University at Buffalo The State University of New York **Lab Animal Facilities**	**STANDARD OPERATING PROCEDURE** **Use of the IVIS Spectrum in BRB 427**	**Quality Form** SOP # **6.A.9** Revision: **02** Last Reviewed:**2/14/23** **Appendix 1**: Anesthesia Machine Operation

1.0 **PURPOSE**:

This describes a standardized procedure for transporting animals to and from the BRB (Biomedical Research Building) and for imaging animals in the IVIS® Spectrum. Specific directions for immunocompromised mice are detailed.

2.0 **SCOPE**:

This applies to Research Staff who will conduct imaging using the IVIS Spectrum in BRB.

3.0 **DEFINITIONS**:

IVIS® Spectrum: In Vivo Imaging System that allows 2D and 3D images of anesthetized live animals after injection of fluorescent or bioluminescent reagents.

4.0 **PROCEDURES**:

4.1 Mice (or rats) are housed in the BEB (Biomedical Education Building) LAF and transported to the adjacent BRB in covered containers as per LAF SOP 1.D.1 Transportation of Laboratory Animals.

4.1.1 If not already in the cage, water bottles are supplied to cages during the day in BRB 427. Autoclaved water must be given to any mice kept under sterile conditions using proper aseptic technique.

4.1.2 Animals must be returned to the BEB LAF at the end of the day (cannot be left overnight in BRB 427).

4.1.3 The IACUC protocol should accompany the animals, so the specifications of procedures are followed.

4.1.4 Upon entering BRB 427, those working with the <u>immunocompromised</u> mice that they have transported should thoroughly wash and dry hands and don new barrier PPE before working with mice in the biosafety cabinet.

a. Wash hands with soap and water. Dry thoroughly.
b. Wear new isolation gown, bouffant cap to fully cover hair, shoe covers, new disposable face mask and new durable exam gloves.
c. Turn on blower for BSC to run for a minimum of 5 minutes and fully disinfect hood and contents.

4.1.5 <u>Immunocompromised</u> mice (or rats) will return to dedicated BEB Barrier Rooms (sterile caging), but <u>not</u> the High Level Barrier Suite (BRB "SPF") which prohibits re-entry of animals. Planning for immunocompromised mouse imaging should involve LAF Veterinarians and the BEB Animal Care Supervisor

4.1.6 Cage cards of immunocompromised mice that undergo imaging must be marked "IVIS" with date of imaging to increase awareness of potential opportunistic pathogen exposure.

4.2 Animals are anesthetized with isoflurane in an induction chamber as per directions posted in the room (Appendix I) and LAF SOP 2.A.26 (Adult Rodent Isoflurane Anesthesia).

4.2.1 Anesthesia for immunocompromised mice must occur inside a biosafety cabinet, and all surfaces contacting the mice must have sufficient contact time with high level disinfectant prior to removal of mice from sterile cages (which must occur inside BSC).

4.3 Bioluminescent reagent will be injected subcutaneously (SC) as per approved IACUC protocol.

4.3.1 Volume, type, needle size, route and frequency will be dictated by approved protocol for specific animals, and must be followed

4.3.2 For immunocompromised mice, injections must occur inside the BSC using aseptic technique.

4.4 Following anesthetic induction /injections, animals are transferred to the IVIS Spectrum, and the animal's nose is placed inside the nose cone manifold within the IVIS Spectrum.

4.4.1 Unused nosecones on the multi-animal manifold are plugged with the dedicated pieces meant to prevent isoflurane from concentrating within the IVIS.

4.4.2 For studies using immunocompromised mice, before mice are moved from the induction chamber to the nosecone, IVIS surfaces and nosecones must be sprayed with appropriate high level disinfectant and allowed sufficient contact time. Gloved disinfected hands should move the animals directly from the induction chamber within the BSC to the disinfected nosecone/surfaces inside the IVIS.

4.5 Supplemental heat is provided inside the imager.

4.6 Mice (or rats) are monitored by observing the images on the computer.

4.7 Depth of anesthesia (1-3% Isoflurane) should be established before imaging begins.

4.8 Frequency of imaging, duration of imaging sessions and number of injections should all be stipulated on the approved IACUC protocol and be followed for a specific group of animals.

4.9 Animals are recovered in a separate cage with supplemental heat.

4.9.1 Animals will receive a subcutaneous bolus of warm, isotonic fluids (0.9% NaCl or LRS) if total anesthesia time has exceeded one hour. (1-3 mL for mice via 25 g needle; 5-10 mL for rats via 22-25 g needle).

4.9.2 Animals will be monitored until able to walk.

4.9.3 Once fully recovered, animals can return to their original cages and be returned to the LAF.

4.10 The heated stage and nose cone is sanitized between groups or animals as follows:

4.10.1 Conventionally housed (immunocompetent) animals:

a. 70% isopropyl alcohol between individual animal groups

b. At the end of an imaging session the IVIS Spectrum Stage will be wiped down with Spor-Klenz or an appropriate cleaner that will not create additional fluorescent background upon drying.

