University Animal Care Committee Standard Operating Procedure		
Document No: 7.33	**Subject: Mouse Irradiation**	
Date Issued: October 30th, 2024	**Revision:** Original	**Page No:** 1

Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the clinical support and care and the standard endpoints following total body lethal irradiation and sublethal irradiation with reconstitution or targeted irradiation studies that result in immune suppression.

1. Introduction and Definitions:

Mice can experience several predictable sequelae following irradiation; these may be mitigated with proper dosing, handling, and supportive care. During the two-week period following irradiation/reconstitution, mice will be susceptible to a wide variety of opportunistic pathogens, with septicemia and acute death being the most frequently observed clinical manifestations. Mice must be maintained under strict sterile conditions during this time. Generally, by two weeks following irradiation, the implanted cells have proliferated to a functional level and the animals will have regained immune function.

The prophylactic use of antibiotics is **not acceptable** as post-irradiation care. The administration of antibiotics requires UACC approval and/or clinical justification.

Mouse age and strain are important factors to consider prior to irradiation. Young mice and certain strains (e.g., NSG and BALB/c) are highly sensitive to irradiation, and the dose applied should be adjusted accordingly. Radiation sickness generally lasts for 5 to 10 days, and recovery usually occurs around 14 days post-irradiation. Approved dose, animal age, and other parameters should be carefully implemented, as outlined in the approved protocol; contact veterinary staff or UACC personnel with any questions.

Graft versus host disease (GVHD), irradiation injuries, and disease relevant to the implanted cell type are other possible adverse events following engraftment. GVHD can occur when genetic differences in histocompatibility haplotype between donor and host stains are present. The primary targets of GVHD are the liver, skin, gastrointestinal tract, and lymphohematopoietic system. The most obvious clinical signs associated with GVHD are diarrhea and skin lesions.

Mice experiencing radiation sickness may develop skin reddening (erythema), ruffling or loss of hair, facial edema, conjunctivitis, watery eyes, hunched posture, diarrhea, or anorexia. Weight loss of more than 15-20% can be common after irradiation and may require supportive interventions or euthanasia.

Please contact ACS for training and refer to SOP 2.38 (X-ray Irradiator Use) prior to irradiator use.

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI,** subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP,** intramuscular **IM**, per os **PO,** per rectum **PR**

2. Materials:

- Sterile caging with water bottle lids
- Sterile water in autoclaved bottles and sippers; stored within a sterile mouse box
- Diet gel
- Sterile Petrie dishes to hold diet gel or moistened chow
- 0.5% Accelerated Hydrogen Peroxide
- Sterile saline (0.9%) or lactated Ringer's solution
- Sterile syringe (1 to 3 mL) and hypodermic needles (25 to 27ga)

3. Procedures:

- Investigators must send in special requests to ACS with a list of supplies required, animal and bedding handling instructions and emergency contact information.
- Prior to the study initiation, all mice must be examined by qualified lab personnel.
    - This exam should look to identify any physical abnormalities including low body condition score or low muscle score, low body weight compared to cage mates, malocclusion, wounds, and other abnormalities that would preclude the inclusion in the study.
    - Baseline behaviour should be noted to aid in clinical scoring during the recovery period.

- A cage card indicating the irradiation treatment should be placed in front of the cage card holder to communicate with husbandry staff. Do not remove this card for at least two weeks following irradiation.
- Prior to and following irradiation, mice must be housed in autoclaved caging with sterile water. These conditions decrease the possibility of introducing pathogenic or opportunistic environmental bacteria during the two-week period following irradiation. Ideally, these conditions will be maintained beyond the first two weeks.
    - Water bottles should be changed twice weekly to avoid contamination.
- Mice should only be handled within a HEPA-filtered biological safety cabinet or cage changing station. All materials that contact the mice must be sterile or cleaned with 0.5% Accelerated Hydrogen Peroxide (e.g., containers used for weighing mice). Gloved hands should be moistened with 0.5% Accelerated Hydrogen Peroxide for a minute prior to handling mice or interior cage components and should remain moist throughout handling.
- Diet gel or moistened chow can stimulate appetite and help maintain hydration during recovery and should be offered starting 2 to 3 days prior to irradiation and through the two weeks following. Introduction prior to irradiation will help prevent food aversion associated with radiation sickness.
    - If using moistened chow, sterile water should be used
    - This supplemental feed should be placed in sterile Petrie dishes and should be replenished daily, or more often if soiled or consumed
- Mice should be monitored at least once daily by lab personnel for the first 14 days, including weekends. Efforts should be made to enter the cage as little as possible, but as often as needed to replenish food, update weights, and provide other supportive care.
    - Starting the day following irradiation, using the table below, a **clinical score should be calculated every other day provided the score is <6**. If the score is 6 or higher, a veterinarian should be notified, and the score should be updated daily. A score of 12 is grounds for immediate humane killing, as death is imminent.
- A baseline, pre-irradiation body weight must be recorded on the corresponding cage card. The weight of each mouse must be updated every other day following irradiation and noted under the baseline weight. Weight loss of 15-20% is justification for euthanasia.

