

**MARSHALL UNIVERSITY
JOAN C. EDWARDS SCHOOL OF MEDICINE
ANIMAL RESOURCE FACILITY
POLICY AND PROCEDURES MANUAL**

Robert W. Coon Medical Education Building
Veterans Administration Medical Center

Director: Billy W. Howard, DVM

Hours: 7:30 a.m. to 3:30 p.m. Monday through Friday
Limited hours on Saturday, Sunday and Holidays

December 2008

INDEX

| <u>Section No.</u> | <u>Title</u> | <u>Page No.</u> |
|--------------------|---|-----------------|
| I. | Introduction | 1 |
| II. | Organization | 1 |
| III. | Principles and Guidelines | 2 |
| IV. | Standard Procedure | 4 |
| | A. Animal Acquisition | 4 |
| | B. Animal Housing | 6 |
| | C. Animal Care | 7 |
| | D. Animal Disposition | 7 |
| | E. Special ARF Services | 8 |
| | F. Billing | 9 |
| | G. Animal Hazards and Zoonotic Diseases | 9 |
| | H. Anesthesia and Analgesics | 15 |

SOP INDEX

| <u>SOP No.</u> | <u>Title</u> | <u>Page No.</u> |
|----------------|--|-----------------|
| 1 | Sanitation - Animal Room Cleaning | 18 |
| 2 | Sanitation - Cage Cleaning Frequency | 19 |
| 3 | Sanitation - Rack Washing Frequency | 20 |
| 4 | Operation of the Cage Washer | 21 |
| 5 | Bedding - Storage and Changing Procedures | 22 |
| 6 | Animal Feed - Monitoring and Storage | 23 |
| 7 | Quarantine Room Procedures | 25 |
| 8 | Caging and Housing Dimensions for Lab Animals | 26 |
| 9 | Dogs | 28 |
| 10 | Security Procedures | 30 |
| 11 | Education and Training | 31 |
| 12 | Equipment Maintenance and Related Activities | 33 |
| 13 | Veterinary Medical Care Program | 34 |
| 14 | Sanitation of Procedure Room | 37 |
| 15 | Operating Procedures for the High-Pressure Spray Cleaner | 38 |
| 16 | Health Monitoring Program | 39 |
| 17 | Exercise for Dogs | 41 |
| 18 | Guidelines for Aseptic Surgery on Rodents | 42 |
| 19 | Occupational Health | 44 |
| 20 | Emergency Plan | 51 |
| 21 | Prolonged Restraint Policy | 53 |
| 22 | Multiple Major Surgical Procedures | 54 |
| 23 | Procedures for Handling Allegations of Mistreatment or Noncompliance Involving Care and Use of Animals | 55 |
| 24 | Rodent Bleeding Policies | 58 |
| 25 | Immunization of Rabbits with Complete Freund's Adjuvant | 61 |
| 26 | Baby Chickens | 64 |
| 27 | Immunocompromised Animals | 66 |
| 28 | Managing Immunocompromised Animals | 67 |
| 29 | Procedures for Threatening Situations for Animals | 69 |
| 30 | Rodent Breeding Colony Management | 71 |

I. **Introduction**

- A. The primary objective of the Association for Assessment and Accreditation, (AAALAC) International accredited Animal Resource Facility (ARF) is to provide professional animal support services to the faculty, staff and students at Marshall University.
- B. The use of animals for teaching and research is a fundamental part of biology and medicine. Suitable animals are required for investigative and teaching purposes. Proper care and management of these animals is both a scientific necessity and a legal requirement.
- C. Animals are sentient beings. Their use is a privilege granted to the scientific community by the public and its policy-making institutions. Along with this privilege goes the ethical responsibility for their humane care and proper use. The University, the individual investigator and each member of his staff must share in this partnership of responsibility.
- D. This guide describes the organization of the ARF, the policies which govern its operations, and its basic rules and regulations. It should be reviewed by all personnel who are involved with the use of animals on this campus. Policies and procedures will be subject to change with changing conditions and new information.

II. **Organization**

- A. The ARF is a centralized service organization responsible for the procurement of animals, cages, feed, supplies and related items. It is responsible for the daily husbandry and care of experimental animals, and their general health.
- B. The Director of the ARF is a veterinarian, whose major responsibility is the efficient functioning of the unit. His professional qualifications and interests lie in the specialty of Laboratory Animal Medicine, and he serves the entire campus in all matters of the Veterinary Sciences. His duties are diversified, including administrative, clinical, teaching, consultative, and research activities.
- C. The ARF Director is responsible for complying with and implementing all aspects of the "Laboratory Animal Welfare Act" (P.L. 89-544 and amendments). He will also comply with the following guidelines.
 - 1. Guide for the Care and Use of Laboratory Animals. NRC
 - 2. "Grants Administration Manual." DHHS
 - 3. Applicable local and state codes.

4. Standards established by other granting agencies, University Policy and appropriate Professional Societies.
- D. The Institutional Animal Care and Use Committee is composed of members qualified through experience and expertise to oversee the institution's animal care programs. The principal charges to this committee are:
1. To review all research protocols in which laboratory animals are used to ensure compliance with the Animal Welfare Act and other local, state and federal rules, regulations and guidelines for humane care and use of animals.
 2. To review the institution's program for humane care and use of animals once every six months. A report of the review is to be submitted to the Institutional Official.
 3. To inspect at least once every six months all of the institution's animal facilities, including satellite facilities and animal study areas. A report of this inspection is to be submitted to the Institutional Official.
 4. To make recommendations to the Institutional Official regarding any aspect of the research facility's animal program, facilities, or personnel training.
 5. To serve as advisory adjudicating body to the Institutional Official or his designee on disagreements on the animal resources operations and animal welfare.
 6. To handle other matters relating to the operation of the animal resource facility and animal welfare and usage as requested by the Institutional Official or his designee.

III. **Principles and Guidelines**

- A. The ARF operates as a centralized laboratory facility in accordance with the operating concepts and procedures in the NRC publication, Guide for the Care and Use of Laboratory Animals, 1996.
- B. The following principles shall be applied for the utilization and care of vertebrate animals used in testing, research and training.
 1. The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et seq.) and other applicable Federal laws, guidelines and policies.

2. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of the society.
3. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered.
4. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
5. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.
6. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly euthanized at the end of the procedure or, if appropriate, during the procedure.
7. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.
8. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.
9. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle 2, by an appropriate review group such as the Institutional Animal Care and Use Committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

IV. **Standard Procedure**

A. Animal Acquisition

1. An "Application for the Care and Use of Laboratory Animals" must be completed by the principal investigator and reviewed by the Institutional Animal Care and Use Committee (IACUC) prior to initiation of a teaching or research project. The Application for the Care and Use of Laboratory Animals is available in the ARF Director's Office or at the ARF Website, <http://meb.marshall.edu/arf/index.htm>. The application and review are a part of our commitment to comply with the amended Animal Welfare Act. The Public Health Service policy of Humane Care and Use of Laboratory Animals and the USDA Animal Welfare Regulations to review protocols involving animals. The completed application and protocol should be submitted to the ARF Director's Office for administrative review, and placed on the agenda of the next IACUC meeting. The application is then reviewed by the IACUC to determine:
 - a. Rationale for involving, and the appropriateness of the species and numbers of animals to be used.
 - b. Procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals.
 - c. The principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals.
 - d. Written assurance has been provided that the activities do not unnecessarily duplicate previous experiments.
 - e. Procedures that cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedatives, analgesics, or anesthetics, unless withholding such agents is justified, in writing, for scientific reasons.
 - f. Animals are humanely euthanized by IACUC approved methods.

There are special sections in the application for surgery, hazardous agents, and prolonged restraint. These must be completed if they are pertinent to the protocol.

Qualifications and experience of the personnel conducting the procedures are included and evaluated by the IACUC.

Applications and amendments may be submitted and considered outside the scheduled meeting; however, every committee member must review the protocol or amendment. There are no expedited reviews. Final review and approval of the full IACUC must be complete and the Principal Investigator notified before the work can begin. All animal use, regardless of funding, undergoes the same review process. All approved studies undergo annual review.

Upon approval of the IACUC, the investigator must complete an "Animal Request Form" in full before animals will be ordered. These forms are available at the Animal Resource Facility office. The ARF will be able to supply cages for the majority of anticipated caging needs. If the need arises for housing that cannot be supplied by the ARF, some arrangements must be made to assure that proper facilities are provided before the animals are ordered.

2. An Animal Request Form is used to order animals through the ARF. This form, properly filled out and approved by the fiscally responsible person, must be received in the office of the ARF Director in sufficient time to allow for acquiring, processing, and delivery of the animals ordered. The ARF will order the animals and the investigator's department will be notified by telephone that the animals have been received and the room number where the animals will be housed after quarantine. All animals are ordered by ARF. This is so preparation can be made for incoming animals. Requests for the most commonly used animals need to be in the ARF office by 12:00 pm on Thursdays to ensure shipment the following week.
3. Make sure that you order what you want, and make double sure that sufficient time is allowed to acquire, process, and receive the order to your specification. With some animals, a minimum time of less than four (4) weeks between placing of orders and desired delivery may prove disappointing. Acquisition of transgenic or mutant animals or conditioned animals may require longer.
4. All new shipments of animals will be held in quarantine for various lengths of time. This is to establish their health status before they are released for investigator use and introduced to our resident animal population.

B. Animal Housing

1. Animal housing and support functions provided by the ARF are directed toward serving the interests of its users. Effective space utilization is generally best accomplished by housing identical species for numerous investigators in the same room, as opposed to assigning each investigator or department an individual room. When research protocol or scientific judgment dictates that the above system of animal housing is unacceptable, exceptions should be made. In such cases, the ARF Director, in consultation with the investigator, can assign ARF room (if available) to the investigator, or else the investigator can provide his own areas for animal housing if this is physically acceptable. In both cases, if the ARF does not provide total animal care, the individual must provide in a humane legally prescribed manner the complete environmental care and sanitation needs of the involved animals as outlined by the ARF Director. The only exception to this would be documented evidence that these procedures will interfere with research objectives.

2. Minimum standards pertaining to space required for each animal have been established. When animals are found in cages which are too small or they have outgrown cage sizes considered adequate when they arrived, they will be moved into adequate sized units. Since this could be disruptive to experiments, it is advisable that all investigators doing long-term work maintain a liaison with the ARF personnel to assure that adequate cage requirements are met.
3. Animals will not be kept in research laboratories overnight unless protocol dictates and prior approval from the ARF Director has been granted.
4. Animals shall be transported from animal rooms in approved carrying cases or cages.
5. The use of animal rooms for purposes other than housing animals is discouraged because of possible hazardous effects on the other animals. There is no provision for investigators' records or equipment to be kept in the animal rooms. For investigator convenience, any qualified investigator may use the procedure room for animal manipulations. It is requested that the use of the room be scheduled through the ARF office. If scheduled, the room will be available to you at the scheduled time. This room will be clean when you use it, and is expected to be clean before you leave it.
6. Casual visitors and pets are not allowed in the animal facilities.
7. Smoking and eating will not be permitted in any of the animal rooms.