4.10.2 Immunocompromised animals (sterile housing required):

a. Prior to and after each group of animals, high level disinfectant must be used on the nosecones and all contact surfaces within the IVIS to prevent opportunistic infections, especially skin infection with *C. bovis.*

References:

Approvals:

Name	**Title**	**Date of Approval**
Jennifer Peirick, DVM	LAF Director/Attending Veterinarian	2/14/2023
Andrew McCall, PhD	Director of Optical Imaging & Analysis Facility	2/14/2023

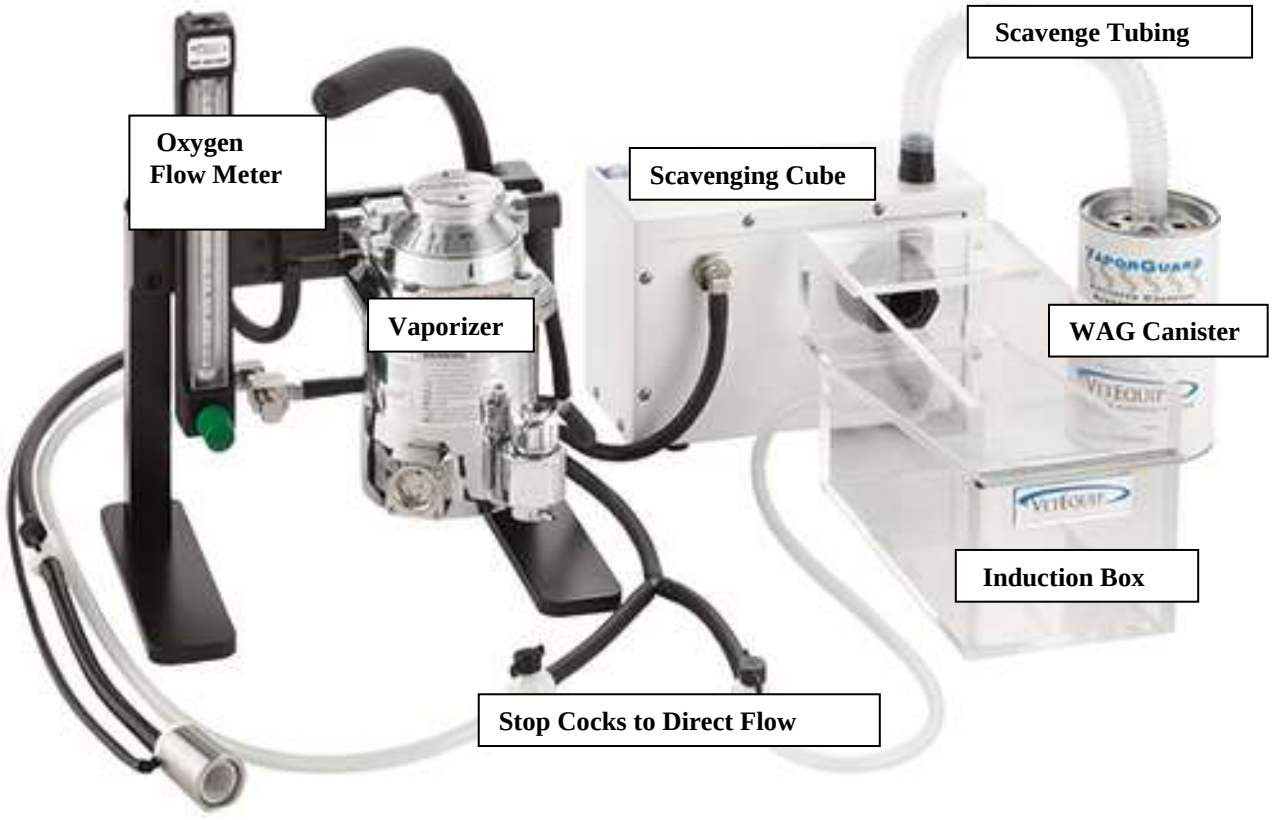
Change History:

Revision #	**Description of Change**	**Effective Date**
01	Location of IVIS Spectrum was updated from BRB 406 to BRB 429. Procedures for immunocompromised mice were added to prevent opportunistic infections. These include: donning new PPE, use of a biosafety cabinet, use of high level disinfectants and marking of cage cards after imaging.	10/14/2022
02	Location change to BRB 427 due to storm damage/remodeling in BRB.	2/14/23

Appendix 1: Anesthesia Machine Operation (V-1 Table Top System)

<u>Ready Machine</u>:

1. Fill vaporizer with isoflurane. Don't overfill. Be sure stainless steel cap is screwed in tightly after filling.
2. Check that Waste Anesthetic Gas (WAG) canister is attached via clear, corrugated scavenge tubing to the white, rectangular scavenging cube.
3. Check the oxygen supply in the green tank and the connection to the flowmeter and anesthetic vaporizer (green tubing).
4. Check that the anesthetic gas tubing is ready for induction. Open stop cock valves that connect to induction box and close valve that connects to IVIS Spectrum. (Turn black stop cocks to parallel with tubing for open and perpendicular to tubing for closed.)
5. Push induction box's black connector into scavenging's cubes black port (front of cube).
6. Be sure back of IVIS Spectrum in properly connected with Gas In/Gas Out lines and ports.



