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CHANGE HISTORY

Revision	Description
	Internal SOP used by SAIP Laboratory
09/17/2019	Standardize SOP for posting to PDMR-TCIA Public website

RELATED SOPS

1.0 PURPOSE/SCOPE

The Standard Operating Procedure (SOP) describes the procedures for animal handling and monitoring, anesthesia, daily MRI QC/QA, and MRI sequences for single and multi-mouse imaging for detection and monitoring of tumors and metastatic lesions. This SOP is used/performed by the Small Animal Imaging Program (SAIP) at NCI-Frederick, Frederick National Laboratory for Cancer Research.

2.0 SAFETY

SAIP treats all patient tissue and mice carrying patient tumors as a possible health threat as the human tissue could still retain human pathogenic agents. Mice are housed in disposable cages located barrier facilities, handled according to ABSL1 procedures, provided by experienced technical staff. The primary mouse strain used is the NOD.*Cg-Prkdcscid Il2rgtm1Wjl*/SzJ (NSG) which are highly susceptible to infection due to their profound immunodeficiency. All materials coming into the barrier facilities are decontaminated by autoclaving, or chemical means for non-autoclavable items. Mice are transported in their cages to the imaging scanners which are located within the barrier facility. Anesthesia is exhausted from nose cones and induction chamber according to local regulations (carbon filter or active system).

3.0 CLEAN-UP

3.1 All materials coming into contact with patient tissue as well as the mice carrying patient tumor samples are treated as a potential health threat (BSL-2 precautions) since the human tissues could retain human pathogenic agents even if they do not replicate in mouse cells (e.g., EBV, HPV, etc).

3.2 Flush/soak any items (e.g., tubes, syringes, petri dishes, lab mats, etc) that were in contact with human tissue with disinfectant (e.g., 10% bleach, Cavicide®, commercial hydrogen peroxide disinfectant, 2% Virkon®) before disposal in biohazard waste or sharps containers (follow institutional guidelines and manufacturer's recommendations).

3.3 For items that can't be rinsed, wipe down thoroughly with bleach-soaked gauze or other appropriate disinfectants.

4.0 EQUIPMENT

4.1 Imaging Equipment

4.1.1 3.0T MRI clinical scanner (Philips Intera Achieva, Best, The Netherlands)

4.1.2 Single mouse volume solenoid receiver coil with 40mm ID

4.1.3 Multi-channel ^{1}H volume array with saddle mouse coils (35 mm ID; coils center-to-center spacing: 65 mm).

4.1.4 Custom-made (3D printed) mouse beds enclosed in transparent plastic tubes. The mouse beds incorporate air flow channels for input and exhaust of anesthesia gas.

4.2 Animal Handling and Monitoring Materials & Equipment

4.2.1 Isoflurane, Anesthesia-vaporizer, Anesthesia Induction chamber

4.2.2 Vented hood

4.2.3 Temperature regulated air heater and monitoring system to maintain animal body temperature (37 ± 0.5 °C).

4.2.4 Pulmonary pad and respiratory monitoring system

4.2.5 Tumor bearing NOD.*Cg-Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice, sex-matched to human patient (PDM SOP50101).

4.3 Personal Protective Equipment

4.3.1 Personal Protective Equipment (PPE) at a minimum laboratory scrubs, latex or nitrile gloves, hair bonnet, facility shoes, and safety glasses.

4.3.2 Anesthesia is vented according to regulations.

5.0 ANIMAL PREPARATION

5.1 Transport cages from vivarium to imaging room and place the cage of mice on a heated blanket (36 - 37 °C).

5.2 Anesthetize mice in the induction chamber with 3% Isoflurane with filtered air (0.2 µm filter) at 1 liter/minute flow rate as the carrier gas. The induction chamber is placed on a heating pad maintained between 36°C - 37°C located within a vented hood.

5.3 Perform the toe pinch test to assure mice are anesthetized, then transport a mouse to the MRI room.

5.4 Prior to transport of mice to MRI, set the MRI scanner isoflurane vaporizer to 2% with oxygen as the carrier gas with a flow rate of 1 l/min.

