

Longwood SAIF Boiler-Plate Language for Animal Protocols

The information below pertains to sedation, imaging modalities, and euthanasia. You can copy and paste the following information into your animal protocol or animal protocol amendment. Please only use the information that is appropriate to the species that you are using. If you have any questions or comments, please contact:

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Introductory Paragraph for your Amendment: The Longwood Small Animal Imaging Facility (Longwood SAIF; www.longwoodsaif.org) provides state-of-the-art molecular imaging to Harvard Medical School (HMS) researchers and their collaborators. Conveniently located at the Beth Israel Deaconess Medical Center, in the center of the Longwood Medical Area of HMS, this 1,100 sq ft custom-built facility performs bioluminescence, multi- and hyper-spectral fluorescence, microCT, microPET/CT, and microSPECT/CT studies on mice, rats, hamsters, guinea pigs, frogs, small rabbits, and new world monkeys. It also houses a satellite animal facility for longitudinal scanning of living animals, a hot cell and associated equipment for custom chemistry, a dedicated cryostat for autoradiography, and several image analysis and image fusion workstations. The Longwood SAIF is a fee-for-service facility run by skilled technicians, and is co-directed by John V. Frangioni, M.D., Ph.D., and Robert E. Lenkinski, Ph.D., who are always available for assistance with experimental design and data interpretation.

How will you sedate the animal?

Animals will be anesthetized in accordance with the Principal Investigator's approved protocol; the table below lists the possible methods that are available on-site.

TYPES OF ANESTHESIA	SIDE EFFECTS	SPECIES
Ketamine	Used with xylazine for anesthesia. No side effects anticipated at the dosage used.	Mouse, Rat, Hamster, Rabbit, Guinea Pig
Xylazine	Used with ketamine for anesthesia. No side effects anticipated at the dosage used.	Mouse, Rat, Hamster, Rabbit, Guinea Pig
Pentobarbital	Used for anesthesia. No side effects anticipated at the dosage used.	Mouse, Rat, Hamster, Rabbit, Guinea Pig
Isoflurane	Used for inhalation anesthesia and titrated to effect. No side effects anticipated at the dosage used.	Mouse, Rat, Hamster, Rabbit, Guinea Pig, Frog
Tricaine	Used for anesthesia. No side effects anticipated at the dosage used	Frog

Anesthesia Doses:

DRUG/SPECIES	MOUSE	RAT	HAMSTER	RABBIT	GUINEA PIG	FROG
<i>Ketamine / Xylazine</i>	100 mg/kg Ket. / 10 mg/kg Xyl. given Intraperitoneal, redose ketamine only at ½ dose	40-60 mg/kg Ket. / 3-5 mg/kg Xyl. mixed together and given Intraperitoneal, redose ketamine only at 1/3 dose	80-100 mg/kg Ket. / 7- 10 mg/kg Xyl. mixed together and given Intraperitoneal	40 mg/kg Ket. / 5-10 mg/kg Xyl. mixed together and given Intramuscular	35 mg/kg Ket. / 5 mg/kg Xyl. mixed together and given Intramuscular	N/A
<i>Pentobarbital</i>	70-80 mg/kg Intraperitoneal	40-50 mg/kg Intraperitoneal	70-90 mg/kg Intraperitoneal	20-40 mg/kg Intravenous	30 mg/kg Intraperitoneal	N/A
<i>Isoflurane</i>	1-3%	1-3%	1-3%	1-3%	1-3%	bubbled into water to effect
<i>Tricaine</i>	N/A	N/A	N/A	N/A	N/A	50-100 mg/liter via skin absorption

Hair Removal:

If hair removal is necessary, i.e., for certain fluorescence or bioluminescence experiments, then insert the following paragraph:

The preferred method of preparing animals for imaging is to shave them with a #10 blade clippers. For complete fur removal, the depilatory Nair (Carter-Horner, Montreal, Quebec) is applied. Nair is applied to the anesthetized animal with a cotton-tipped swab or cloth for 1-2 min then fur is wiped off. Some strains require a second 1-2 min application.

