SOP FOR IVIS

Overview/Purpose

The objective of this SOP is to describe the procedure for conducting bioluminescence and fluorescence imaging performed with the IVIS Lumina II (PerkinElmer Inc., Waltham, MA, USA) system located in BRT-0020.

Owner of SOP and Responsibilities: Kimerly Powell is responsible for ensuring that the SOP is followed, updated as needed (including personnel changes) and that personnel conducting the procedures are appropriately trained.

Species - Mice and rats

Agent Administration

<table>
<thead>
<tr>
<th>Agent Name</th>
<th>Vehicle</th>
<th>Dose</th>
<th>Route</th>
<th>Volume</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>O2/air/ 95% O2 + 5% CO₂</td>
<td>-.5-%</td>
<td>Inhalation</td>
<td>As needed</td>
<td>during imaging</td>
</tr>
<tr>
<td>Depilatory cream</td>
<td>None</td>
<td>As needed</td>
<td>Topical</td>
<td>As needed</td>
<td>1x imaging</td>
</tr>
<tr>
<td>Luciferin</td>
<td>Sterile saline</td>
<td>50-200 mg/kg</td>
<td>Intraperitoneal, Intravenous, Subcutaneous</td>
<td>As needed</td>
<td>1x imaging</td>
</tr>
<tr>
<td>Commercially Available Fluorescent agents</td>
<td>Sterile saline</td>
<td>As recommend by vendor</td>
<td>Intraperitoneal, Intravenous, Subcutaneous</td>
<td>As needed</td>
<td>1-2x imaging</td>
</tr>
<tr>
<td>Developmental contrast agent *</td>
<td>As needed*</td>
<td>As needed*</td>
<td>As needed*</td>
<td>As needed*</td>
<td>1-2x imaging</td>
</tr>
</tbody>
</table>

(*) Details will be provided in PI protocol

Procedure

1. If luciferin is to be used, it will be administered to the animal ~0-15 minutes before induction of anesthesia and the start of imaging.

2. Luciferin and Fluorescent agents are not available in a pharmaceutical preparation and therefore will be used in accordance with the IACUC policy http://orrp.osu.edu/files/2011/10/Use-of-Pharmaceutical-and-Non-Pharmaceutical-Grade-Compounds-in-Animals-and-Labeling-Expectations.pdf

3. Fur may be removed, prior to imaging, over appropriate part of animal body for better quality images (due to better light penetration).
Details of hair removal:
Animals will be kept under isoflurane anesthesia for this procedure. Hair will be removed using an electrical animal shaver and/or depilatory cream (eg. Nair®). After 30 to 90 seconds, the depilatory cream will be wiped off and any remaining agent will be thoroughly removed using wet tissue to prevent burns.

4. Animals will be anesthetized prior to imaging and depth of anesthesia will be monitored by lack of response to noxious stimuli, such as toe pinch and loss of spontaneous movement. Once a sufficient plane of anesthesia has been reached, animals will be positioned securely on non-fluorescent black paper on the heated imaging platform (maintained at 37°C) and positioned for imaging. The platform can deliver anesthesia up to 5 animals using a precision vaporizer, providing controlled consistent anesthesia for multiple animals during one imaging session. Unused nose cone are closed prior to beginning of air/anesthesia flow.

5. IVIS imaging lasts 5-30 minutes. Fluorescence imaging lasts 5-30 minutes and may be repeated up to a maximum of 6 times a day.

6. Physiological monitoring during IVIS imaging is as follows:
   - An optical picture will be taken, when possible, to validate position of animal and depth of anesthesia
   - The platform is heated up to 37°C to keep the animal at normal physiological temperature during the procedure

7. Following imaging, animals will be monitored until fully recovered from anesthesia (walking, active, etc.) prior to return to routine husbandry/housing.

Personnel conducting procedure must be up to date on all IACUC study team requirements. Personnel not associated with an active protocol must create a profile that is up to date on IACUC study team requirements including a training narrative providing detail on experience for the techniques described in the SOP.

1. Anna Bratasz
2. Michelle Williams
3. Kimerly Powell
4. Katie Gallagher
5. Personnel listed on protocols referencing this SOP may perform this activity

Potential adverse effects
None anticipated related to activities covered on this SOP.

Early Removal Criteria
In case of unexpected issues with animals at any time the PI/study team will be notified by the core staff and will be responsible for further animal monitoring, treatment or euthanasia as per the IACUC protocol.

Any unusual/unexpected symptoms or anesthetic deaths will be reported to the ULAR veterinary staff for consultation.

History of Revisions
401-00 - new SOP approved by IACUC on 08/17/2018
401-01 – Revisions include a new SOP title and adding fluorescence imaging activities. Approved by IACUC on 10/19/2018