C. Animal Care

1. The total maintenance program of all animals housed in the ARF will be conducted by trained ARF personnel. This program includes daily cage cleaning, feeding and watering along with the routine cage washing, treatment for naturally occurring diseases and other needs that may arise in regard to good animal husbandry practices. The ARF daily maintenance includes all routine care by personnel. Included are feeding of standard diets, ad lib; watering daily, ad lib; provision of bedding; routine cleaning and sanitizing of cages, and routine cleaning of the rooms.
2. Special diets provided by investigators can be fed by ARF personnel *ad lib*, or in prepackaged units or in easily measured quantities. This applies to situations where very little, if any, record of consumption or spillage is required. Potentially hazardous agents will be fed by ARF personnel only after approved by the IACUC.
3. Any animal that is sick, diseased, or injured must be provided with veterinary care or humanely destroyed. When therapy is indicated, the investigator is notified before recommended treatment is begun. If it is impossible to contact the investigator within a

reasonable period of time, critically ill or injured animals will be euthanized. The only exceptions are those disabilities which are an integral part of an appropriately approved project. These procedures must be carefully supervised by the principal investigator or other qualified scientist. The scientist in charge of the experiment must be prepared to terminate it whenever its continuation may result in unnecessary injury or suffering to the animal.

D. Animal Disposition

1. After completion of an animal experimentation project, investigators will be responsible for the disposition of the animals. The ARF Director will be available for consultation on the accepted methods of euthanasia. There is no place in the ARF where ether can be used safely. If animals are euthanized with ether, under no circumstances should the carcasses be placed in the cold room (B21) before the odor of ether has been dissipated in a hood. No animal should be discarded until death is certain.
2. After investigators complete their animal experimentation projects, they should place all animal carcasses or animal parts that are no longer needed in a plastic bag and deposit them in the cold room (B21). ARF personnel will be responsible for the disposition of the animal carcasses.
3. In the event an experimental animal should die, ARF personnel will place it in the cold room (B21) and an "Animal Death Report" will be completed. The responsible investigator will be notified as soon as possible. The animal carcass will be held in the cold room (B21) for 24 hours and then disposed of. If an investigator desires to have his animal held for a longer period of time, he should contact the ARF Director.
4. Research animals are considered state property and none may be retained or given as pets.

E. Special ARF Services

1. Veterinary consultation is encouraged and will be available for an investigator who desires this service for University related teaching or research needs. Consideration might be given to such things as:
 - a. Suitable animals for the experimental design.
 - b. Sources of animals and equipment.
 - c. Necropsy results.
 - d. Per diem charges.

- e. Unusual environmental requirements.
 - f. Special diets and care.
 - g. Pre- and post-operative care.
 - h. Special nursing care.
 - i. Analgesics.
 - j. Other professional, technical, and ancillary services.
2. ARF personnel will be available for assistance in handling and restraint of some of the larger and more difficult animals for short periods of time.

F. Billing

1. The ARF will prepare monthly billings for investigators who have utilized the services. The statement will be itemized to indicate the number of board days per species, animal purchases, and other charges for specific items that have been requested. This statement will be forwarded to the Investigator's Department where the amount of each investigator's charges is encumbered by the Department Secretary on the Banner System.
2. The School of Medicine is absorbing many of the costs inherent to the operation of the ARF, such as transportation from the airport to the school, maintenance of the facility, disposal of animals, etc.

G. Animal Hazards and Zoonotic Diseases

1. The hazards associated with handling animals can be loosely placed in three categories. First, physical injuries occur from bites or scratches (rodents, rabbits, dogs, cats, swine, primates, and others), kicks (horses, cows), and direct injuries such as butts from goats and punches from apes. The key to prevention of these types of injuries is proper training of research personnel by the animal care staff or other qualified individuals. Second, there are serious allergic hazards associated with breathing or contacting allergens such as animal dander or urine. The safest policy is to prevent exposure by always wearing protective clothing (such as face masks, gloves, and a lab jacket) when handling animals. Third, the possibility of zoonotic diseases must always be considered. Zoonotic diseases are those that can be transmitted from animals to humans. Although zoonotic diseases are not common in modern facilities, the prevention, detection, and eradication of zoonotic diseases from the animal facility is a primary concern of the entire animal care staff. Remember that zoonotic diseases can frequently be transmitted by tissues as well as the living animals.

Although humans usually are not susceptible to infectious diseases suffered by animals, there are some important exceptions. Infections of animals may sometimes produce severe disease in humans even when the animals themselves show little, if any, sign of illness. A pathogen in the normal flora of a healthy animal may cause a serious disorder in a person exposed to it because the animal has developed "resistance" to these microorganisms, whereas humans with no previous exposure to the agent lack this protective immunity. Therefore, one should always be aware of possible consequences when working with each species of animal, and take precautions to minimize the risk of infection. In the event that you do become ill with a fever or some other sign of infection, it is important to let the physician caring for you know that you work with animals.

Some of the specific diseases and the animals associated with those disorders are described below.

There are some common sense steps that can be taken to lessen the risk of infection in general. These include no eating, drinking, or applying cosmetics or contact lenses around animals or animal care areas, wearing gloves when handling animals or their tissues, taking care not to accidentally rub your face with contaminated hands or gloves, and hand washing after each animal contact. Research personnel can protect themselves against self-inoculation by wearing gloves, substituting manually operated pipettes for needles and syringes, taking enough time to give injections properly, anesthetizing animals prior to inoculation with infectious agents, and using a two-person team to inoculate animals. Do not recap used needles! Instead, discard them promptly in a biohazard "sharps" container. For procedures such as necropsies, bedding changes, and tissue and fluid sampling, physical containment devices such as biological safety cabinets, full-face respirators or other personal safety gear should be used as indicated.

The scope of possible zoonotic infections is quite large, and only a few examples will be described here. However, all personnel should be aware that laboratory animals are sources of potent allergens to sensitized persons. Further details are available from the Division of Occupational and Environmental Health.

- a. If you work with rodents (e.g., Gerbils, Guinea Pigs, Hamsters, Mice, Rats)
 - 1) Contact with rodents requires precautions against such diseases as tapeworm infection, lymphocytic choriomeningitis (LCM), salmonellosis, and "ringworm" fungal skin infections. Additional concerns for investigators using some rodents are leptospirosis and bubonic plague. Attention should also be paid to the possibility of allergic reactions.

To protect against these agents, care should be taken to limit exposure to soiled bedding containing feces (salmonellosis, tapeworms) and urine (LCM and leptospirosis). Gloves and masks not only limit exposure to soiled bedding, but also help prevent transmission of diseases such as ringworm and fur mites when rodents are handled.

There are infectious agents that can be transmitted to humans through rodent bites, but the incidence of these agents in modern rodent colonies is virtually non-existent.

b. Occupational Health and Safety Program

Our health program may include the following, depending on results of a risk assessment by the occupational health personnel. A TB test is done. If TB test is positive, employee will be given a chest radiograph. Tetanus protection status is evaluated and brought up to date. A complete blood count without a differential is done. The rabies prophylactic series using Human Diploid Cell Rabies Vaccine is offered, as well as the prophylactic series for Hepatitis B. The blood work and the urinalysis is done annually, as well as the TB test. This program is mandatory for all of the animal care staff. The program is offered to all research investigators and research technicians who have substantial animal contact. The records are kept in the Occupational Health office and also sent to the employee. The Occupational Health Office monitors the program. For personnel potentially exposed to hazardous agents such as infectious virus, a vaccination program is offered if vaccines are available.

H. Procedures for reporting exposure and potential hazards.

Personnel should notify their supervisor of potential or known exposures and of suspected health hazards and illnesses. The supervisor should record this exposure or illness and contact the emergency room for instructions. If the employee's supervisor is not available, he should contact the IACUC Chairman or the Veterinarian for instructions.

Suspected health hazards should be reported to the supervisor, IACUC Chairperson, or the Veterinarian for corrections.

**ANALGESICS FOR RESEARCH ANIMALS
DOSAGE IN MG/KG**

| DRUG | RATS, MICE, HAMSTERS?, GERBILS? | GUINEA PIGS |
|---------------------------|---|------------------------|
| Aspirin | 20 S.Q. | 20 S.Q. |
| Pentazocine (Talwin®) | 10 S.Q. (Rat) 4 hours | 10 S.Q. 4 hours |
| Butorphanol (Stadol®) | 1 – 5 S.Q. (Mice) 5 – 10 (Rat) 4 hours | |
| Meperidine (Demerol®) | 20 S.Q. 2 – 3 hours | 20 S.Q. 2 – 3 hours |
| Morphine | 10 S.Q. 2 – 4 hours | 10 S.Q. 4 hours |
| Buprenorphine (Buprenex®) | 2.0 S.Q. (Mice) 0.01 – 1.0 S.Q. (Rat) 0.02 12 hours | 0.05 S.Q. 12 hours |
| Codeine | 20 S.Q. (Mice) 60 S.Q. (Rat) 4 hours | |
| Flunxin (Banamine®) | 2.5 S.Q. (Mice) 1.1 S.Q. (Rat) 1.2 12 hours | |

**ANALGESICS FOR RESEARCH ANIMALS
DOSEAGE IN MG/KG**

| DRUG | DOG | RABBIT |
|---------------------------|-------------------------------------|-----------------------------------|
| Morphine | 0.5 – 1.0 S.Q. 2 – 4 hours | 5.0 S.Q. 2 – 4 hours |
| Meperidine (Demerol®) | 5.0 – 10.0 I.M. 6 hours | 10.0 S.Q. 2 – 3 hours |
| Butorphanol (Stadol®) | 0.05 – 0.1 I.M., S.Q. 6 12 hours | 0.1 – 0.5 I.V. 0.2 4 hours |
| Buprenorphine (Buprenex®) | 0.01 – 0.02 S.Q. 0.02 12 hours | 0.03 – 0.05 S.Q. 0.04 12 hours |
| Aspirin | 10 P.O. 6 – 8 hours | |
| Flunxin (Banamine®) | | 1.1 S.Q. 1.2 12 hours |

ANESTHETICS AND TRANQUILIZERS FOR
RESEARCH ANIMALS

DOSAGE IN MG/KG

| DRUG | MOUSE | RAT |
|-------------------------------------|---|---------------------------------|
| Thiopental | 25 – 50 IV | 30 IV |
| Pentobarbital | 40 IP Sedation 50 – 90 IP Anesthesia | 50 IP |
| Ketamine plus Xylazine | 200 IM 10 IP | 40 – 80 IM, IP 5 – 10 IM, IP |
| Ketamine plus Acepromazine | 100 IM 2.5 IM | 40 – 80 IP 2.5 IM, IP |
| Ketamine plus Diazepam | 200 IM 5 IP | 40 – 80 IP 5 – 10 IP |
| Urethane 50% Concentration | | 1000 – 2000 IP |
| Chloral Hydrate 5% Concentration | | 300 – 450 IP |
| Acepromazine | 1 – 2 IM | 1.0 IM |

ANESTHETICS AND TRANQUILIZERS FOR
RESEARCH ANIMALS

DOSAGE IN MG/KG

| DRUG | GUINEA PIG | HAMSTER |
|-------------------------------|------------------------|---------------------------|
| Thiopental | 20 IV | 20 IV |
| Pentobarbital | 35 – 40 IP | 50 – 90 IP |
| Ketamine plus Xylazine | 40 – 80 IM 4 – 5 IM | 100 – 200 IP 7 – 10 IP |
| Ketamine plus Acepromazine | 125 – 150 IM 5.0 IM | 150 IM 5.0 IM |
| Acepromazine | 5 – 10 IM | 1 – 2 IM |
| Ketamine plus Diazepam | 100 IM 5 IM | |

ANESTHETICS AND TRANQUILIZERS FOR
RESEARCH ANIMALS

DOSAGE IN MG/KG

| DRUG | DOG | CAT |
|---------------------------|-------------------------------------|------------------|
| Thiopental | 10 – 20 IV | 10 – 15 IV |
| Pentobarbital | 25 – 30 IV | 25 – 30 IV |
| Ketamine plus Xylazine | 5.0 IV 1 – 2 IV | 15 IM 1.0 IM |
| Acepromazine | 0.05 – 0.1 IM 0.06 0.55 – 2.2 SQ | 0.1 – 0.2 IM, SQ |

ANESTHETICS AND TRANQUILIZERS FOR
RESEARCH ANIMALS

DOSAGE IN MG/KG

| DRUG | RABBIT |
|-------------------------------|--------------------------------------|
| Thiopental | 30 IV |
| Pentobarbital | 45 IV |
| Ketamine plus Xylazine | 35 IM 5.0 IM less for sedation |
| Acepromazine | 1 – 5 IM |
| Ketamine plus Acepromazine | 75 IM 5.0 IM |
| Ketamine plus Diazepam | 25 IM 5.0 IM |

STANDARD OPERATING PROCEDURE #1

Sanitation - Animal Room Cleaning

Purpose: To set forth proper procedure to insure that animal rooms are maintained in a clean and sanitary manner.

