

Title:
Small Animal Molecular Imaging Facility Procedures

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A. Purpose

This S.O.P. describes the range of small animal imaging procedures that are provided by the Jefferson Small Animal Molecular Imaging Facility (SAMI, 387 JAH) in mouse and rat species.

B. Introduction

The mission of SAMI at TJU is to facilitate basic and translational research in which usage of small animals plays a prominent role. SAMI can monitor and record biological interactions at molecular and cellular level in a living system and thereby allow longitudinal studies in the same species without having to sacrifice scores of them for statistical evaluation of data. These animals can serve as their own controls and allow investigators to monitor molecular interactions in vivo that may up regulate or down regulate disease processes. In most cases this type of imaging requires animals to be injected with a radioactive biomolecule that targets a key biomarker that controls the disease process.

The imaging aspect of SAMI therefore is associated with two institutional jurisdictions. First the animal care and use committee (IACUC) and the second, the radiation safety office (RSO). This S.O.P. is designed for better understanding of small animal imaging procedure, the animal care issues and the approvals the investigators need to seek, prior to imaging.

Currently, the facility includes microPET (positron emission tomography), microSPECT (single photon emission computerized tomography), and microCT (computerized tomography). Configurations of all micro-imaging devices, including these, limit the imaging of animals to mice and rats (entire body) and rabbits and small non-human primates (head only). This S.O.P. only addresses the imaging procedures for mice and rats as these are the most commonly used species. The procedures for imaging rabbits and non-human primates may vary depending upon the need of a given investigator and therefore the S.O.P. for these procedures are not included here.

C. Radioactive Imaging Agents

To enable PET and SPECT imaging a trace amount of a radionuclide is administered to the animals, usually intravenously. The radiopharmaceutical to be used is chosen depending on biological process to be measured.

The radionuclides which are the integral part of the radiopharmaceuticals to be used and which facilitate imaging are divided into three classes based on their half-life.

C.1. Class A ($t_{1/2} \leq 2.4\text{h}$)

The radionuclides with half-lives less than 2.4 hours will decay to background levels in 24 hours (10 half-lives, <0.05 mR/hr). Animals injected with these radionuclides will be housed in the imaging facility for 24 hours post injection, but not longer, allowing decay of the radioactivity to background levels. Animals will then be returned to the investigators. Class A radionuclides include:

F-18 ($t_{1/2} = 109\text{min}$)

F-18 is a positron emitting radioisotope with a 109 minute half-life and is used for PET imaging. Common commercially available radiopharmaceuticals labeled with F-18 include bioprobes to measure processes such as glucose metabolism, cell proliferation and inhibition, dopamine synthesis, hypo or hyperoxia, and apoptosis.

Recommended doses of F18 labeled radiopharmaceuticals, administered i.v., are,

Mice: $\leq 1.0\text{mCi}$ in ≤ 0.2 ml

Rats: $\leq 5.0\text{mCi}$ in ≤ 2.0 ml

Currently only one F-18 labeled radiopharmaceutical, F-18–Fluorodeoxyglucose (F-18-FDG), is commercially available. However, it is expected that other F-18 labeled compounds may be available commercially in the future. TJU does not have a facility to prepare these compounds in house. Other useful radionuclides for PET imaging that fall in this group are O-15 ($t_{1/2} = 2$ min), N-13 ($t_{1/2} = 10$ min), C-11 ($t_{1/2} = 20$ min). Their short half life prevents them from commercial availability and TJU does not have the facility to produce them in house. These require a in-house cyclotron. Ga-68 ($t_{1/2} = 68$ min) and Rb-82 ($t_{1/2} = 20$ min) can be produced on commercially available “generators”. In this group no radionuclides are commercially available that can be used for SPECT imaging.

C.2. Class B ($2.4\text{h} < t_{1/2} \leq 24\text{h}$)

Radionuclides in this group have half-lives between 2.4 to 24 hours. These radionuclides will require from between 24 hours to 10 days to decay to background levels (10 half-lives, <0.05 mR/hr). Animals injected with these compounds will be housed in the imaging facility for a maximum of 24 hours and then returned to the care of the investigators. As these animals will still have radioactivity greater than background levels, the investigators are required to have the imaging isotope on their radiation license and have secured proper housing arrangements with LAS for the remainder of radiation decay. Furthermore, these animals will require a Radiation Safety Office transfer of activity from Dr. Thakur’s license to the investigators license. Class B radionuclides include:

Cu-64 ($t_{1/2} = 12.4\text{h}$)

Cu-64 is a positron emitting radio isotope with a 12.4 hour half-life to be used in PET imaging. Cu-64 in ionic form is available commercially. Pharmaceuticals labeled with Cu-64 used for PET imaging are synthesized in Dr. Thakur's laboratory and include bioprobes to measure processes such as oncogene expression, hypoxia, and cell proliferation.