5.5 Place the mouse on the imaging platform, supine position, and tape the respiratory pad around their abdomen. Then slide the imaging platform into the plastic tubes for placement within the center of the coil.

5.6 Repeat steps 4.1-4.3 for the remaining mice (if multi-mouse imaging is to be performed).

5.7 Place the coil array in the center of the magnet.

6.0 ANIMAL MONITORING DURING IMAGING PROCEDURE

6.1 Animal monitoring

6.1.1 Animal body temperature is maintained at 37°C ± 1°C by supplying warm air around the mouse holder with a temperature sensor (as feed-back) located within the coil array.

6.1.2 The animals' respiration is monitored by a pulmonary pad / physiology monitoring system and the animal's percent isoflurane from the vaporizer is modified to maintain a constant respiration rate (40-50 bpm).

7.0 MRI IMAGING PROCEDURE

7.1 Daily Imaging Quality Test is performed prior the imaging session:

7.1.1 One 50 ml mineral oil phantom tube is used for single mouse coil and three 15 ml mineral oil phantom tubes are used for multi-channel mouse coil. A 2D T1 weighted Fast Field Echo (T1w-FFE) sequence is performed and the images are visually inspected for ghosting, lines and other artifacts.

7.2 Multi-channel coil (multi-mouse):

7.2.1 T1w-FFE survey scan is performed in three planes (sagittal, coronal and transverse) and used for the planning of the imaging volume.

7.2.2 Reference T1-FFE scan is used with a large field of view of 260 mm x260 mm x 60 mm and short echo and repetition time, TR/TE: 4.3/1.0 ms

7.2.3 Multislice T2 weighted turbo spin echo (T2w-TSE) imaging sequence is applied in coronal direction to cover the whole body of the 3 mice.

7.2.4 A Spectral Presaturation with Inversion Recovery (SPIR) fat suppression technique is used to suppress the fat component to help distinguish fat from cystic, metastatic and tumor masses.

7.2.5 T2w-TSE sequence parameters:

7.2.5.1 FOV: 78 x 160 x18 mm^3

7.2.5.2 In plane resolution: 0.180×0.180 mm^2

7.2.5.3 Slice thickness: 0.5 mm

7.2.5.4 Repetition time (TR): 5230 ms

7.2.5.5 Echo time (TE): 45 ms

7.3 Single channel coil:

7.3.1 T1w-FFE survey scan is performed in three planes (sagittal, coronal and transverse) and used for the planning of the imaging volume.

7.3.2 Multislice T2w-TSE imaging sequence applied in coronal view with respiratory triggering to minimize motion artifacts.

7.3.3 A Spectral Presaturation with Inversion Recovery (SPIR) fat suppression technique is used to suppress the fat component to help distinguish fat from cystic, metastatic and tumor masses.

7.3.4 T2w-TSE sequence parameters:

7.3.4.1 FOV: 70 x 30 x18 mm^3

7.3.4.2 In plane resolution: 0.180×0.180 mm^2

7.3.4.3 Slice thickness: 0.5 mm

7.3.4.4 Repetition time (TR): 5333 ms

7.3.4.5 Echo time (TE): 65 ms

8.0 POST-IMAGING PROCEDURE

8.1 At conclusion of an imaging session

8.1.1 Move the MRI image bed to provide access to the mice and coils.

8.1.1.1 Remove plastic tube containing the mouse and the imaging bed from the coil.

8.1.1.2 Remove mouse from imaging bed system and remove pulmonary pad.

8.1.2 Transport the mice to their respective cage located on a warming pad maintained at (36°C and 37°C). Monitor their breathing until they completely recovered from anesthesia.

8.1.3 Transport cages to their rack located in the animal holding room.

APPENDIX 1: EXTENDED METHODOLOGY

Additional methodology, reference images, or bibliographic references outside of the details in the SOP can be inserted here