1.0 OBJECTIVE

1.1 The objective of this procedure is to describe standard laboratory and surgical practices for Animal Biosafety Level 2 (ABSL-2) work at the MMRI Animal Research Facility.

2.0 SCOPE

2.1 This procedure applies to all work done with Biosafety Level-2 (BSL-2) agents including non-replicating viral agents such as Adenovirus (AV) and Adeno-Associated Virus (AAV) vectors.

2.2 Viral or other DNA vectors will be replication incompetent, i.e. not truly "infectious" but they contain recombinant DNA and procedures for the handling of BSL-2 appropriate samples will be adhered to. These vectors have all functional viral genes removed. There are no known cases of accidental human infection or recombination to date. AV and AAV are not known to cause any human disease. Animals will be treated with replication incompetent viral vectors, or plasmids alone. Some types of native Adenoviruses can cause human infections, including colds or pneumonia but types and amounts used for vectors in animals are not known to be hazardous to humans. Plasmid DNA is not known to present risk.

3.0 POLICY

3.1 It is the policy of MMRI to establish written and approved procedures to ensure that the health and well being of employees is protected, and that potentially hazardous procedures are performed in a safe manner.

4.0 RESPONSIBILITIES

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

4.2 It is the responsibility of all nonclinical research and development personnel working with ABSL-2 agents to follow these procedures. It is the responsibility of the supervisor of personnel working with ABSL-2 agents to ensure that individuals are trained and understand and follow these procedures.

5.0 PROCEDURES

5.1 General

5.1.1 Perform all work in a limited access; ABSL-2 designated room(s).

5.1.2 Label the door(s) to the ABSL-2 room (S) with a sign including the universal biohazard symbol, the infectious agent in use, the name and telephone number of the laboratory supervisor and/or other responsible person(s), and special requirements/procedures for entering room.

5.1.3 Only persons who have been advised of the potential hazard and who have received appropriate training (including annual updates) documented in written records, and meet specific entry requirements should enter room.

5.2 Laboratory

5.2.1 Do not eat, drink, chew gum, or handle contact lenses, apply cosmetics, store food for human use, or smoke in the lab.

5.2.2 Use disposable laboratory coats. For work with BSL-2, double glove. Change gloves frequently to prevent skin contact through small holes or tears that might develop. Change outer pair of gloves prior to touching any surface that is not labeled with a standard biohazard label.

5.2.3 Perform procedures carefully to minimize creation of aerosols. Perform procedures (i.e. dose preparation, syringe filling) in a biological safety cabinet whenever possible.

5.2.4 Cover work surfaces with disposable absorbent pads. When heating pads are in use place additional absorbent pads under and over pad(s).

5.2.5 Report any spills or accidental exposure week days and weekends to the Biological Safety Officer.

5.2.6 Have an appropriate disinfectant near work area. The disinfectant will be freshly prepared or not expired. It will be suitable to kill the biohazardous agent being used. For example: consumer bleach solution, 1:50 dilution, prepped within 24 hr of use can be used to kill M. tuberculosis with a 10 min contact time.

5.2.7 Use extreme caution with any potentially contaminated sharp objects such as syringes, glass pipettes and slides. Whenever practical, avoid use of sharp objects and use syringes that re-sheath or needle-less systems.

5.2.8 DO NOT recap needles or directly handle broken sharp objects. Place all contaminated sharps in biohazard sharps container.

5.2.9 Use mechanical pipetting aids fitted with filters and pipettemen with filtered tips for ABSL-2 agents. **DO NOT PIPETTE BY MOUTH!**

5.2.10 Place cultures, tissues, or specimens of body fluids in a primary container that prevents leakage during collection, handling, processing, storage or transport. The use of secondary containment is helpful during storage or transport within the building.

5.3 Animal

5.3.1 Use care to ensure that outside of animal cages do not touch any surface that may come into contact with BSL-2 agents.

5.3.2 After administration of BSL-2 agent, all contact with animal is potentially contaminating. Change gloves and absorbent pads that have contacted excrement and dispose of contaminated materials in two nested red autoclave biohazard bags.

5.3.3 Return animal to a cage/container within the surgical suite or room during recovery. Avoid touching the outside of the cage with contaminated gloves or any other instruments that may have contacted the BSL-2 agents, especially when opening and closing cage. When the animal has sufficiently recovered, return cage to designated ABSL-2 animal room or transfer from container to cage in animal room.

5.3.4 Label animal cages with biohazard warning label and the name of the agent in use.

6.0 DISPOSAL AND CONTAMINATION PROCEDURES.

6.1 Dry Waste

6.1.1 Dispose of syringes and needles in biohazardous sharps container.

6.1.2 Place all disposable surgical items in covered biohazard waste container.

6.2 Equipment

Immediately after completion of surgery, immerse all surgical instruments in an appropriate disinfectant (e.g.: 10% of a consumer bleach solution). Follow manufacturers instruction for minimum contact time.

6.3 Cages/Husbandry Equipment

Follow procedures outlined in SOP for handling and decontaminated soiled/infectious husbandry equipment.

6.4 Carcasses and Animal Tissue

Place animal carcasses and or tissues inside two nested red biohazard bags, then tape each bag closed. Place carcasses/tissues in an appropriately labeled freezer. Carcasses/tissues will be removed for incineration.

6.5 Surfaces

When all moveable instruments, cages, and waste have been cleared, decontaminate all surfaces in surgical suite, heating pads, and any non-autoclave equipment with appropriate disinfectant (e.g.: 10% of a consumer bleach solution). Follow manufacturers instruction for minimum contact time.

6.6 Personal Decontamination

6.6.1 After completing procedures 6.1 – 6.5, remove all disposable personal protective equipment, place in red autoclave biohazard bags.

6.6.2 Thoroughly wash hands and arms with disinfectant soap. If direct exposure to ABSL-2 agent had occurred to eyes or skin, decontaminate the area using appropriate eyewash, and/or agent (eg, 70% alcohol or betadine scrub) and notify direct supervisor or biosafety officer. Autoclave the bagged personal protective equipment before disposal.

7.0 DEFINITIONS

Biosafety Level 2 agents are those agents that pose a moderate to potential hazard to personnel and environment. These agents are associated with human disease that can be contracted via inoculation, ingestion, or mucous membrane exposure

8.0 REFERENCES

8.1 MMRI Safety Manual and Guidelines

8.2 SOP # ANP031, cleaning and storage of animal caging and equipment.

8.3 SOP# ANP005, Euthanasia of Rats and Mice

8.4 *Biosafety in Microbiological and Biomedical Laboratories*, 4th edition. Washington, D.C.: US Department of Health and Human Services, Public Health Service, Centers of Disease Control and Prevention, and National Institutes of Health, 1999.