Imaging Modalities Available:

The following is a description of each imaging modality available:

i) **Fluorescence:** Fluorescence light imaging is rapid, painless, and harmless to the animal. The typical experiment involves shining excitation light of the desired wavelength on the animal at a fluence rate of approximately 4-20 mW/cm². This fluence rate is about the same as a brightly lit room, or outside on a sunny day. The emission light reflected from the animal is then imaged with a camera. A typical image takes 1second to 1 minute to acquire.

ii) **Bioluminescence:** Bioluminescence imaging is a high-sensitivity, low-noise, non-invasive technique used for visualizing, tracking, and monitoring specific cellular and genetic activity in an animal. This specificity comes from the ‘tagging’ of the target gene or cells with a luciferase enzyme. When the genes are activated, they emit light, which the system can passively detect and record expression of the gene. For those experiments that require the Luciferin substrate to be injected, a typical dosage for a 10g mouse would be 100 µl of a 15 mg/ml stock to deliver 1.5 mg of Luciferin given intraperitoneally. Experiments needing the coelenterazine substrate would typical have an intravenous injection of 100 µl of a 28 mg/ml stock to deliver 2.8 mg coelenterazine. A typical image can take 1 to 5 minutes to acquire.

iii) **Computed Tomography:** Computed tomography is performed routinely on humans and involves a test subject (in this case the anesthetized rodent) lying motionless on a table for a period of 1-60 minutes while tomographic x-rays are used to “see” inside the animal.

iv) **Single Photon Emission Computed Tomography (SPECT):** SPECT imaging involves the injection of a radioisotope, usually intravenously, followed by imaging with a gamma camera that is rotated around the animal to form a 3D image. SPECT is used routinely in humans for studies such as bone scans (using technetium-99m-methylene diphosphonate) and heart scans (using technetium-99m-MIBI). Depending on the experiment, 0.1 to 10 mCi of radioisotope will be injected into the anesthetized mouse or rat. After a “washout” period determined by the results of a preliminary biodistribution and clearance, typically 1 hour for small molecules of less than 5,000 Da and 6 hours for molecules of 40,000 Da, the animal is imaged on a Siemens InVeon SPECT scanner. For studies involving repeat scanning, animals will be housed in the Longwood SAIF satellite animal facility between scans.

v) **Positron Emission Tomography (PET):** PET imaging involves the injection of a radioisotope, usually intravenously, followed by imaging with a coincidence camera, which is a stationary ring of photon-sensitive detectors. PET is used routinely in humans for studies such as bone scans (using sodium fluoride-18) and tumor scans (using fluorine-18-deoxyglucose). Depending on the experiment, 0.1 to 10 mCi of radioisotope will be injected into the anesthetized mouse or rat. After a “washout” period determined by the results of a preliminary biodistribution and clearance, typically 30 minutes for small molecules of less than 5,000 Da and 2 hours for molecules of 40,000 Da, the animal is imaged on a Siemens InVeon PET scanner. For studies involving repeat scanning, animals will be housed in the Longwood SAIF satellite animal facility between scans.

Disposition of Animals Following Study:

Will all animals described in this protocol be euthanized upon completion of this experimentation?

For BIDMC animals: Dependent on the Principal Investigator’s protocol

For Non-BIDMC animals: All animals will be euthanized after completion of experiment

All protocols (regardless of answer to above question) must include a euthanasia plan for each animal species if needed.

The following method(s) of euthanasia are available:

Agent Used and Dose per Species:

DRUG/SPECIES	MOUSE	RAT	HAMSTER	RABBIT	GUINEA PIG	FROG
<i>Pentobarbital</i>	200 mg/kg Intraperitoneal	200 mg/kg Intraperitoneal	200 mg/kg Intraperitoneal	120 mg/kg Intravenous	120 mg/kg Intraperitoneal	120 mg/kg Intraperitoneal
<i>Carbon Dioxide</i>	Asphyxiation	Asphyxiation	Asphyxiation	N/A	Asphyxiation	N/A
<i>Tricaine</i>	N/A	N/A	N/A	N/A	N/A	100-200 mg/liter via skin absorption

Individual(s) performing euthanasia: Elaine Lunsford, B.S., John V. Frangioni MD, Ph.D., Robert E. Lenkinski Ph.D.