Methods: All animal rooms are cleaned weekly. This cleaning includes the following:

1. All trash cans are emptied and a clean plastic liner inserted daily; containers are sanitized once per week.
2. Sinks and table tops are washed daily using a detergent disinfectant solution (Bio-Kleen) using one ounce in three gallons of water.
3. Rooms will be swept daily and mopped once or twice per week, or as needed.
4. All air vents will be cleaned weekly or as often as necessary.
5. Corridors are swept and mopped at least once per week, and walls and ceilings are cleaned once per month.
6. Room doors and windows are cleaned once per week.
7. Two rooms will be stripped of all equipment and will be washed down (including walls, ceiling, pipes, air ducts, floors) each week, following removal of animals, and as needed. On this schedule, all rooms will be cleaned once per month. The room will be sanitized using a detergent-disinfectant solution.

NOTE:USE OF CLEANING AGENTS CONTAINING ODOR COUNTERAGENTS IS PROHIBITED.

STANDARD OPERATING PROCEDURE #2

Sanitation - Cage Cleaning Frequency

Purpose: To establish the frequency and the procedures for cage changing so that animals are housed in a clean environment.

- Methods:**
1. All small animals are transferred to clean cages at least twice per week.
 2. Clean cages, outfitted in the same manner as the one with which they are exchanged, are brought into the animal room. Starting at the left top of each rack, each animal and its cage are transferred.
 3. Special procedures are in place for maintenance of dogs (See SOP #9)
 4. Guinea pig cages have an alkaline urine deposit - usually in the corners. These deposits are removed with a urinary de-scaler before they are sanitized in the cage washer. Rabbit pans and cages are cleaned with Lime-Away de-scaler also.
 5. Wire stainless steel tops are changed and sanitized at least once per week.
 6. Water bottles are changed at least twice a week.

STANDARD OPERATING PROCEDURE #3

Sanitation - Rack Washing Frequency

Purpose: To establish procedures to assure racks are maintained in a clean condition.

- Methods:
1. Racks in use are washed once per week; all racks are wiped down twice per week; carts and racks are dusted daily or as often as necessary.
 2. A detergent-disinfectant is used to sanitize racks and carts.

STANDARD OPERATING PROCEDURE #4

Operation of the Cage Washer

Purpose: To insure proper operation of the cage washer.

- Methods:
1. Jets on impellers are checked daily and cleaned as necessary.
 2. Cage washer will be cleaned out daily; both screens in the cage washer are cleaned daily.
 3. After the cages have been dumped and rinsed, the cages are put into the cage washer.
 4. The detergent is added by the operator at the beginning of the cycle.
 5. The desired cycle times are selected and the machine is started.
 6. After the cycle has been completed, the cages are placed on racks to dry. When dry, they are taken to the storage room, filled with bedding and stored for later use.
 7. Quality Control reports are conducted monthly on a random sampling of water bottles and cages. The bottles/cages chosen are swabbed onto RODAC plates and then incubated to detect colony growth. The plates are incubated for 48 hours and the colonies are counted and recorded. Copies of the report are kept in the Animal Resource Facility Office.
 8. All stainless steel in the cage wash room will be cleaned and polished once per week.
 9. The cage washer is drained at the end of the day and the cage wash room is swept and mopped with a detergent-disinfectant at the end of every working day.

STANDARD OPERATING PROCEDURE #5

Bedding - Storage and Changing Procedures

Purpose: To insure proper storage and instructions for the proper use and disposal of bedding material.

Methods: A. Receiving and Storage

1. Verify the amount received.
2. Refuse any bags that are torn and leaking. Patch immediately any bags torn during the unloading process or empty them into bedding container, provided that the bedding has not become wet or contaminated. Dispose of spilled or contaminated bedding. Do not accept wet bags.
3. Plastic containers, lids, and roller base for storage of daily use bedding are sanitized once per week in the cage washer.
4. Pallets for storage of bulk quantities are cleaned quarterly or more often as necessary with the high pressure sprayer. The bulk feed and bedding storage area is swept weekly.

B. Dispensing

Bedding is to be placed in clean animal cages only in the Bedding room or the clean cage storage room. It is not to be done in the animal rooms. Filling of cages causes bedding particles to become dust particles in the rooms. The bedding can be placed in clean animal cages and placed in clean cage storage or bedding storage rooms.

C. Removal of Spent Bedding

After changing animals to clean cages containing clean bedding, the dirty cages containing dirty bedding are taken to the cage cleaning area. There the bedding is dumped into the Garbel disposal sink, the cage scraped, brushed, and sprayed out, and then placed on a rack for subsequent cleaning in the cage washer. If washing by hand instead of the cage washer, wash with water containing a detergent-disinfectant, rinse with hot clear water, allow to dry, and store in clean cage storage room.

STANDARD OPERATING PROCEDURE #6

Animal Feed - Monitoring and Storage

Purpose: To insure that animal feeds purchased by the ARF are received and stored until time of use in wholesome condition.

Methods: A. Receiving and Storage

1. Verify the amount and type of food. Check each lot for the date of manufacture. Inventory on a weekly basis, quantities on hand.
2. Refuse and return all bags that are:
 - a. Torn and leaking food.
 - b. Not stamped on the bag with a milling date (hand written milling date are unacceptable).
3. Patch immediately any bags torn during the unloading process or empty them into a feed container, provided that the feed has not been contaminated. Dispose of any spilled or contaminated feed, and bags which are wet or show evidence of having been wet.
4. Do not accept feed if more than two months have elapsed since manufacture or if older, mark each bag "Use by (date)" allowing six months from milling date, except guinea pigs food, which is three months.
5. Place accepted feed in the food storage room. Stack the feed on shelves in such a manner as to allow easy access to that food with the oldest milling date so it can be used first.
6. Rotate the older feed to the front and use prior to the new shipment.
7. Plastic container, lids, and roller base for storage of daily use food are sanitized once per week in the cage washer.

B. Feed Use

Technicians and animal care personnel using the feed are to check the general appearance and odor of the feed and to watch for contaminants.

Milling dates should be checked: guinea pig feed is not to be used if more than three months old; all other six months. Outdated or suspect feed is set aside and the Veterinarian notified.

C. Feeding

1. Animals on regular diets are fed daily, ad lib. Special diets provided by investigators can be given to the animals by the ARF personnel ad lib, in prepackaged units or in easily measured quantities. This applies to situations where very little, if any, records of consumption or spillage are required. Potentially hazardous agents will be fed by ARF personnel only after approval by the Animal Studies Subcommittee and Research Safety/Bio-Safety Subcommittee.
2. All feed bowls and hoppers are cleaned and sanitized in the cage washer once per week or more often if necessary.

STANDARD OPERATING PROCEDURE #7

Quarantine Room Procedures

Purpose: To control the spread of disease from newly acquired animals into the general public.

- Methods:
1. Entry into the Quarantine Room is restricted to ARF personnel.
 2. Entry should be as infrequent as possible and the Quarantine Room should be entered last.
 3. An isolation gown must be worn in the Quarantine Room and removed as you leave. This gown will be worn in this room only.
 4. Wash hands upon entering room and as you leave the room.
 5. Items (animals, cages, etc.) will not be brought into or taken from the Quarantine Room without approval of ARF personnel.
 6. The Quarantine Room and everything in it (cages, racks, etc.) will be thoroughly cleaned after each group of animals is removed.
 7. The species of animals quarantined determines the cleaning schedule. These schedules will be the same as when the animals are housed in the holding room.
 8. Quarantine rooms will be empty except for the racks, cages, and bottles needed to care for the animals in quarantine.

Animals of the same species delivered directly to the facility by vendor truck may, after completing an initial inspection, be placed in existing holding rooms. These holding rooms must house the same species of animals from the same vendor. All newly received animals should be allowed a stabilization period prior to their use.

Animals arriving at the facility by any other means, or with incomplete histories, will be placed in quarantine. Quarantine time will vary depending on the species and the source. Ten days for most animals and up to four weeks for random source animals. These animals will be examined and released by the veterinarian at the completion of the quarantine period.

STANDARD OPERATING PROCEDURE #8
Caging and Housing Dimensions for Laboratory Animals

| Cage Dimensions | Animals per Cage |
|--|-------------------------|
| Rats in 20" X 16" X 8" plastic cages | |
| Weight of Rat (grams) | Number in Cage |
| Up to 100 | 15 |
| 100 – 200 | 11 |
| 200 – 300 | 8 |
| 300 – 400 | 6 |
| 400 – 500 | 4 |
| Over 500 | 3 |
| Rats I 19" X 10½" X 8" plastic cage | |
| Weight of Rat (grams) | Number in Cage |
| Up to 100 | 8 |
| 100 – 200 | 6 |
| 200 – 300 | 4 |
| 300 – 400 | 3 |
| 400 – 500 | 2 |
| Over 500 | 1 |
| Guinea Pigs in 20" X 16 X 8" plastic cage | |
| Weight of Guinea Pig (grams) | Number in Cage |
| Up to 350 | 4 |
| Over 350 | 2 |
| Guinea Pigs in 19" X 10½" X 8" plastic cage | |
| Weight of Guinea Pig (grams) | Number in Cage |
| Up to 350 | 2 |
| Over 350 | 1 |
| Mice in 11½" X 7½" X 5" plastic cage | |
| Weight of Mice (grams) | Number in Cage |
| Up to 10 | 11 |
| 10 – 15 | 8 |
| 15 – 25 | 5 |
| Over 25 | 4 |
| Mice in 19" X 10½" X 5" plastic cage | |
| Weight of Mice (grams) | Number in Cage |
| Up to 10 | 24 |
| 10 – 15 | 18 |
| 15 – 25 | 12 |
| Over 25 | 9 |

MU MEB Policy & Procedures Manual

| Hamsters in 20" X 16" X 8" plastic cage | |
|--|----------------|
| Weight of Hamster (grams) | Number in Cage |
| Up to 60 | 25 |
| 60 – 80 | 19 |
| 80 – 100 | 16 |
| Over 100 | 13 |
| Hamsters in 19" X 10½" X 8" plastic cage | |
| Weight of Hamster (grams) | Number in Cage |
| Up to 60 | 13 |
| 60 – 80 | 10 |
| 80 – 100 | 8 |
| Over 100 | 7 |
| Rabbits in 4 foot square stainless steel cage | |
| Weight of Rabbit (pounds) | Number in Cage |
| <11.88 | 1 |
| Rabbits in 5 foot square stainless steel cage | |
| Weight of Rabbit (pounds) | Number in Cage |
| >11.88 | 1 |

STANDARD OPERATING PROCEDURE #9

Dogs

Purpose: To establish and maintain proper procedures for the care and use of dogs in laboratory animal housing.

