Creighton University
Institutional Animal Care and Use Committee
Animal Resource Facility

STANDARD OPERATING PROCEDURES

Creighton
UNIVERSITY

Animal Resource Facility
http://www2.creighton.edu/fileadmin/user/ResearchCompliance/IACUC/SOP/Animal_Resource_Facility_SOPs.pdf
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1.0 Introduction

1.1 BACKGROUND

The Creighton University animal care and use program has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) since 2005. This process provides continuing voluntary peer review of Creighton University's program by internationally recognized experts. The institution is registered as a research facility by the United States Department of Agriculture (USDA) under the Animal Welfare Act. The care and use of animals used for research at Creighton University is based on national guidelines and Federal Regulations including:

- The U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training;
- The USDA implementing regulations (9CFR) of the Animal Welfare Act;
- U.S. Public Health Service Policy Assurance for the Care and Use of Animals negotiated with the Office of Laboratory Animal Welfare (OLAW); and
- The *Guide for the Care and Use of Laboratory Animals* (*The Guide*) (see the Creighton University IACUC Policies and Procedures, for care of animals housed outside the Creighton University Animal Resource Facility).

Creighton University is committed to providing its research community with a safe, clean, humane, and efficient Animal Resource Facility (ARF). The Creighton University ARF Standard Operating Procedures (SOPs) are intended to provide guidance to ARF Personnel, Principal Investigators, and Research Personnel at Creighton University regarding the following:

- Animal Health and Care;
- Veterinary Medical Care;
- ARF Maintenance; and
- Occupational Health and Safety of ARF Personnel.

These SOPs are designed to promote and sustain a safe and high-quality ARF that supports the University's research mission and ensures the humane care and use of laboratory animals in accordance with Federal requirements and the standards of Creighton University's Institutional Animal Care and Use Committee (IACUC). While the SOPs focus primarily on the care of animals housed in the ARF, they also serve as a

resource in addition to *The Guide*. All Principal Investigators approved for animal research at Creighton University have received a paper copy of *The Guide.*

These SOPs ensure the growth and success of a comprehensive animal care program at Creighton University. All personnel involved in animal research or animal care at Creighton University are expected to become familiar with and follow the procedures set forth in these SOPs. Failure to follow these SOPs may result in unsafe or unhealthy conditions for animals and/or personnel as well as the violation of Federal and Creighton University policy. In addition, adverse affects on research protocols may result. If necessary, appropriate corrective action will be taken to address any noncompliance (see section 6.0). Creighton University reserves the right to amend, modify or update these ARF SOPs (see http://www2.creighton.edu/researchcompliance/iacuc/about/index.php).

1.2 EDUCATION PROGRAM

Training and Certification for Animal Care Technicians – Animal Care Technicians are trained and/or certified to meet training mandates of federal agencies according to one or more of the following:

- **Purina Mills, Inc. Exam** – All new ARF Animal Care Technicians must complete the self-study PMI Laboratory Animal Care Course and meet passing criteria within the first 90 days of employment (the probationary period);

- **American Association for Laboratory Animal Science (AALAS) Certifications** – Animal Care Technicians are encouraged to further their knowledge by seeking certification through AALAS. The three levels of certification are Assistant Laboratory Animal Technician (ALAT), Laboratory Animal Technician (LAT) and Laboratory Animal Technologist (LATg). The ARF will pay for one test per year per technician. Furthermore, an incentive program is in effect to recognize and reward the Animal Care Technicians for obtaining AALAS certification. The ARF subscribes to the AALAS Learning Library (ALL). This resource provides training that emphasizes the appropriate handling, care, and use of animals and is designed to assist in studying for AALAS certification. ALL subscriptions include access to the Animal Care and Use Library and The Contemporary Topics Continuing Education Unit Test Library. The Animal Care and Use Library offers courses on certification, regulatory mandates, bioethics, biomethodologies, biosafety, and management. The Contemporary Topics Continuing Education Unit Test Library offers the opportunity to earn continuing education units by taking the self-administered test based on the scientific articles in *Contemporary Topics* online. Following completion of a unit, a transcript is provided for documentation. In addition, AALAS training material is available from the ARF;

- **Species-Specific Training** – Animal Care Technicians must be trained in handling the specific species housed in the ARF. The ARF Manager has extensive experience in handling a diverse variety of conventional laboratory animal

species, such as mice, hamsters, rats, guinea pigs, rabbits, dogs, cats and non-human primates, as well as livestock species, such as swine, calves and sheep. The ARF Manager trains new technicians to ensure they are able to handle the species housed in the ARF. Species-specific educational videos available from the ARF to train technicians as needed. If a species is brought to the ARF that the ARF Manager or Attending Veterinarian is not trained to work with, the ARF Manager will obtain training videos and/or arrange for technicians to receive training from someone experienced in handling the species;

- **Microisolator Technique Training** – All Animal Care Technicians are trained in proper microisolator technique prior to changing cages in rooms where mice are housed. The ARF utilizes Thoren caging;

- **Animal Occupational Health and Safety** – All Animal Care Technicians receive Animal Occupational Health and Safety Program training through Creighton University in order to address specific concerns related to working with animals. All individuals working with animals must complete the Animal Exposure Risk Assessment and submit it to the Animal Occupational Health and Safety nurse for review on an annual basis; and

- **Tuition Remission** – In addition to the above training and certification, full-time employees are eligible to receive tuition remission for up to two Creighton University classes per semester, to be scheduled outside of regular working hours.