Recommended doses of Cu-64 labeled radiopharmaceuticals, administered i.v., are,

Mice: $\leq 1.0\text{mCi}$ in $\leq 0.2\text{ ml}$

Rats: $\leq 5.0\text{mCi}$ in $\leq 2.0\text{ ml}$

Tc-99m ($t_{1/2} = 6.0\text{h}$)

Tc-99m is a single photon emitting isotope with a 6.0 hour half-life used in SPECT imaging. This radionuclide and several radiopharmaceuticals labeled with it are available commercially and can be used for SPECT imaging of thyroid, certain types of cancers, brain and cardiac perfusion, bone metastases, and renal function to name a few.

Recommended doses of Tc-99m labeled radiopharmaceuticals, administered i.v., are,

Mice: $\leq 1.0\text{mCi}$ in $\leq 0.2\text{ ml}$

Rats: $\leq 5.0\text{mCi}$ in $\leq 2.0\text{ ml}$

I-123 ($t_{1/2} = 13.2\text{h}$)

I-123 is a single photon emitting isotope with a 13.2 hour half-life used in SPECT imaging. This radionuclide is available commercially. Pharmaceuticals labeled with I-123 used for SPECT imaging can be synthesized in Dr. Thakur's laboratory. These include bioprobes to measure dopamine neuron function, thyroid function, tumor imaging ect..

Recommended doses of I-123 labeled radiopharmaceuticals, administered i.v., are,

Mice: $\leq 1.0\text{mCi}$ in $\leq 0.2\text{ ml}$

Rats: $\leq 5.0\text{mCi}$ in $\leq 2.0\text{ ml}$

C.3. Class C ($t_{1/2} > 24\text{h}$)

Radionuclides in this group have half-lives greater than 24 hours. These radionuclides will require 10 days or longer to decay to background levels (10 half-lives, $<0.05\text{ mR/hr}$). Animals injected with these compounds will be housed in the imaging facility for a maximum of 24 hours post-injection and then returned to the care of the investigators. As these animals will still have radioactivity greater than background levels, the investigators are required to have the imaging radio isotope on their radiation license and have secured proper housing arrangements with LAS for the radiation decay. These animals require a Radiation Safety Office transfer of activity from Dr. Thakur's license to the investigators license. Class C radionuclides which is available commercially include:

I-124 ($t_{1/2} = 4.2d$)

I-124 is a positron emitting radio isotope with a 4.2 day half-life used in PET imaging. This radionuclide is available commercially. Pharmaceuticals labeled with I-124 used for PET imaging can be synthesized in Dr. Thakur's laboratory. These include bioprobes to measure processes such as oncogene expression, hypoxia, cell proliferation, thyroid function and tumor imaging.

Recommended doses of I-124 labeled radiopharmaceuticals, administered i.v., are,

Mice: $\leq 1.0mCi$ in $\leq 0.2 ml$

Rats: $\leq 5.0mCi$ in $\leq 2.0 ml$

D. Non-Radioactive Imaging Agents

To enable CT imaging tissue contrast agents may be employed. Contrast agents to be used in the lab include:

Isovue-M 300: An iodinated vascular contrast agent with fast clearance.

Recommended doses, administered i.v., are,

Mice: 0.1 ml / 25 g

Rats: 0.1 ml / 25 g

Fenestra VC: An iodinated vascular contrast agent with slow clearance.

Recommended doses, administered i.v., are,

Mice: 0.3 ml / 20g

Rats: 0.3 ml / 20 g

Fenestra LC: An iodinated hepatobiliary contrast agent with slow clearance.

Recommended doses, administered i.v., are,

Mice: 0.3 ml / 20 g

Rats: 0.3 ml / 20 g

E. Anesthesia

To facilitate either imaging compound injections or imaging (without motion) the animals may be anesthetized. One of four methods of anesthesia administration may be used.