Methods: A. General

1. During the initial conditioning period, physical examination and treatment of dogs will be performed by the veterinarian.
2. Dogs will not be moved outside of the holding room without a leash or other restraint.

B. Feeding and Watering

1. All dogs are to be fed first thing in the morning, according to specified amounts and diet requirements. After feeding, wait approximately one hour before cleaning.
2. All dogs will be provided with fresh food and water daily.
3. Feed bowls will be changed daily and water bowls will be replaced twice each week with clean sanitized bowls.

C. Cleaning

1. Cage floors and pans will be flushed into the wall gutter when cleaning in the morning and in the afternoon before going home. Cage floors and pans will be flushed only once per day on weekends and holidays.
2. With care, the cage may be hosed with the dog in it. Care must be taken not to get the dog wet.
3. Fecal material may be removed with a shovel before cleaning is started.
4. Flush wall gutters to floor drain after cleaning cages. Gutters are cleaned daily.

5. Dogs will be removed from the cages and the cages cleaned with the high pressure sprayer once a week. The cage floor and pan will be washed in the cage washer at this time.
6. The room floor under and around cages will be mopped daily.
7. Animal room doors are to be kept closed except during movement of cages, supplies, or personnel.

D. Handling

1. Dogs will not be taken outside of the Animal Research Facility by animal caretakers.
2. Dogs should never be allowed to jump off a table or from the top cages.
3. Every dog has the potential to become unmanageable or to inflict serious harm to the handler. Always use caution.
4. If a dog appears unmanageable, consult the veterinarian before handling the animal.
5. For exercise of dogs, see SOP #17.

STANDARD OPERATING PROCEDURE #10

Security Procedures

Purpose: To establish security procedures for the Animal Resource Facility.

- Methods:**
1. Unauthorized entry into the Animal Resource Facility is strictly prohibited. Special attention to physical security is warranted by the threat of property destruction and theft by groups opposed to the use of animals in research. The outside doors to the building remain locked after normal working hours and on weekends and holidays. Issue of keys to the area is stringently controlled. The external doors of the ARF remain locked at all times. The doors to individual rooms in the ARF remain locked after normal duty hours and on weekends and holidays, unless the ARF staff is present in the room.
 2. Requests for tours of the ARF by the media and persons claiming to represent animal rights and animal welfare organizations must be approved by the Institutional Official. If approved, such tours will be conducted by the Director of the Animal Resource Facility.
 3. Inquiries regarding lost pets should be handled with caution and sensitivity and will be referred to the Veterinarian.

STANDARD OPERATING PROCEDURE #11

Education and Training

Purpose: To outline the training program regarding the functions, operation, and use of the Animal Resource Facility and animal subjects; and to outline the Institutional Animal Care and Use Committee (IACUC) responsibilities regarding instruction available and assessment of the capabilities of investigators and staff to perform the investigation.

- Methods:**
1. Newly recruited staff are required to have training regarding the functions and operations of the Animal Resource Facility and use of animal subjects. Newly recruited animal care technicians undergo an intensive training program under the direction of the veterinarian. Work is closely, carefully, and frequently monitored. Each duty is explained and demonstrated. Reference material, Standard Operating Procedures, and the Policies and Procedures manual are current and are maintained in the ARF office for all employees who work with animals.
 2. The training program includes, but is not limited to animal husbandry, sanitation procedures, AAALAC guidelines, Animal Welfare Act and other regulatory guidelines, guidelines for adherence to protocols, normal/abnormal behavior, signs/symptoms of illness, handling and manipulation of various species, records and reports, ethical considerations, including testing methods that minimize the number of animals required to obtain valid results and minimize animal distress, occupational health and safety, and general operating guidelines for an Animal Resource Facility.
 3. In addition to personal interaction with the veterinarian, audiovisuals, journals, bulletins, and magazines are used as training aids. The veterinarian also provides training and instruction to all research technical staff and investigators as appropriate. The IACUC will assess the capabilities of each investigator and his/her staff as part of the review process before the study is permitted to begin. Specific areas of training include:
 - A. Humane and scientifically sound practices of animal care and use, including:
 - the basic needs of each species of animals.
 - proper handling and care for the various species of animals used.
 - proper pre- and post-procedural care of animals.
 - aseptic surgical methods and procedures.
 - the importance of strict adherence to protocols in terms of

procedures and medications

- B. The concept, availability, and use of research or testing methods which limit the use of animals or minimize animal distress.
 - C. Proper use of anesthetics, analgesics, and tranquilizers for any species of animal used by the facility.
 - D. Methods whereby deficiencies in animal care and treatment are reported, by any employee of the facility. No facility employee, Committee member, or laboratory personnel shall be discriminated against or be subject to any reprisal for reporting violations of regulations or standards under the Animal Welfare Act. See SOP #23.
 - E. Utilization of services (i.e. National Agricultural Library, and National Library of Medicine) available to provide information:
 - on appropriate methods of animal care and use.
 - on alternatives to the use of live animals in research.
 - unintended or unnecessary duplication of research involving animals.
 - the intent and requirements of the Animal Welfare Act.
 - F. Specialized instruction for use of ANY radioactive, biohazardous, or other hazardous substance(s).
 - G. Any special skills required in performance of animal manipulative procedures employed in the course of experimentation.
 - H. Employee rights to a safe and healthy workplace under the Occupational Safety and Health Act, Public Law 91-596 and Executive Order 12196.
4. To remain useful and current, the training program is reviewed annually and updated as necessary to fit the needs of the individuals involved, and to meet the legal requirements of regulatory agencies.

STANDARD OPERATING PROCEDURE #12

Equipment Maintenance and Related Activities

Purpose: To establish an equipment and facility maintenance program for the Animal Resource Facility.

Methods: The following is the maintenance program governing the operation of the Animal Resource Facility.

Equipment used for animal experimentation is checked/inspected by Biomedical Engineering annually or more often if necessary.

Temperature is monitored and recorded on the room census sheet daily by the ARF staff during daily examination of animals. High and low temperatures for the previous day are recorded from the Hi-Low thermometers attached to the animal room wall.

The light cycle in each room is controlled by timers and is set for 12 hours light and 12 hours darkness, unless the protocol requires a different cycle.

Macro environments are monitored by a centralized computer program which records humidity levels, intake air temperature, and exhaust air temperature.

The cage washer spray nozzles are checked daily and cleaned as needed.

The emergency power generator is inspected monthly by our Engineering staff and after each use.

Emergency Eyewash stations are checked on a weekly basis.

STANDARD OPERATING PROCEDURE #13

Veterinary Medical Care Program

Purpose: To outline the veterinary medical program of this facility.

- Methods:
1. Veterinary care is provided by Billy W. Howard, DVM, Director, Marshall University School of Medicine, Animal Resources Division. He has been involved with laboratory animal medicine since 1985.
 2. The veterinarian's major responsibilities are the appropriate and efficient functioning of the Animal Resource Facility (ARF), overseeing all professional aspects of the ARF program, including, but not limited to monitoring care and use of laboratory animals, establishment of policies and procedures for veterinary care, reviewing protocols, animal husbandry and animal welfare, monitoring occupational health and conducting educational programs for ARF staff and other research technicians.
 3. His professional qualifications and interest lie in the specialty of Laboratory Animal Medicine and he serves the School of Medicine in all matters of Veterinary Science. The veterinarian will be responsible for complying with and implementing all aspects of the "Laboratory Animal Welfare Act: (PL 89-544)" and its subsequent amendments. He stays informed in order to comply with future legislation that may affect the use of laboratory animals.
 4. The veterinarian will serve on the Institutional Animal Care and Use Committee and inform the committee on matters dealing with laboratory animal care, use, etc. Veterinary care will be available for any investigator who desires this service for research or for which the IACUC mandates veterinary oversight. Consideration will be given to such things as:
 - a. Suitable animals for the experimental design.
 - b. Sources of animals and equipment.
 - c. Necropsy results.
 - d. Unusual environmental requirements.
 - e. Special diets and care.
 - f. Pre and post-operative care.

- g. Special nursing care.
- h. Analgesics and anesthesia.
- I. Other professional, technical, and ancillary services.

5. Veterinarian Location

The Veterinarian is located in Room 112 of the Byrd Biotechnology Science Center and is immediately accessible by telephone. He provides training and guidance to the animal care technicians. Additionally, he meets with all investigators who submit research protocols utilizing animal subjects to discuss the study and animal species required. A back-up veterinarian, Dr. Jan M. Ball, is available to provide care in the absence of the veterinarian.

6. Primary Duties of the Veterinarian

The Primary Duties of the veterinarian include, but may not be limited to:

- a. Directing the design and operation of the animal facility to ensure compliance with current animal welfare laws, regulations and policies, and to support R&D activities using animal subjects.
- b. Providing professional guidance and technical support to the health care facility's investigators and technicians in planning, executing, and directing R&D activities using animal subjects.
- c. Initiating or reviewing requests for equipment used in the animal facility and plans for animal facility construction and renovation.
- d. Serving as a member of the Institutional Animal Care and Use Committee (IACUC). The IACUC oversees all animal research facility activities, including review of all protocols utilizing animal subjects, policies and procedures, physical facilities, and operations.
- e. Reviewing all proposals that include the use of live vertebrate animals before consideration by the IACUC.
- f. Participating in semi-annual inspections of animal facilities.
- g. Contributing to the promotion of favorable community relations and

increased public appreciation of the importance of animal studies in improving patient care.

- h. Consideration for humane aspects of animal experimentation such as the proper use of anesthetics, analgesics and tranquilizers.
- i. Implementation of measures to alleviate unacceptable levels of pain or distress to animal subjects.
- j. Provides training and guidance to animal care technicians and annual training of all staff. The veterinarian will conduct training sessions with the Animal Resource Facility Staff and with the research technicians and investigators for each new protocol or modification of an existing protocol, outlining the salient points of the protocol. This training will be conducted upon the receipt of the first shipment of animals and will be specific to each protocol or modification thereof.

STANDARD OPERATING PROCEDURE #14

Sanitation of Procedure Rooms

Purpose: To establish sanitation procedures for Procedure Rooms.

- Methods:
1. Use of the Procedures Room must be scheduled in advance with Animal Care Staff.
 2. All rooms will be swept and mopped with a cleaner/disinfectant and after every procedure and/or removal of animal(s).
 3. All equipment will be cleaned once per week with a cleaner/disinfectant and after every procedure and/or removal of animal(s).
 4. All tables (examining, etc.) and counter tops will be cleaned once per week and after every procedure and/or removal of animal(s).
 5. All equipment will be removed once per month and walls, ceiling, pipes, and floors will be cleaned and disinfected with Bio-Kleen®, and after every procedure involving a large animal.
 6. Trash will be emptied daily or as needed and new liners inserted. Trash containers will be sanitized once per week and after every procedure in the cage washer.

NOTE: ALL INVESTIGATORS WHO USE THE PROCEDURE ROOM ARE RESPONSIBLE FOR CLEANING UP AFTER THEMSELVES. ALL HEAVY CLEANING/DISINFECTING WILL BE PERFORMED BY ARF STAFF.