    - If weight loss is noted, warmed subcutaneous fluids (saline or lactated Ringer's, 40 to 60 mL/kg) can be administered after consultation with veterinary staff.
- Mice that lose more than 20% of their starting body weight, appear quiet, dull, lethargic, cold, dyspneic (increased respiratory rate or effort), or hunched, or those that are continuing to lose body weight after 10 days should be euthanized or examined by a veterinarian.
- After the first two weeks, mice should be monitored once weekly long-term, or more frequently if warranted, and weight and body condition score should be recorded. Ongoing clinical issues may include tumours (related to the radiation dose or cell line used), malocclusion from damage to the tooth root(s), progressive weight loss, or conjunctivitis. Mice displaying these signs or other concerns should be euthanized or examined by a veterinarian.
    - Alopecia and/or greying of the hair coat may also occur.

1. 4. **Clinical monitoring (modified from Koch et al. 2016)**

Appearance	Normal (smooth coat, clear eyes/nose)	0
	Hunched and/or fluffed	1
	Ocular discharge and/or edema	3
	Pale, white mucus membranes/skin	6**
	Blue mucus membranes/skin (cyanosis)	12*
Respiratory Rate	Normal breathing	0
	Increased breathing (double normal rate; rapid; shallow)	6**
	Abdominal breathing +/- gasping or open mouth breathing	12*
General Behaviour	Normal (based on baseline observations)	0
	Decreased mobility	2
	Ataxia, wobbly, weak	6**
	Inability to stand	12*
Provoked Behaviour	Normal (moves when cage is disturbed, runs from hand)	0
	Subdued; responds to stimulation (moves away briskly)	1
	Subdued even to stimulation (moves away slowly)	3
	Unresponsive to gentle prodding	6**
	Does not right within 5 seconds when placed on side	12*
Weight Loss	<20%	0
	20-25%	3**
	26-30%	6**
	>30%	12*

Score	Clinical interpretation
<6	Normal: continue to monitor at least once daily
6-11	Heightened concern: monitor at least 3 times per day; contact a veterinarian immediately
≥12	Moribund: must be humanely killed immediately
**** Regardless of score, notify a veterinarian immediately * Regardless of score, mouse should be humanely killed as death is imminent**	

Date							
Initial Body Weight							
Current Body Weight							
% Body Weight Loss							
A. Appearance							
B. Respiratory Rate							
C. General Behaviour							
D. Provoked Behaviour							
E. Weight Loss							
TOTAL SCORE (add A to E)							

References:

Abrams HL. 1951. Influence of Age, Body Weight, and Sex on Susceptibility of Mice to the Lethal Effects of X-radiation.∗. Proc Soc Exp Biol Med **76**:729–732.

Dubé P. 2017. "Considerations for Rodent Irradiation." Taconic Biosciences. Online. https://www.taconic.com/resources/rodent-irradiation-considerations. Accessed 25/07/2024.

Duran-Struuck R, Dysko RC. 2009. Principles of bone marrow transplantation (BMT): providing optimal veterinary and husbandry care to irradiated mice in BMT studies. J Am Assoc Lab Anim Sci **48**:11–22.

Grahn D, Hamilton KF. 1957. Genetic variation in the acute lethal response of four inbred mouse strains to whole body x-irradiation. Genetics **42**:189–198.

Iwakawa M, Noda S, Ohta T, Ohira C, Lee R, Goto M, Wakabayashi M, Matsui Y, Harada Y, Imai T. 2003. Different Radiation Susceptibility among Five Strains of Mice Detected by a Skin Reaction. J Radiat Res **44**:7–13.

Koch A, Gulani J, King G, Hieber K, Chappell M, Ossetrova N. 2016. Establishment of Early Endpoints in Mouse Total-Body Irradiation Model. PLoS ONE **11**:e0161079.

Mims BM, Enriquez J, Santos AP dos, Jones-Hall Y, Dowd S, Furr KL, Grisham MB. 2021. Antibiotic administration exacerbates acute graft vs. host disease-induced bone marrow and spleen damage in lymphopenic mice. PLoS ONE **16**:e0254845.

Mori N, Okumoto M, Morimoto J, Imai S, Matsuyama T, Takamori Y, Yagasaki O. 1992. Genetic Analysis of Susceptibility to Radiation-induced Apoptosis of Thymocytes in Mice. Int J Radiat Biol **62**:153–159.

Massachusetts Institute of Technology. VC-Irradiation-005. "SOP: Clinical Management of Mice in Lethal/Sublethal Irradiation/Reconstitution Studies." Revised January 2024.

Toronto Centre for Phenogenomics. SOP PR021. "Irradiation: Medical and Husbandry Requirements." Revised February 2020.

SOP Revision History:

Date	New Version
10/30/2024	Protocol Created