center of the work area where the surgery will be performed.
   b. Place layer of underpads where the transport container will be place.
18. Vector transport container with ice and vials of the selected vectors.
19. Insulin syringe 1cc U-100 28g1/2(Becton Dickinson, reorder no. 329410)
20. Gloves.

Anesthesia:
1. Verify isoflurane level in vaporizer is adequate and scavenger canister is properly connected.
2. Check tubing connections and adjust flow meter for approximately 0.5 liters per minute.
   a. VIP 3000 Vaporizer, Matrix Medical Inc., 145 Mid County Drive, Orchard Park, NY, 14127-1737, 1-800-847-1000, 1-716-662-6650, fax 716-662-7130, made in England, serial no. 98VAP1215
   b. Visi-Float flow meter, Dwyer Instruments, Inc. 102 Highway 212, Michigan City, IN. 46360, 219-879-8000, fax 888-891-4963, e-mail: info@dwyer-inst.com, http://www.dwyer-inst.com. Cat. no. VFA-22-BV.
   c. AErrane (isoflurane, USP) 100 ml NDC 0856-0773-40. Manufactured for Fort Dodge Animal Health, Fort Dodge, Iowa 50501 USA. Mfg. by: Ohmeda Caribe Inc. Guayama PR 00784
   d. Omnicon f/air canister, reorder cat. no. 80120, A.M. Bickford Inc., 12318 Big Tree Road, Wales Center, New York 14169. 12 hour use life.
3. Place rat in induction chamber with 5% isoflurane setting for approximately 5 minutes. Respirations should be present but will become slow and shallow as induction of anesthesia occurs.
   a. Switch isoflurane flow to ventilator and clamp tubing to induction chamber.

Intubation
1. Intubate rat and place on ventilator rate of 50 to 60 and peak inspiratory pressure of 15-25. Peak inspiratory pressure should be lowest level that achieves adequate chest wall excursion.
a. A 2% lidocaine dipped Q-tip may be used to clear secretions from the laryngeal area to help visualize the vocal cords.
b. With direct laryngoscopy visualize breathing tube passing between vocal cords.
c. Place back on ventilator to allow anesthesia level to equilibrate again.

2. Quickly disconnect rat from ventilator and place on blue underpad and shave left thorax.
a. Brush off cut hair and swab sequentially with povidone iodine, 70% ethyl alcohol, and dry with gauze.
b. Place back on ventilator with isoflurane 3% setting.

Incision
1. Make an incision over third intercostal space of left thorax. With blunt dissection of muscle carefully enter third interspace and widen opening to allow rib spreaders to be placed.
a. Be careful not to damage lung tissue with rib spreaders, and open intercostals space to allow adequate visualization of heart.
b. Wean isoflurane to 1.5 to 2% and titrate to maintain anesthesia while minimizing effect on cardiac inotropy and chronotropy.

2. Use forceps and hemostatic forceps to delicately open pericardial sac.
a. Use Q-tip dipped in saline to push lung out of surgical field and lift apex of heart into intercostal space.

Injection
1. Delicately place superficial 6-0 suture at apex of heart. Gently use this suture to hold gentle traction on heart for positioning heart for vector/vehicle injection.

2. Superficially insert insulin syringe into left ventricular free wall to the left of the left anterior descending artery.

3. Slowly inject while simultaneously withdrawing syringe to enhance area of spread of the injection.

4. Use Q-tip to gently position heart properly within thorax.

Suture
1. Suture incision site with 4-0 silk.
a. Suture rib layer first, placing chest tube between first and second suture loops, and continue until incision is closely approximated. Do not tighten so much as to cause overlap of ribs or intercostals muscle.
b. Suture muscle layer so as to approximate muscle edges without tightening so much as to cause overlap of muscle.
c. Suture skin layer so as to approximate skin edges without tightening so much as to cause overlap of skin edges.

2. Attach 10 or 20 cc syringe to chest tube and apply continuous suction until air is evacuated from pleural space.
a. At peak inspiration quickly remove chest tube while continuing to apply continuous suction.
b. Apply petrolatum skin protectant over incision to enhance air seal.

Extubation
1. Wean isoflurane to 0%.
2. Mark rat-tail to corresponding rat number.
3. Disconnect ventilator from breathing tube and observe for adequate spontaneous respiratory effort. If inadequate, place back on ventilator for a few minutes. Then remove again from ventilator and observe for adequate spontaneous respiratory effort.
4. Weight animal and place in cage. As animal wakes up remove breathing tube from mouth.

Clean-up
1. Place brown water bottle with tetracycline (0.5 mg/quart distilled water) in cage along with food.
   a. Label each cage with date of surgery, injection vector, date to change water bottle and other appropriate information.
   b. Place cages in appropriate animal rooms.
2. Clean laboratory area.
   a. Bleach instruments and virus contaminated waste in beakers.
   b. Dispose of virus contaminated underpads in appropriate biohazard bag.
   c. Dispose of any virus contaminated animals in appropriate biohazard animal storage area (on third floor) with appropriate identification.
   d. Wipe any potentially virus contaminated surface or area with Lysol.
   e. Immediately wash and dry instruments and place in autoclave packet. Place in autoclave prior to leaving work.
   f. Wash induction chamber.
   g. Wipe vaporizer clean and dry. Verify airflow is turned off and that vaporizer is turned off.
   h. Make sure all sharps have been properly disposed off.
3. Inspect area and enter animal data in notebook.