STANDARD OPERATING PROCEDURE #15

Operating Procedures for the High-Pressure Spray Cleaner

Purpose: To establish operating guidelines for the High-Pressure Spray Cleaner

- Methods:
1. When animal rooms are stripped and cleaned, the walls and floor will be sprayed with the sprayer containing a cleaning/disinfectant solution
 2. Dog cages will be sprayed with the sprayer after the cages are hand scrubbed and the animal housed in that cage has been removed, as well as any animal(s) in any surrounding cages that may be exposed to the over spray. The cleaning solution will be the same as used in the animal rooms. (Note: All parts of the dog cages that will fit in the cage washer, will be sanitized in it.)
 3. All stainless steel carts with hanging baskets will be sprayed with the sprayer using the same cleaning solution as is used in the animal rooms.
 4. The sprayer is cleaned after each use.

STANDARD OPERATING PROCEDURE #16

Health Monitoring Program

Purpose: To establish a health monitoring program for animals housed in the Animal Resource Facility.

Methods:

1. The veterinarian is notified immediately when animals display signs of illness or injury.
2. When uncrating shipments of new animals, special attention is given to the level of activity of the animals and feces are observed for signs of illness. All animals are visually observed for gross lesions and/or symptoms of illness or disease each day.
 - a. Every animal will be checked once a day for overt signs of illness and/or injury. This includes weekends and holidays.
 - b. Any signs of unusual behavior, unhealthy appearance (not eating or drinking, diarrhea, lack of feces, bloody urine, etc.), or obvious signs of injury are immediately reported to the investigator or technician. The ARF personnel will then contact the veterinarian. The investigator and veterinarian will decide if treatment is warranted or if the animal should be euthanized. If disease is suspected in a dead or moribund animal, the veterinarian will conduct the examination or necropsy. At the discretion of the veterinarian, cultures or tissues will be taken for histopathology. If necessary, steps can also be taken to isolate the animal.
3. If an animal displays signs of illness or disease, it is immediately removed from the colony and quarantined. The veterinarian and Principal Investigator (PI) are contacted. The veterinarian examines the animal(s) and prescribes the necessary treatment or recommends euthanasia. Necropsy is performed and further diagnostic testing ordered if deemed necessary by the veterinarian or requested by the PI. If an entire colony displays symptoms of disease or illness, the entire colony is quarantined. If other animals are housed with this colony in the same room, the sick colony is removed and placed in quarantine.
4. During servicing of ill or suspected ill animals, ARF staff wears a gown, shoe covers, mask, and gloves. This procedure is followed when servicing animals being held in quarantine as well. Upon removal of an ill colony (or

suspected ill), the animal room is stripped and disinfected.

5. If an animal is unexpectedly found dead, a necropsy is performed and diagnostic testing is performed if deemed necessary by the veterinarian or requested by the PI.
6. All serology on animals will be done on recommendation of the veterinarian. Routine serology is done every six months on animals in long-term projects and breeding colonies. Serology for diagnostic purposes will be accomplished as deemed necessary in the professional opinion of the veterinarian. Serology is performed by an outside contract laboratory.
7. Diagnostic microbiology is performed by the VA Microbiology Laboratory. Pathology is performed by the Pathology Department at the Marshall University School of Medicine.
8. Health monitoring reports are obtained quarterly from all vendors from whom animals are purchased.
9. Microbiological cultures are performed monthly on random samples of cages and water bottles.
10. Virus Free and Non-Virus Free Animals: Specific guidelines for prevention of the spread of viruses to viral-free colonies are contained in paragraph #4 of this SOP. These include wearing a mask, gown, shoe covers, and gloves. These animals are serviced last each day. Protective clothing is discarded after leaving the room.

STANDARD OPERATING PROCEDURE #17

Exercise For Dogs

Purpose: To establish an exercise program, approved by the veterinarian and the Institutional Animal Care and Use Committee (IACUC) to provide dogs with the opportunity for exercise in accordance with the Department of Agriculture, APHIS, Animal Welfare Standards-Final Rule, February 1991.

- Methods:**
1. Dogs over 12 weeks of age housed individually in enclosures that provide less than two times the required floor space per dog will be provided additional opportunities for exercise.
 2. Opportunity for additional exercise will be provided to each dog three times per week on Monday, Wednesday, and Friday, for a period of thirty (30) minutes each day.
 3. Dogs will be removed from their cages and released together to facilitate social interaction.
 4. When possible, two compatible dogs of the same sex will be released together to facilitate social interaction.
 5. One or more persons will observe and positively, and physically interact with the dogs during the opportunity for exercise. This will facilitate socialization with humans and minimized conflict between incompatible dogs.
 6. The exercise room will be hosed, cleaned, and sanitized after each exercise period.
 7. All other enclosures for housing dogs that individually provide more than two times the required floor space per dog; or for group housed dogs that provide in total at least 100% of the required space for each dog, additional opportunity for exercise is not required.

STANDARD OPERATING PROCEDURE #18

Guidelines for Aseptic Surgery on Rodents

Purpose: To establish guidelines for aseptic surgery on rodents.

The National Research Council's Guide for the Care and Use of Laboratory Animals states that "Survival Surgery on rodents does not require a special facility, but should be performed using sterile instruments, surgical gloves, and aseptic procedures to prevent clinical infections." As required by the U.S. Public Health Service and the Animal Studies Subcommittee all vertebrate animal-use protocols, for research or teaching, regardless of funding source, must comply with the guidelines stated in the Guide.

Listed below are guidelines, which are consistent with the Institutional Animal Care and Use Committee's interpretation of the N.R.C. guidelines and which provides satisfactory aseptic conditions. Investigators who feel that their vertebrate animal experiments require significant exceptions to these guidelines should contact the veterinarian for assistance. Otherwise, investigators will be expected to follow these guidelines.

- Methods:**
1. Surgery will be conducted on a clean, uncluttered lab bench or table surface. The surface will be wiped with a disinfectant before and after use and/or covered with a clean drape.
 2. Hair should be removed from the surgical site with clippers or a depilatory. The surgical site should be treated with an antiseptic scrub and then with an antiseptic solution (chlorhexidine or povidone iodine solution).
 3. All instruments will be sterilized. The method is determined by the surgical instruments or devices being used. Acceptable techniques for cold sterilization include soaking in 2% glutaraldehyde for ten (10) hours, in 8% formaldehyde, or in 6% stabilized hydrogen peroxide for six (6) hours.
 4. The surgeon should wash his hands with an antiseptic surgical scrub preparation and then aseptically put on sterile gloves. If working alone, the surgeon should have the animal anesthetized and positioned and have the first layer of the double-wrapped instrument pack opened before putting on sterile gloves.
 5. The surgeon should wear a facemask. A cap and sterile gown are recommended, but not required.

6. Multiple surgeries present special problems. After the first surgery, the sterilized instruments may be kept in a sterile tray containing or isopropyl alcohol. The alcohol should be replaced when contaminated with blood or other body fluids. Sterile gloves should be changed between surgeries if the surgeon touches the non-sterile surfaces, alternatively, surgeons may wipe their gloves for 30 seconds with sterile gauze pads soaked in isopropyl alcohol or non sterile surfaces may be handled aseptically with sterile gauze pads.
7. The abdominal or thoracic body wall should be closed with absorbable suture material. The skin should be closed with staples or with a non-absorbable suture material in a simple interrupted pattern. Skin sutures or staples should be removed in 7 to 10 days after surgery.
8. Rodents should be recovered from anesthesia in a warmed environment. Antibiotics may or may not be indicated after surgical procedures.
9. Access to the operating area should be restricted and the entire procedure should appear professional.
10. Analgesics should be administered one day prior to surgery and for 5-7 days after surgery.

Questions about aseptic surgical techniques may be addressed to the veterinarian.

STANDARD OPERATING PROCEDURE #19

Occupational Health

Purpose: To ensure that all employees who handle animals maintain in good health.

- Methods:
- A. Pre-employment physical and periodic screening examinations may be conducted. For animal caretakers and animal handlers, the physical may include a TB Skin Test, a Physical Examination by a physician, a Urinalysis, an EKG if indicated by age or health history, Blood tests: pre-employment screening blood tests may include a CBC and other tests as deemed necessary by the examining physician, a Tetanus Injection, a Rabies and other vaccinations as required by assigned duties. Personnel health records are maintained by the Occupational Health Office of Marshall University Medical Center. All Animal Resource Facility employees are vaccinated against tetanus and have annual physicals.
 - B. A risk assessment of employees will be performed using the criteria established by the Office of Laboratory Animal Welfare (OLAW). A questionnaire for risk assessment is attached as Table 1. "Marshall University Animal Handler's Health Questionnaire." These criteria will be used to determine the procedures to be performed on each employee to insure the Occupational Health Program is utilized appropriately. Accident reporting (including animal bites, etc.) is required and proper treatment and records are maintained by personnel.
 - C. Protective equipment such as caps, masks, gloves, gowns, scrub suits, restraining cages, mesh gloves, etc. are provided to all staff. Outer garments worn in animal rooms will not be worn outside the animal facility.
 - D. Injury prevention is an integral part of the orientation process for new employees and is routinely discussed at monthly staff meetings. Animal care technicians are instructed by the veterinarian in methods in handling animals, use of mesh gloves to prevent bites, and use of restraining cages for manipulations.
 - E. Experiments involving hazardous agents are monitored closely by the appropriate committees and the IACUC approval is not granted unless adequate facilities are available and appropriate safeguards are observed.
 - F. Other appropriate immunizations are available to Animal Resource Facility personnel as determined by the personnel health physician, veterinarian, and

nature of animal research projects.

- G. All Animal Resource Facility employees receive initial training under the direction of the veterinarian regarding occupational health, including zoonoses. The education program regarding zoonoses, personal hygiene, and other considerations is under the direction of the veterinarian.
- H. Transporting animals into or through areas used by visitors is to be avoided when feasible. When essential to do so, all reasonable means of minimizing health risks to visitors will be observed.
- I. An eating and drinking area has been designated. Eating or drinking is not permitted in the Animal Resource Facility.
- J. Smoking is not permitted within the MU Medical Education Building.
- K. Shower facilities are provided for animal care technicians.
- L. Hand washing is mandatory upon entering and leaving any animal holding area or working with animal tissues.

