1.0 OBJECTIVE

1.1 The Objective of this Standard Operating Procedure (SOP) is to describe the Procedures for the handling and care of Immunodeficient animals to be housed at Molecular Medicine Research Institute (MMRI) Animal Facility (AF)

2.0 SCOPE

2.1 This SOP applies to all animal rooms in AF and all immunodeficient animals housed within those rooms. This SOP embodies guidelines for their cage preparation, cage changing and documentation duties

3.0 POLICY

3.1 It is the policy of MMRI to establish written and approved procedures to assure that the animals are treated in a humane manner in accordance to Guide for the Care and Use of Laboratory animals and to educate personnel utilizing animals in research

4.0 RESPONSIBILITY

4.1 It is the responsibility of Manager of Animal Research or designated alternate to implement this SOP and revise it when necessary

5.0 REFERENCES

5.1 The Guide for the Care and Use of Laboratory Animals, 8th edition, National Research Council, National Academy Press, 2011

5.2 Guide for the Care and Use of the Nude (thymus-deficient) Mouse in Biomedical Research, ILAR News, Volume XIX, Number 2, 1976

5.3 Use of Ultraviolet Lights in Biological Safety Cabinets: A Contrarian View. Applied Biosafety, 11(4):222-227, 2006

5.4 Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets. Applied Biosafety, 11(4):228-30, 2006

6.0 PROCEDURES

6.1 Preparation of Biosafety hood to be used for immunodeficient animal operations. This is the exclusively assigned Laminar flow hood for immunodeficient animal operations

6.1.1 Turn on ventilation flow of assigned Laminar flow biosafety hood and let it run for 15 minutes before spraying disinfectant such as 70% alcohol or quaternary ammonia solutions (See MMRI SOP ANP015)

6.1.2 Thoroughly spray disinfectant over all of the inside surfaces and sides of the hood and allow 10 minutes before use while wearing dedicated Personal Protective Equipment (PPE): Gown / Smock, Hair Bonnet, Shoe Covers, Gloves, Mask and Eye Shield / Glasses

6.1.3 Work in the center of hood, away from sides, so as to not interfere with airflow pattern

6.1.4 When finished working, thoroughly spray inside surfaces and sides of hood with disinfectant and allow 10 minutes before wiping dry using clean paper towels

6.1.5 Turn off hood after 15 minutes

6.1.6 NOTE: Use of UV light is NOT recommended (See references 5.3 & 5.4). If it is to be used, sash must be down, hood must be completely empty and light should only be on when no personnel are occupying the room. A 60 minute exposure time will result in sufficient sterilization.

6.2 Preparation of Innovive disposable cages for housing immunodeficient mice

6.2.1 Wearing disposable PPE spray disinfectant liberally over front and back of gloves and whenever a break in sterility is suspected.

6.2.2 Thoroughly mist disinfectant such as 70% alcohol or quaternary ammonia solutions (See MMRI SOP ANP015) over the entire outside surfaces of double plastic bagged gamma irradiated Innovive cage bottoms, cage lids, feeders and prefilled water bottles

6.2.3 After spraying, carefully remove inner bags and spray them with disinfectant over all surfaces. Move them inside of the Biosafety hood. Retain outer bags so unused cage assemblies can be repacked in double bagged fashion

6.2.4 Once placed in the Biosafety hood the seals of inner plastic bags may be cut open

6.2.5 Prepare needed number of complete cage set-ups one at a time and dock these onto Innovive rack running in positive pressure mode (so as to isolate immunodeficient animals from nonsterile vivarium room air)

6.2.6 Spray disinfectant liberally over front and back of gloves each time before placing them back in the Biosafety hood for the next activity

6.2.7 When finished, reseal all cage assembly bags while still inside the Biosafety hood, removed the sealed bags, place them back in to the second plastic bag, and twist closed

6.3 Use of Innovive disposable cages for housing immunodeficient mice

6.3.1 Spray disinfectant over all surfaces of the animal shipping crate and sealed envelope of irradiated chow (e.g. Teklad 2920) prior to placing them inside the Biosafety hood

6.3.2 One at a time, bring over a preassembled empty cage from the rack to the biosafety hood. Spray a mist of disinfectant on all outer surfaces of the cage prior to removing lid

6.3.3 Open shipping crate and use long blunt tweezers to transfer mice by their tails from the shipping container to preassembled cage.

6.3.4 Add irradiated chow into feeder and provide sterile antibiotic tablets, such as SCID MD's from Bio-Serv (bacon flavored Trimethoprim / Sulfamethoxazole: 12 mg / 60 mg) on the cage floor. Alternatively, prophylactic antibiotics can be provided in irradiated rodent chow or added to drinking water

6.3.5 Once all animals are transferred into a cage, replace the lid and press down on all four corners to assure a seal with the cage bottom

6.3.6 Place a new prefilled Innovive water bottle into the recess in the cage lid, making sure to remove the tab from the bottle top. Gently press at the base of the bottle to confirm water flow

6.3.6.1 Innovive bottles are filled with water that is carbon filtered, passed through reverse osmosis, steam distilled, UV exposed, microfiltered (0.2 um), ozonated and acidified to a pH of 2.5-3.0. Every production run is sent for microbial and pH testing.

Therefore, prefilled Innovive water bottles can be used for immunocompromised animals "as is"

6.4 Animal Care Personnel, Vivarium equipment and Miscellaneous coming in contact with Immunocompromised animals:

6.4.1 Personnel will use disposable PPE for all work with immunocompromised animals. PPE will be discarded when work with immunodeficient animals is completed

6.4.2 Sterile cages will only be opened once they are placed inside the dedicated laminar flow Biosafety hood

6.4.3 **ANY** supplies or equipment entering the Biosafety hood will be sprayed thoroughly with disinfectant

6.4.4 A dedicated cart will be used for transport of immunocompromised animal cages and temporarily placement of required supplies

6.4.5 Bedding (Alpha Dry or corncob) is already placed inside Innovive cages prior to irradiation. Innorichment sheets (e.g. nesting material) can also be provided inside of the cage bottom along with the bedding, so as to require no additional preparation / additions

6.4.6 Irradiated rodent chow (See 6.3.1) and antibiotics (See 6.3.4) are provided in re-sealable containers or envelops. These will only be opened once inside the Biosafety hood and will be sealed before removing from the hood and placed inside a second plastic bag

6.4.6.1 If space permits, sealed materials may be stored inside a secondary bin on the shelf underneath the Biosafety hood. Materials should NOT be stored inside the hood, as this may interfere with proper sterilization of the hood

6.5 Cage Changing Schedule

6.5.1 Cage bottoms, lids and feeders will all be changed at the same time, at least once within a 14-day period. Depending on cage density or health status of the animals, more frequent cage changes may be required (no greater than once per week)

6.5.2 Irradiated food and/or water bottles will be replaced as needed, but may only be done so inside of the Biosafety hood (See 6.4.3 & 6.4.4) or open front safety hood if animals are not inoculated with human tumors (See

6.5.4 below). Therefore, the ideal time for these activities is during the scheduled cage change

6.5.3 Similarly, a new antibiotic tablet will be provided on an "as needed" basis (e.g. when none of the prior tablet remains). This will necessitate opening the cage and therefore will only be done within the Biosafety hood

6.5.4 Note: When receiving and transferring animals before tumors are implanted immunodeficient animals can be transferred using the open ventilating hood and not the biosafety cabinet, **if and only if** the hood is dedicated ONLY for work with immunocompromised animals

7.0 Documentation

7.1 Initial the corresponding box on the vivarium checklist for the task performed