1. Either an i.p. or s.c. injection of a mixture of Ketamine (**150-200** mg/kg), Xylazine (**6-10** mg/kg), Acetpromazine (**1-2** mg/kg) to achieve deep sedation.

2. Either an i.p. or s.c. injection of a mixture of Ketamine (**70-80** mg/kg), Acetpromazine (**0.3-0.4** mg/kg) to achieve light sedation for short procedures or when the increase of glycemic index due to Xylazine is to be avoided.

3. A i.p. injection of a mixture of Pentobarbital (**30-45** mg/kg) and Atropine (**0.15-0.25** mg/kg).

4. Inhalation of Isoflurane (1.5%) mixed with Oxygen (98.5%) supplied by a precision vaporizer. To initiate anesthesia the animals will be placed in an induction chamber. During injections and imaging, sedation will be maintained either through a nose cone or the animal will be placed in an air tight holder. Carbon filters will be used on exhausted air to remove isoflurane.

F. Imaging Procedure

F.1. Injections

The primary route for imaging compound administration is i.v. Injections will either be conducted in the laminar flow hood or on the palette of the scanner with the animal in the imaging bore. Animals will be injected when either awake or anesthetized. Mice and rats will be placed in appropriately sized restraining devices, that allow access to the tail, and lateral tail vein injections will be administered. Where appropriate, 27G catheters will be placed *in a lateral tail vein* to facilitate injection of the imaging compound. To facilitate tail vein injection, the animal's tail is warmed with an infrared heating lamp held at a safe distance of one foot for approximately 10 min. This dilates the tail vein for an easy venous injection.

F.2. Holders

For imaging animals will either be placed on an aseptic palette or in an appropriately sized cylindrical stereotactic holder. During imaging animals will be observed for adequate sedation and visually monitored for vital signs. If required a heating lamp *held at a safe distance* will be used to maintain animal body temperature *at 37 +/- 2 °C during imaging. Skin surface temperature will be monitored with the use of an infrared non-contact thermometer.*

F.3. Imaging

Images will be acquired either immediately following injection or at a suitable time point post injection (0 to 24 hours) to allow for proper distribution and uptake of the imaging compound. Images will be acquired for 5 minutes up to 1 hour depending on the amount of tracer injected and imaging task. Imaging is performed while animal remains undisturbed in the bore of the scanner. After imaging animals are removed carefully, placed in a clean cage, and are covered *with a blanket of sterile gauze* to maintain body heat until the anesthesia effects wear off.

F.4. Cleanup

Following all imaging procedures the injection stage, hood, animal holder, and scanner are washed with an anti-septic cleaning agent such as Saniplex. Sample swipes are taken on the animal holder and imaging pallet to ensure radioactive decontamination.

F.5. Care and Feeding

During their stay in the imaging facility, at 1018 BLSB, cages will be placed in a laminar flow hood. Food and water, supplied by the investigator, will be monitored and

maintained. *For some studies animals may be fasted from solid foods the night before imaging to reduce glucose and insulin levels, which interfere with the bio-distribution of some imaging compounds (notable F-18-Fluorodeoxyglucose). Water will always be available ad libitum.* The room temperature will be maintained at 68 °F and ambient light will be on during the day and off at night.

F.6. Release Animals to Investigators

After a maximum of 24 hours after imaging radionuclide injections the animals will be released back to the investigator. For Class A radionuclides the animal, bedding, and cage will be surveyed with a Geiger counter to insure that radioactivity is at or below background levels and recorded. For Class B and Class C radionuclides the remaining radioactivity (calculated by decay from injection time) in the animal, bedding, and cage will be transferred, as required by the Radiation Safety Office, to the radiation license of the investigator. Animals will be returned to the investigators and subsequent care, euthanasia, and carcass disposal are the responsibility of the investigator.

G. Protocol Inclusion

For investigators to make use of the imaging facility they will need state the following in their Animal Use Protocol (RO5) or Amendment:

1. Method and route of transport of animals to and from the imaging facility
2. Reference that imaging will be performed according to this S.O.P.
3. Radionuclide and above Class to be used per imaging session
4. Type of imaging (*CT, PET, and/or SPECT*) to be performed per imaging session
5. Frequency and interval of imaging sessions. If multiple sessions are planned then report interval animal housing plans. Office of Animal Resources will assist investigators with housing arrangements.
6. If Class A or Class B radionuclides are to be used, the investigator will need to provide a letter *of their individual protocol* approval from the Radiation Safety Office.

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