Table 1

**MARSHALL UNIVERSITY
ANIMAL HANDLER'S HEALTH QUESTIONNAIRE**

Date: _____

Name: _____

Supervisor: _____

Department: _____

Age: _____ Sex: Male Female

OCCUPATIONAL HISTORY Answer these questions about your present job:

1. Job title: _____
2. Number of years employed at this facility: _____ years
3. How many months/years at your present position: _____
4. Description of duties (briefly): _____

5. With which laboratory animals do you work?

| Animal | Yes | No | Approximate contact hours/week |
|---------|-----|----|--------------------------------|
| Rat | | | |
| Mice | | | |
| Gerbils | | | |
| Other | | | |
| | | | |
| | | | |

6. Do you feel you are allergic to any animals? Yes No Please list those to which you feel you are allergic.

7. Did you work with laboratory animals prior to employment at this facility? Yes No If yes, how long? _____ years What species of animals? _____

8. Do you use or wear any of the following items when working with animals?

| | Yes | No |
|------------------------|-----|----|
| Protective eye glasses | | |
| Mask/respirator | | |
| Lab coat | | |
| Gloves | | |

HOME ENVIRONMENT INFORMATION

9. Do you have any indoor pets? Yes No If yes, which animals and for how long?

| Animal | 1-2 years | 3-4 years | over 4 years |
|--------------|-----------|-----------|--------------|
| Dogs | | | |
| Cats | | | |
| Other (List) | | | |

10. Do you regularly have any of the following symptoms? Yes No
Please indicate if the symptom is present and the year of onset. Also check in what location or time "period" the symptom(s) is/are present.

| Symptom | Present | Year of onset | Symptoms are present | | | |
|----------------------|---------|---------------|----------------------|---------|-------------|---------------|
| | | | At work | At home | On vacation | No difference |
| Cough | | | | | | |
| Sputum production | | | | | | |
| Shortness of breath | | | | | | |
| Wheezing | | | | | | |
| Chest tightness | | | | | | |
| Asthma | | | | | | |
| Nose congestion | | | | | | |
| Runny nose | | | | | | |
| Sneezing | | | | | | |
| Itchy eyes | | | | | | |
| Sinus problems | | | | | | |
| Hay fever | | | | | | |
| Frequent colds | | | | | | |
| Hives | | | | | | |
| Skin rash | | | | | | |
| Swelling -eyes/lips | | | | | | |
| Eczema | | | | | | |
| Difficult Swallowing | | | | | | |

Immunization History:

| Vaccine | Yes/No | Date | Vaccine | Yes/No | Date |
|-------------|--------|------|--------------------|--------|------|
| Hepatitis B | | | Tetanus-Diphtheria | | |
| Hepatitis A | | | Rabies | | |
| Comments: | | | | | |

11. Were you ever told by a doctor that you had allergies? Yes No

12. Have you ever been skin tested for allergies? Yes No If so, to what substances were you found to be allergic or sensitized? _____

13. Have you ever received allergy (desensitization/immunotherapy) shots? Yes No

14. Has a doctor ever said you have asthma? Yes No

If yes, when did your asthma start? _____(year)

Are you currently taking medication (either over the counter or by prescription) to control your asthma? Yes No

15. Has a doctor ever told you that you have a medical condition caused by your working conditions? Yes No

16. Do any of your blood relatives (grandparents, parents, brothers/sisters) have allergies or asthma? Yes No

17. Are you under a doctor's care for any other illnesses? Yes No If yes, please list illnesses (optional - however, if you are immunosuppressed due to treatment or illness, please let Occupational Health and your supervisor know so that proper protective equipment can be provided in accordance with your physician's recommendations).

18. Do you regularly use "over the counter" (non-prescription) nose drops or nose sprays, e.g. Afrin, Neosynephrine? Yes No

Comments: _____

Further evaluation needed by physician: NO - YES -

Additional Personal Protective Equipment (PPE's) needed (beyond lab coats and gloves):

NO - YES -

Recommended additional PPE's: _____

Reviewed by: _____ Date: _____

OCCUPATIONAL HEALTH QUESTIONNAIRE WAIVER

At this time, I decline to participate in the Occupational Health Questionnaire for persons having contact with animals.

Signature Date

I wish to have my personal physician administer my Occupational Health Program

Signature Date

Laboratory Animal Allergies

Why it's important:

When working with animals it is important to be aware of the risk of developing allergies to the animals you work with or even to your own pets. If you become allergic to the animals you work with, your job can become quite uncomfortable and even unhealthy. If you have asthma, working with animals to which you are allergic can be a significant health risk.

Symptoms:

Allergy symptoms can range from minor to severe. People who are having an allergic reaction can get a runny nose, runny or itchy eyes, asthma (characterized by wheezing and shortness of breath), a skin rash or bumps, or even gastrointestinal (GI) disorders. It is important that you notify Employee Health if you have any of these symptoms that last more than a few days or if your symptoms are severe.

How it happens:

People who work with or even near animals can be allergic to any animal species. The allergens are proteins that are found in animal body fluids and skin. These substances can stick to animal hair and dust particles and can float around in the air. Allergens are unique in each animal species. That's why a person can be allergic to mice but not rats or cats but not dogs. It's also possible to be allergic to more than one species. In fact, if you are allergic to something (an animal species or anything else) you're more likely to become allergic to something new than a person who isn't allergic to anything.

Because people can become allergic to any animal species, you may become allergic to the species you work with or any other species that is housed in the ARF or is taken to a lab nearby yours.

Relative Risk:

The incidence of animal allergies among people who work with animals may be as low as 10% or as high as 30%. This means that most people who work with animals are not allergic to them. But, this also means that if you work with animals your risk of allergy to them is as much as three times higher than people who don't work with animals.

Prevention:

The most important part of preventing animal related allergies is to minimize exposure to animals as much as possible. The following practices may help you to reduce your exposure to animals:

- Work with your animals in a ventilated hood or Biosafety cabinet when possible.
- When not working in a hood, make sure that the room where you're working with animals is well ventilated. Contact the Engineering Department if you have any questions about the air handling in your lab.
- Don't wear your street clothes when working with animals. Always wear a lab coat or scrubs, or even a disposable gown or jumpsuit.
- Don't take your lab coat or scrubs home for washing. See your supervisor about where to launder lab coats and scrubs.
- Always wear gloves when handling small animals.
- Wash your hands frequently and always after handling animals.
- Avoid touching your face when working with animals.
- Keep your work area clean.
- Be considerate of others - keep animal cages properly covered when moving them through common hallways. Do not touch common items (such as door handles) with animal-use gloves or unwashed hands.

Treatment:

If you think you're allergic to animals you work with or around, contact Occupational Health at 691-1178. Your symptoms may be controlled by medication or increased measures to reduce exposure. Also speak with your own physician about your allergy symptoms. Some people have such severe symptoms they cannot continue to work with or near animals.

If you require personal protective equipment to protect yourself from animal allergens, notify your supervisor and have him/her contact the safety office.

STANDING OPERATING PROCEDURE #20

**MARSHALL UNIVERSITY SCHOOL OF MEDICINE
ANIMAL RESOURCE FACILITIES
DISASTER PLAN**

The following has been prepared as a guide for handling an emergency in the Animal Resource Facility that places the health and well-being of research animals at risk.

Emergency/Disaster During Regular Duty Hours

Employees will follow existing procedures for emergency/disaster during regular duty hours. Any and/or all animals will be rescued if possible, without endangering human life or health. Animals are housed in the basement of the Medical Education Building. In the event of disaster, attempts shall be made to evacuate animals to the Huntington Veteran Affairs Medical Center Animal Resource Facility located adjacent to the Medical Education Building, if the VAMC is capable of accepting these animals. For animals housed in the BBSC, safe areas will be utilized for evacuation sites. If there are no safe areas in the BBSC, attempts will be made to evacuate the animals to the Medical Education Building ARF. If no suitable areas are available, the animals will be monitored for injury or distress and, if needed, be humanely euthanized. Animals that are injured or in distress will be humanely euthanized by the means appropriate for that species, in accordance with the latest AVMA Euthanasia Recommendations. Care will be supervised by the ARF Director.

Emergency/Disaster After Regular Duty Hours

Responders will follow existing procedures for emergency/disaster after regular duty hours. Any and/or all animals will be rescued if possible, without endangering human life or health. Animals are housed in the basement of the Medical Education Building. In the event of disaster, responders are directed to attempt rescue of the animals and to promptly notify the ARF Director and the Animal Care Staff. Provisions have been developed for temporary housing facilities in the Huntington Veteran Affairs Medical Center Animal Resource Facility located adjacent to the Medical Education Building. . For animals housed in the BBSC, safe areas will be utilized for evacuation sites. If there are no safe areas in the BBSC, attempts will be made to evacuate the animals to the Medical Education Building ARF. If no suitable areas are available, the animals will be monitored for injury or distress and, if needed, be humanely euthanized. Animals that are injured or in distress will be humanely euthanized by the means appropriate for that species, in accordance with the latest AVMA Euthanasia Recommendations. Care will be supervised by the ARF Director.

MU MEB Policy & Procedures Manual

Emergency Telephone Numbers

| | <u>Office</u> | <u>Home</u> |
|--|---------------|-------------|
| Billy W. Howard, DVM ARF Director | 696-7374 | 654-9817 |
| Monica Valentovic, Ph.D Chairman, IACUC | 696-7332 | 697-0321 |
| Jan M. Ball, DVM Backup Veterinarian | 525-5121 | 429-5148 |
| Tara Runyon Animal Technician | 696-7373 | 525-6867 |
| Lonny Muncy Animal Technician | 696-7373 | 453- |

STANDARD OPERATING PROCEDURE #21

Prolonged Restraint Policy

PURPOSE: This policy establishes procedures for prolonged restraint of any animal.

METHODS: Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished naturally or with devices such as restraint stocks or squeeze cages. It is important that such devices be suitable in size and design for the animal being held and operated properly to minimize stress and avoid injury to the animal.

Prolonged restraint of any animal should be avoided unless essential to research objectives and requires approval of the IACUC. The following are important guidelines for the use of restraint equipment:

- *Animals to be placed in restraint equipment should be conditioned to such equipment prior to initiation of the research.

- *The period of restraint should be the minimum required to accomplish the research objectives. Prolonged restraint for any reason must be approved by the IACUC.

- *Restraint devices are not to be considered "normal" methods of housing, although they may be required for specific research objectives.

- *Restraint devices must not be used simply as a convenience to investigators in handling or managing animals. When such devices are used, their use must be specifically approved by the committee.

- *Attention must be paid to the possible development of lesions or illnesses associated with restraint, including contusions, decubital ulcers, dependent edema, and weight loss. If these or other problems occur, veterinary care must be provided to treat the animal, which if necessary must be temporarily or permanently removed from the restraint device.

STANDARD OPERATING PROCEDURE #22

Multiple Major Surgical Procedures

PURPOSE: This policy establishes procedures for multiple major survival surgical procedures on a single animal.

METHODS: Multiple major survival surgical procedures on a single animal are discouraged. However, such procedures may be performed with the approval of the IACUC after careful deliberation of potential benefits and assurance of minimization of stress to animals.

The procedure for securing approval of multiple survival surgeries is the same as for any other protocol, with the following additional requirements: the investigator must attend the IACUC meeting and thoroughly explain his/her protocol to the full Committee. He/She must present and defend the need for multiple survival surgeries. Cost savings alone is not an adequate reason for performing multiple survival surgical procedures.

STANDARD OPERATING PROCEDURE #23

Procedures for Handling Allegations of Mistreatment or Noncompliance Involving Care and Use of Animals

PURPOSE: One of the basic functions of the IACUC is to review and, if warranted, investigate concerns involving the care and use of animals at the facility resulting from public complaints received and from reports of non-compliance received from laboratory or research facility personnel or employees. This policy establishes procedures for reporting, receiving and handling allegations of animal mistreatment or other noncompliance.

METHODS: a. Definitions

Mistreatment: Any action, physical or psychological, which results in wrongful or abusive treatment of an animal (e.g., inadequate or improper care or housing of animals).

Noncompliance: Violation of University procedures or policies, which encompass those of the Public Health Service and the Animal Welfare Act. Examples include unauthorized use of animals for an activity or procedure; failure to have an active IACUC approval for an activity involving animals; continuing an activity past its authorized expiration date.

