

Shock Center Protocol

Protocol: Ultrasonography, including Echocardiography -

Date: 2/8/17

Originator: J Neal

Note:

Following is the FGB protocol for PIXI. The FGB performs PIXI for the Shock Center.

1. Procedure Description

Include exact details of any/all chemical, biological, radiation, or physical agents as well as route(s)/ dose(s)/ volume(s)/ frequency and duration

Ultrasonography will be performed using a VisualSonics Inc. (VSI) Vevo 770/2100 high-frequency ultrasound with 30 and 40 MHz probes. The mice will be anesthetized with Isoflurane at concentrations up to 5% and flow rate of 0.8 – 2.0 L/min in oxygen. Ophthalmic ointment is placed on the eyes to prevent drying of the cornea while the mouse is anesthetized and tested. After anesthetic induction the animals will be placed on a thermostatically-controlled heated platform where isoflurane anesthesia will be maintained by delivery through a close fitting face-mask. The delivered isoflurane concentration will be the minimal necessary to keep the animals immobile for the duration of the examination; generally this will be between 0.5 and 2%. During the examination the animal's heart rate will be monitored through use of an electrocardiograph. Once in position the appropriate area of the animal's skin overlying the area of interest will be cleared of hair either by the use of clippers or a depilatory agent, such as "Nair". If a depilatory is used, it will be allowed to remain in contact with the animal's skin for as brief a time as possible to achieve the desired effect. After this time, remaining agent will be wiped off and the skin rinsed with water to remove any trace amounts.

Once prepared in this way, heated ultrasound transmission gel will be applied to the animals skin to act as a coupling-medium. The ultrasound probe is then brought into contact with the animal's skin through the gel and appropriate images acquired according to the experimental design. Echocardiography uses pulsed Doppler sonography, applied through the ultrasound probe, to measure blood flow rates and volumes. At completion of the examination the transmission gel will be removed by gently wiping the animal down after which the mice are allowed to recover from anesthesia in a heated environment (either with a heating pad and/or a heat lamp) until ambulatory. Then they will be returned to their normal housing.

When contrast agents are required, PolySon microbubbles (Miltenyi Biotec, Inc.) will be introduced into the vascular stream via tail vein injection or through a jugular catheter. Commercially available microbubbles are composed of a perfluorocarbon gas core encapsulated by a lipid shell, stabilized and shielded by a layer of polyethylene glycol, suspended in normal saline or PBS. The microbubbles are generally 0.5-5µm in diameter and therefore do not leave the vascular space.

Animals are anesthetized and prepared as described above except that they are anesthetized with isoflurane diluted in 30% oxygen rather than 100% oxygen. High concentrations of oxygen will reduce the half-life of circulating microbubbles. Once prepared in this way, the animal will be injected with a slow bolus of 50µL of microbubbles into the lateral tail vein using a 25-30G needle. If necessary, contrast agent will be delivered through the previously placed jugular catheter. Bubbles are cleared between 10-20 mins and if there is successful delivery then no more than 2 injections per animal will be necessary. A maximum of 4 injections

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may occur per one mouse, with no more than 200uL of microbubbles injected. Bubbles will be re-injected once they have cleared from the system and the image has returned to baseline.

For imaging the colon, mice will receive a corn oil enema (~ 1.0-1.5 ml) as a negative contrast agent, as in Pickhardt et al, 2005, after anesthesia.

Imaging and micro-injecting neonates is also possible using isoflurane or deep hypothermia, also referred to as cryoanesthesia as described in . By using the ultrasound and rail system to image, the user can more accurately inject into the desired sites (heart, brain, etc).

2. Anesthetic/Analgesic Regimen

- a. Please list all anesthetics/analgesics used in this procedure in the following table.

If not applicable, please check here NA

Anesthetic Agent	Diluents Used	Dose & Route of Administration (e.g. 1mg/kg I.V.)	Volume
Isoflurane	Oxygen	By inhalation to effect : 1-5%	1-5% @ 1.5L/min

Analgesic Agent	Dose & Route of Administration (e.g. 1mg/kg I.V.)	Volume

- b. Supportive care while animal recovers from anesthesia:

After imaging mice are placed in a recovery box. Half of the box is warmed to 80-86°F and animal has the opportunity to move away from the heat source. To avoid overheating the temperature inside the cage is monitored with a thermometer at rodent level prior to animal placement, and frequently thereafter. When the animals have fully recovered they are returned to cages and checked once again before the end of the day.

3. Post Procedure Care

Describe post procedure care, including frequency of observations, schedule for removal of sutures/clips, etc...

During the time the mouse is recovering from anesthesia, with supportive care as described above, guidelines on supportive care while animal recovers from anesthesia (found in the "Standards for Rodent Survival Surgery at The Jackson Laboratory") will be followed.

4. References if applicable:

Pickhardt, PJ, Halberg RB, Taylor AJ, Durkee BY, Fine J, Lee FT Jr and JP Weichert, Microcomputed tomography for polyp detection in an *in vivo* mouse tumor model. PNAS (2005), 102 (9), 3419-22.