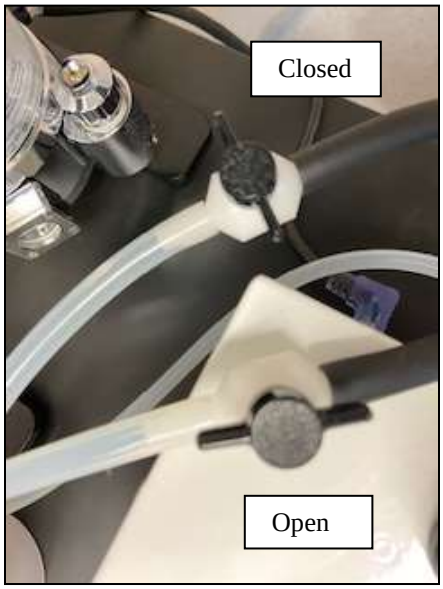
Nose cones inside IVIS Spectrum. Plug if not used.

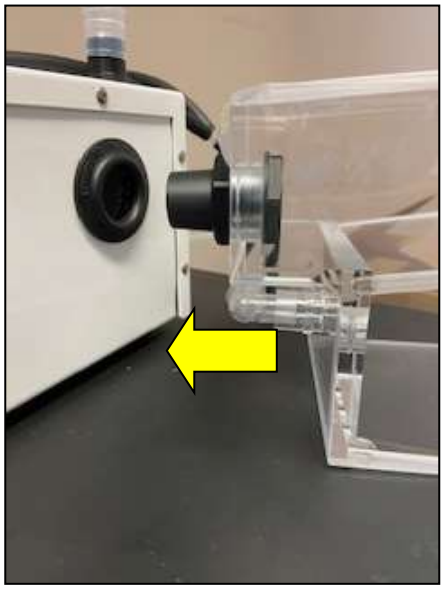
Induction:

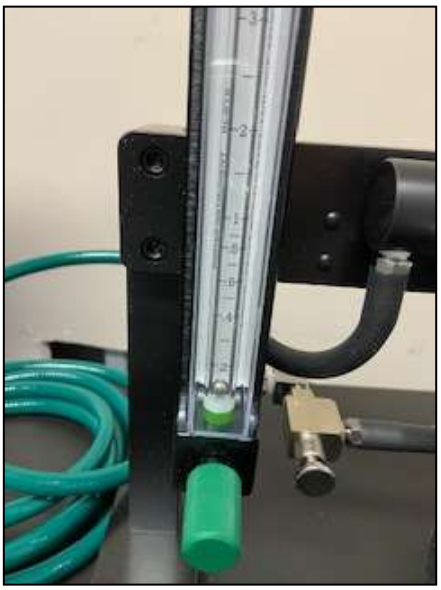
1. **For immunocompromised mice, induction should occur inside hood.**

Back of IVIS Spectrum: "Gas Out" and "Gas In" Ports to connect fresh gas and waste gas tubing.

2. Double check that black stop cock valves are positioned for induction.
3. Push the induction box's black connection piece into the scavenging cube's port.
4. Turn on the oxygen tank, and turn flow meter to 2 LPM.
5. Place mouse (or rat) into induction box.
6. Turn isoflurane vaporizer up slowly from **0.5** % to 4% while constantly monitoring the mouse. If the mouse will require extended time inside the induction box, do not exceed 3 %.







Valves: Open to induction
Closed to nose cones

Push induction box into scavenge cube.

Oxygen Flow Meter: Turn to 2 LPM

Maintenance Anesthesia:

1. Turn oxygen flow meter down to 1 LPM.
2. Turn isoflurane down to between 1-3 %.
3. Switch stop cock valves to divert flow to nose cones inside IVIS Spectrum.
4. Remove mouse from induction box and place onto nose cone inside IVIS Spectrum.
5. Monitor mouse until steady and appropriate anesthetic planes is achieved. This may require adjusting isoflurane % to between 1-3%.

6. Close door of IVIS Spectrum and monitor via video until procedure is finished.
7. After anesthesia is no longer needed, turn off isoflurane and maintain mouse on 100% oxygen until mouse begins to move. Then, continue recovery in individual cage with supplemental heating until mouse is walking.