Reporting allegations: It is not always obvious at what level of alleged mistreatment or noncompliance the IACUC should become involved. Frequently the attending veterinarian, animal care personnel, and investigators can work together to prevent or resolve a problem. However, serious or repeated problems always require the involvement of the IACUC. If in doubt it is better to submit a report, as this may well protect the institution, the complainant, the alleged violator(s), and, of course, the animals.

b. Reporting Procedures

Alleged allegations may be reported in conversation with, or correspondence (letter, FAX, email) with members or staff of the IACUC, the veterinary staff, or the Institutional Official (President of the University). There shall be no restrictions on who can report an alleged incident and there can be no threat of reprisals against anyone reporting the perceived mistreatment or noncompliance. Any information should be quickly relayed to the

Chairperson of the IACUC for action. All complaints brought to the attention of the IACUC will be fully documented under signature. There must be sufficient substance to the complaint for the Chair to proceed further. An allegation has no substance until proven, and should remain confidential to the extent possible to protect all concerned. If the complainant has freely identified him/herself, it is appropriate that receipt of the allegation be acknowledged.

c. IACUC Procedures for the Investigation of a Complaint

The Chairperson may elect to bring the matter as a whole before the committee or may appoint a subcommittee to investigate the complaint. In either case the results of the investigation must be considered IACUC actions and all members must have the opportunity to present their views. The person(s) against whom the complaint has been raised should have an opportunity to explain their position. As much documentation as is reasonably needed will be collected. This may include animal receiving records, housing and health records, billings, memos, and other written materials. It may also be necessary to interview persons or to carry out an inspection of the facilities. The results should be made available to all parties involved, including the Institutional Official who is ultimately responsible for instituting corrective action.

d. Institutional Responses

This is influenced by legal requirements, institutional policy and the nature of the investigative findings. If the violation is verified by the IACUC, the IACUC is empowered under USDA Regulations and PHS Policy to suspend a previously approved project. If the activity is supported by PHS funds, the IACUC, through the Institutional Official, must file a full report to the National Institutes of Health, Office for Laboratory Animal Welfare (OLAW). In cases where there is sufficient evidence of serious noncompliance, it may be prudent for the IACUC to suspend an activity pending the outcome of a full investigation. In these cases, a preliminary report will be sent to OLAW and the USDA, through the Institutional Official, on the understanding that a full report will be submitted upon completion.

The Institutional Official, in consultation with the IACUC, has the power to impose further sanctions on an investigator found to be responsible for mistreatment or noncompliance. Each case must be considered individually and may result in precedents being set, therefore, the implications of these

should be considered. The institution must also consider whether to announce its findings publicly.

Standard Operating Procedure #24

RODENT BLEEDING POLICIES

PURPOSE: In many experimental protocols, it is necessary to obtain blood from rats, mice, gerbils, hamsters, and guinea pigs. The Marshall University School of Medicine Institutional Animal Care and Use Committee endorses the following techniques as acceptable means for routine blood collections (species variations are noted).

POLICY:

A. Cardiac Puncture

This technique is generally employed when a larger quantity of blood is necessary in a short amount of time. However, it is a relatively difficult technique that is best performed by experienced, trained personnel. Also, it is a technique to be performed on anesthetized rodents only. Due to the possibility of causing cardiac tamponade and subsequent death, it is generally best employed as a terminal procedure.

B. Retro-orbital (Eye) Bleeding

In this procedure, the orbital sinus of rats, mice, hamsters, or gerbils is punctured in order to obtain a small blood sample. This procedure requires the animal to be anesthetized. When properly performed on the anesthetized animal, the eye is not damaged.

However, the eye will be damaged when repeated bleeds are done using the same eye frequently. Therefore, at least two weeks should lapse between bleedings from the same eye. A bland ophthalmic ointment should be applied at the end of the procedure to prevent corneal drying. Pressure over the orbital sinus after the bleeding tube is withdrawn will prevent further bleeding complications. This technique is not recommended in guinea pigs.

C. Venipuncture

Direct access into a vein is a common method for both blood collection and drug injection. The site of venipuncture will vary according to the species.

In mice, tail vein and jugular vein bleeding are the best choices. Proper restraint is essential for collection from both sites. Tail vein bleeding is facilitated by transillumination of the tail, warming the tail and/or the whole animal, occlusion of the veins at the base of the tail, and/or applying oil of winter green or alcohol. Xylene/Xylol is not recommended because of the skin irritation and slough that they can cause. For jugular vein bleeding, proper animal positioning is crucial in a conscious mouse, some hair clipping is necessary, and occlusion of the opposite jugular vein facilitates blood collection. A 27 gauge or smaller needle is recommended for mouse venipuncture.

Rats, in addition to the mouse sites and techniques noted above, can also be bled from the dorsal metatarsal vein or the saphenous vein (requiring a hair clip, aseptic preparation and a 27 gauge or smaller needle), the sublingual vein which requires anesthesia and a 26 gauge needle. For tail bleeding, a 19 gauge or smaller needle is recommended.

Hamsters and gerbils fall somewhere in between the mouse and the rat techniques with tail bleeding not usually attempted in the hamster.

Blood collection in the guinea pig is relatively difficult due to the small sized peripheral veins. Ear (auricular) vein puncture with a 25 or 26 gauge needle may be possible after warming of the ears and/or the whole animal. Blood collection is also possible from the lateral or medial metatarsal veins as well as the lateral saphenous vein.

At any venipuncture site, direct pressure applied to the site after the needle is withdrawn, is required to control hematoma formation. If venipuncture needs to be repeated on a frequent basis (whether for blood collection or injection), alternative routes of bleeding/injection should be considered, including implantation of a chronic indwelling intravenous catheter.

D. Decapitation

This technique can be used to collect blood from smaller rodents. A guillotine or autopsy shears are used to perform the decapitation.

This technique should be used in research settings only when scientifically justified by the user and approved by the IACUC. Personnel employing this technique should receive appropriate preparation and training from a person experienced in this technique.

Blood collections in this manner will be contaminated with tracheal and salivary

secretions.

This method is commonly used to collect blood from neonatal rodents.

E. Exsanguination

This is a terminal procedure that must be carried out under surgical anesthesia. Exsanguination can be carried out in a variety of ways, i.e., cardiac puncture, severing of major blood vessels, aspiration of the major blood vessels with a syringe and needle, etc. Verification of the rodent's death is necessary before disposal of the carcass. This may be done by: physical means, i.e., decapitation, thoracotomy, cervical dislocation; chemical means, i.e., CO₂, barbiturate overdose; and/or by observation of cessation of heart and lung movements.

F. Other Methods

Other methods of rodent bleeding may be acceptable, i.e., tail snips, which will be considered on an individual basis by the veterinarian and approval by the IACUC. The ARF Director should be consulted during the formulation of the research plan if there are any questions regarding proper blood collection techniques.

Additionally, consideration should be given to total blood volume withdrawn within a given time frame. Over-bleeding can leave animal hypovolemic, anemic, and weak. As a rule, up to 25% of total blood volume can be collected in a two-week period with replacement fluid (sterile 0.9% saline) recommended for larger collections.

Standard Operating Procedure #25

IMMUNIZATION OF RABBITS WITH COMPLETE FREUND'S ADJUVANT

General Background

Immunization with Freund's complete adjuvant (FCA) is a widely used procedure to stimulate antibody production. It is often associated with undesirable side effects such as granuloma formation, tissue necrosis and sloughing, abscessation and fever.

These lesions are generally caused by:

1. Instillation of too much inoculum at the injection site.
2. Injection sites which are too close together.
3. Use of improper injection sites.

Undesirable local lesions can be prevented by carefully limiting the quantity injected at each site and widely scattering the injection sites to ensure adequate barriers of normal skin. Trials have shown that using 1:1 suspensions of FCA and aqueous antigens, intradermal (I.D.) injections of 0.05 ml per site and subcutaneous (S.Q.) injections of 0.1 ml per site in rabbits produce palpable lumps that can be monitored for complications.

The limited quantity and scattering sites requires more time because more of the back and flanks must be clipped and prepared for injection, and more training and skill to instill small quantities of inoculum. However, an advantage of higher antibody production is gained by broader area of stimulation reacting with more regional lymph nodes and by the creation of many small foci of local cell interactions. The favorable results more than compensate for the greater time and skill required.

The peritoneal cavity and the footpad are considered improper sites for the use of FCA. These sites are determined as not acceptable.

FCA is considered a human biohazard, since accidental self-inoculation or splashing in the eye has been shown to cause painful sequelae not readily amenable to treatment, as well as sensitization to tuberculin.

Immunization Procedures

The majority of rabbits can be immunized using only manual restraints since the inoculation causes only minor or transient pain. The back, forequarters and flanks of the rabbit should be clipped using a #40 surgical clipper head. This allows clear visualization of the injection sites and a clean work

field. Loose hair should be brushed away and the skin prepared for injection using 70% alcohol. A rabbit restrainer is not generally used since the injection field covers more surface area than is generally accessible through the restraint device.

Freund's Complete Adjuvant is mixed 1:1 with a suspension of the antigen and is used for the initial immunization only. Each subsequent booster injection will be done with Incomplete Freund's Adjuvant (IFA). The initial immunization may be a total of 2.0 ml (1.0 ml of FCA plus 1.0 ml of antigen). The adjuvant and antigen are mixed into a cloudy suspension using two syringes and a double-headed single-barreled micro-emulsifying needle.

A mixture of intradermal and subcutaneous injection sites may be used with no more than 0.05 ml of suspension inoculated I.D. at each site and no more than 0.1 ml of suspension inoculated S.Q. at each site. Be sure to allow at least 2 centimeters between I.D. sites. Best results are obtained using a 23 gauge 1 inch needle with the bevel up. This is generally tolerated very well by the rabbits with minimal signs of discomfort. It is recognized that the large number of sites involved and the small amount of inoculum delivered requires more time, a larger field and better technique control than most standard inoculation protocols. The favorable results, reflected in the high titers of antibody produced and the clinical well-being and appearance of the rabbits, fully justifies the additional time and care required.

The following schedule is typical of those used in the Neurology Institute (NINCDS) at NIH for producing antibody to synthetic peptides; schedules may vary depending upon the antigen.

Week #1 -- Initial immunization with Freund's Complete Adjuvant (FCA).

Week #3 -- Boost with Incomplete Freund's (IFA).

Week #5 -- Boost with Incomplete Freund's (IFA).

Week #6 or #7 -- Bleed to check serum titer (usually 5 ml), if titer is favorable, follow with a larger bleed.

Follow this with monthly boosts with Incomplete Freund's Adjuvant, bleeding three or four weeks after the boost for 25 to 30 ml.

Current practices in humane care recognize that these animals are monitored closely for reactions by the ARF staff and the Principal Investigator. Reactions will be treated by current accepted veterinary medical procedures. Rabbits that develop lesions that do not respond to treatment may have to be euthanized.

Bleeding Rabbits

A maximum of 1% of body weight may be collected every three weeks. (For example, 25 cc of blood could be collected from a 2.5 kg rabbit.)

Blood may be collected by cannulation of the marginal ear veins or central ear artery. Cardiac puncture should be reserved for terminal procedures, and requires a surgical plane of anesthesia.

Using tranquilizing agents to relax the rabbit and promote vasodilation is advised. Acepromazine tranquilizer at 1.0 mg/kg S.Q. prior to blood collection is one method.

Application of Xylene to dilate ear vessels is not recommended. This substance may cause scarring of the ears.

Standard Operating Procedure #26

BABY CHICKENS

A. General

1. Daily record the high and low temperature for the last 24 hours on the census sheet.
2. House in 19" x 25" x 10" metal cages.
3. Regulate temperature in the cage as follows:

| AGE | Degrees C | Degrees F |
|------------|------------------|------------------|
| 1 Day | 32°C | 90°F |
| 7 Day | 29°C | 84°F |
| 14 Days | 26°C | 79°F |
| 21 Days | 23°C | 74°F |
| 28 Days | 20°C | 68°F |

B. Feeding and Watering

1. Chicks are to be provided adequate diet on a daily basis.
2. Fresh water will be provided to all chicks daily. Each waterer is to be rinsed out and filled daily with fresh water.
3. All feeders and waterers should be checked daily before leaving to assure adequate feed and water is present to last until the next day.

C. Cleaning

1. Chick drop pans and screens are cleaned and cage washer sanitized at least once a week.
2. Chick cages, feeders, and waterers are changed once a week with cage washer sanitized cages, feeders, and waterers.

3. Daily rinse out water cups and add fresh water.
4. Daily add only enough food to last 24 hours. If feeder is soiled, replace with a clean one. Do not fill feeder to the top.
5. When changing cage, be sure to transfer cage card.
6. Weekends are the same as weekdays. Rinse out waterer and refill. Check feeder and replace if necessary. Add enough food for 24 hours.

D. Handling

To remove a chick from a cage, use both hands to lift it out. Place the hands over the wings to hold them close to the body and prevent flapping.

Standard Operating Procedure #27

Immunocompromised Animals

A. General

1. Entry into the animal rooms containing immunocompromised animals is restricted to ARF personnel and the Principal Investigator and staff.
2. Entry should be as infrequent as possible and the immunocompromised animal room should be entered first, before any other procedures are performed in the ARF.
3. An isolation gown must be worn in the immunocompromised animal room and removed as you leave. This gown will be worn in this room only.
4. Autoclaved cages and bedding, water bottles and water, and any special husbandry materials will be provided by the Principal Investigator and staff.

B. Cleaning

1. The immunocompromised animal room and everything in it will be thoroughly cleaned after each group of animals is removed.
2. The species of animals housed in the immunocompromised animal holding rooms determines the cleaning schedule. These schedules will be the same as immunocompetent animals of the same species, unless a different schedule is agreed upon by the Veterinarian and Principal Investigator.

Standing Operating Procedure #28

MANAGING IMMUNOCOMPROMISED ANIMALS

A. General

This SOP describes methods for maintaining immunocompromised rodents to prevent infections from adventitious pathogens.

B. Methods

1. Preparing the microisolation housing unit.
 - a. Assemble the microisolation housing unit by placing bedding into the cage, placing the wire bar lid, water bottle, and the filter top on the cage.
 - b. Autoclave the assembled microisolation housing unit in a vacuum autoclave set to the appropriate gravity setting.
 - c. Autoclave a flask filled with water on the liquid cycle to fill the bottles at the time they are put in use.
2. Preparing the operator and workbench
 - a. Put on a disposable lab coat and gloves. Pull the gloves over the cuff of the lab coat and tape to form a tight seal.
 - b. Place the autoclaved microisolation housing unit, water flask, and animals near the laminar-flow workbench.
 - c. Spray arms and gloves with disinfectant. (Gloves should be damp with disinfectant at all times while working in the workbench.)
 - d. Wipe down the internal surfaces of the workbench with disinfectant and paper towels. Allow disinfectant to sit for three minutes.
3. Load the microisolation housing unit.
 - a. Place the autoclaved microisolation housing unit in the workbench and remove the filter top. Place the filter top upside-down on the surface of the workbench. (This procedure prevents dust particles from blowing from

the top of the filter top into the cage bottom.)

- b. Spray the water flask with disinfectant and place in the workbench. Fill the water bottle, add the stopper with sipper tube, and place in the cage.
- c. For filter-crated animals (obtained from a specific pathogen-free colony), inspect the crate carefully for any holes or breaks in its integrity. If completely intact, thoroughly spray the crate (including the filter) with disinfectant and place in the workbench. Open the crate. If a tool is used, it must be kept in a tray containing disinfectant in the workbench.
- d. If animals are currently in a microisolation unit, place the unit in the workbench and open as in 3.a. above. Do not spray the cage or cage filter.
- e. Take a pair of totally submerged rubber-tipped forceps from the forceps tray in the workbench and shake off excess disinfectant in the hood. Lift the wire bar lid, grab animals by the tail, carefully transfer to the cage, and replace the filter top on the cage bottom. The unit is now ready and can be placed on the animal rack
- f. Spray and wipe workbench with disinfectant after all animals have been transferred to clean microisolation units and placed on the animal rack.
- g. Remove soiled microisolation units to the cage wash room and used shipping crates to the dumpster.

STANDING OPERATING PROCEDURE #29

PROCEDURES FOR THREATENING SITUATIONS FOR ANIMALS

A. General:

This SOP describes procedures to follow during situations where animal health and safety is threatened.

B. Methods:

1. Whenever a threatening situation, other than fire, is discovered (temperatures 80°F or above or 64°F or below, lack of air flow, etc.) the Veterinarian and the Maintenance Section Chief will be contacted. If there is no response from the Veterinarian and/or the Maintenance Section Chief, the Animal Care Technicians and the alternate Maintenance Section designee will be contacted. These individuals will assess the situation (i.e., check animal room temperatures and air flow) and take corrective measures to insure the animal health and safety is not compromised.
2. The Veterinarian will compile a list of emergency telephone numbers from each investigator. This list will be maintained in the Veterinarian's office and the Animal Care Technicians' office.
3. Emergency telephone numbers for the Veterinarian, Animal Care Technicians, and Maintenance Section will be posted on the bulletin board outside the Animal Care Technicians' office, room B-19, and in the Maintenance Section office, room B-40.
4. The Principal Investigators will be notified by the Veterinarian of the situation and what immediate corrective measures are being taken. The Veterinarian will answer any questions the Investigator may have concerning the safety of their animals and the research implications involved. The Principal Investigator will then apprise the Veterinarian of any alternative measures their animals may require.
5. The Veterinary and Maintenance staffs will remain on location until the situation is resolved.
6. This SOP will be posted on the bulletin board outside room B-19 in the ARF and should be used as part of any orientation for all new animal users.

Emergency Telephone Numbers

| | <u>Office</u> | <u>Home</u> |
|--|---------------|-------------|
| Billy W. Howard, DVM ARF Director | 696-7374 | 654-9817 |
| Monica Valentovic, Ph.D Chairman, IACUC | 696-7332 | 697-0321 |
| Jan M. Ball, DVM Backup Veterinarian | 525-5121 | 429-5148 |
| Tara Runyon Animal Technician | 696-7373 | 525-6867 |
| Lonny Muncy | 696-7373 | 453- |

STANDING OPERATING PROCEDURE #30

RODENT BREEDING COLONY MANAGEMENT

A. General

This SOP describes procedures to follow for the management of rodent breeding colonies.

B. Methods

1. The cleaning and sanitation frequency for rodent breeding colonies will be the same as for rodent holding rooms.
2. The Principal Investigator will be responsible for setting-up the mating scheme and breeding scheme to insure the resulting offspring are as expected, i.e., maintaining either an outbred or inbred colony or if a mutant or transgenic, that the parents carry the desired mutation or transgene.
3. Sick or poor-doing neonates will be euthanized utilizing halothane as the euthanatizing agent, or by physical means complying with the current recommendations of the “AVMA Euthanasia Guidelines.”
4. After the age of 4 weeks, the neonates will be counted as adults for census records.
5. The majority of “normal” offspring will be euthanized. Several of these offspring will be utilized as sentinel animals. They will be kept until they are four months of age, bled for serology testing, euthanized, and gross necropsy performed. Tissues will be collected for histological examination if any appear abnormal.

STANDING OPERATING PROCEDURE #31

SENTINEL ANIMAL MANAGEMENT

A. General

This SOP describes procedures to follow for the management of sentinel animal cages.

B. Methods

1. The cleaning and sanitation frequency for sentinel animals will be the same as for rodent holding rooms.
2. The sentinel animal cage will be the last cage changed on the rack.
3. When changing other animals on the rack, a small amount of dirty bedding from these cages will be placed into the cage to be used for the sentinel animals.
4. After the sentinel animals have been placed into the new cage, this cage will be placed on the bottom shelf of the conventional rack, closest to the exhaust air vent. For cages in an individually ventilated rack, the cage will be placed closest to the exhaust plenum.

STANDING OPERATING PROCEDURE #32

Cage Card Information

A. General

This SOP describes procedures to follow for the completion of the animal cage cards. This information is used to provide accurate identification for all animals.

B. Methods

1. Upon entry into the vivarium a cage card will be completed for all animals.
2. The information completed on the card is as follows:
 - a. Species of animal(s)
 - b. Sex of animal(s)
 - c. Weight of animal(s)
 - d. ID number (if has collar, ear tag, etc.)
 - (1) Rodents and lower species may or may not be assigned an individual identification number.
 - (2) Species above rodents will be assigned an individual identification number.
 - (3) If an identification number was assigned by the vendor, this number will continue to be used by SOM vivarium.
 - e. Account number the animals are charged to (i.e. grant number or department account).
 - f. Date animal(s) was (were) received
 - g. Source of the animal(s)
 - h. The number of animals in the cage.
 - i. Investigator's name
 - j. Investigator's department
 - k. The IACUC assigned protocol number
3. The cage card is placed on the animal's cage.
4. As animals are removed from or added to the cage, the number of animals marked on the cage card will be changed by the person who adds or removes the animal(s) from the cage.

STANDING OPERATING PROCEDURE #33

Euthanasia Chamber

A. General

This SOP describes procedures to follow for euthanizing small rodents using carbon dioxide.

B. Methods

1. Only medical grade carbon dioxide from a gas cylinder will be used.
2. Insure the chamber is empty and clean.
3. Open the valve on the cylinder.
4. Adjust the flow rate to 20 on the glass tube.
5. Allow gas to enter the chamber five (5) minutes prior to placing any animals into the chamber.
6. Place the animals into the chamber, insuring the animals are not overcrowded.
7. After observing no signs of breathing or movement from the animals, remove the animals. Insure the animals have been euthanized by applying cervical dislocation or bilateral thoracotomy.
8. Close the valve on the cylinder.
9. Place the animals into a bag and remove them to the cold room for disposal.
10. Clean and disinfect the chamber.

STANDING OPERATING PROCEDURE #34

TAIL SNIPPING IN MICE

PURPOSE:

To describe procedures used to obtain tissue needed for transgene identification in mice.

APPLICATION:

This procedure applies to colony breeders, researchers and technicians who collect tissue samples from mice for transgene identification.

NOTES:

PCR techniques may require less tissue and allow use of auricular flap tissue obtained during the ear punch identification procedure. Southern Blot testing may require more material and need tissue from the tail.

Standard Techniques:

Ear Punching: Ear punching does not require anesthesia in mice thru 21 days of age. Several tissue samples approximately 0.5 mm in diameter can be obtained.

Tail Snipping: Anesthesia is not required in mice through 21 days of age if less than 1 cm of skin is taken (skin can be pushed down toward the tip of the tail so that the vertebrae are avoided). Innervation of the tip of the tail is minimal at this age (Pain Category B).

Tail tip samples greater than 1 cm in length will probably damage the coccygeal vertebrae and will require anesthesia in mice of any age. Anesthesia is required for any tail snipping if animals are greater than 21 days old (Pain Category C).

Anesthesia:

Local: FluorEthyl™, cetyl chloride, or similar hypothermic methods.

Injectable: Ketamine Hydrochloride/Xylazine mixture

Inhalant: Inhalant anesthetics (halothane or isoflurane, with appropriate safety precautions for personnel) or CO₂

PROCEDURES FOR TAIL SNIPPING

Gloves should be worn when handling laboratory animals

Anesthetize mouse (if required).

Gently, but securely, restrain mouse.

Swab tail with alcohol (povidone iodine or chlorhexidine solutions may interfere with the DNA identification tests).

Push skin toward tip of tail.

Snip skin sample (with sterile instrument[s]).

Cauterize and/or apply gentle compression until hemostasis occurs.

Release mouse.

Observe mouse for bleeding or abnormal behavior.

Check tail daily to ensure tip is healing.