Education Programs for Investigators and Research Personnel – Creighton University requires that all Principal Investigators using animals in research or teaching complete an educational program in the care and use of these animals. Creighton University has developed the Education Program for the Use of Animals in Research and Teaching to assist Principal Investigators/Research Personnel in meeting federal regulations and Creighton University's education requirements. The program is designed to:

- Assist Principal Investigators/Research Personnel in understanding the special requirements associated with the use of animals in research and teaching;

- Clarify the responsibilities of those involved in animal research/teaching and the IACUC;

- Provide education on the protection of animals as mandated by the federal regulations including the *Animal Welfare Act* [7 USC, 2131-2156] and the *Federal Animal Welfare Regulations* [Title 9, Volume 1, Parts 1 to 199]; and

- Provide education on the Creighton University IACUC Policies and Procedures, ARF SOPs and Animal Occupational Health and Safety Program.

In addition and in accordance with PHS Policy and USDA regulations, training addresses

the following areas:

- Humane methods of animal maintenance and experimentation, including the basic needs of each species of animal, proper handling and care for the various species used at Creighton University, and proper pre-procedural and post-procedural care of animals;

- Research and testing methods that minimize the number of animals required to obtain valid results and minimize animal distress;

- Proper use of anesthetics, analgesics, and tranquilizers for any species of animals housed in the ARF; and

- Methods whereby deficiencies in animal care and treatment are reported, including deficiencies in animal care and treatment reported by any employee of Creighton University.

All Principal Investigators who use animals in research or teaching are required to complete the Education Program for the Care and Use of Animals in Research and Teaching. This training is coordinated by the Research Compliance Education Coordinator. Initial certification will be provided when investigators have met the following criteria:

- Attendance at a Creighton University IACUC Policies and Procedures and ARF Education Seminar;

- Attendance at a Creighton University Animal Occupational Health and Safety Program Seminar and annual completion and submission of the Animal Exposure Risk Assessment;

- Curriculum Vitae (CV) – on file with the Research Compliance Education Coordinator's Office;

- Training forms on file with the Research Compliance Education Coordinator's Office;

- Verification of receipt of (or access to) the Creighton University *Investigators' Manual for the Care and Use of Animals in Research*; and

- CITI Training – 'Working with the IACUC' and species modules as appropriate.

In addition to the initial education, formal re-certification training is required every three years. Creighton University Principal Investigators are notified when they must be re-certified. The re-certification process includes seminars on specialized topics and periodic updates to inform Principal Investigators/Research Personnel of changes in federal regulations or Creighton University requirements.

User Orientation Program – The ARF User Orientation Program is designed for new Principal Investigators and Research Personnel and is coordinated by the ARF Manager. Training will cover ARF SOPs and will include:

- Security,
- Elevator Usage,
- Gowning Requirements,
- Record Keeping Requirements,
- Animal Ordering,
- Clean/Dirty Caging Procedures,
- Euthanasia and Carcass Disposal,
- Rodents at Home, and
- Microisolator Technique Training (for mouse users).

Media-based Training for Animal Care Technicians and Research Personnel – The ARF has a comprehensive library of training media, in videotape and DVD format, available for checkout to all personnel who desire or require additional regulatory, bio-methodology, or technique training. These include the following titles and modules from the Laboratory Animal Training Association (LATA):

Base Module – Topics include laws, regulations, ethics, alternatives, IACUC and ARF Personnel responsibilities, and information sources. All ARF Personnel, regardless of species handled, will require this module. Titles include:

- The Humane Care and Use of Laboratory Animals

Species Module – Topics include housing, social needs, nutrition, health considerations, handling, restraint, experimental techniques, environmental practices, and euthanasia. This module will be required as needed depending on the level of existing training and species of animal to be handled. Titles include:

- The Humane Care and Use of the Mouse, Rat & Hamster;
- The Humane Care and Use of the Guinea Pig and Rabbit;
- The Humane Care and Use of the Dog and Cat;

- The Humane Care and Use of the Nonhuman Primate;
- The Humane Care and Use of Fish; and
- The Humane Care and Use of Swine.

Techniques Module – These modules demonstrate current acceptable practices and techniques and are utilized where needed for specific technique training. Titles include:

- Aseptic Surgery of Rodents;
- Anesthesia and Analgesia of Rodents;
- Necropsy of Rodents;
- Sanitation – Practices and Procedures; and
- Microisolator System in the Conventional Colony (required of all ARF Personnel and research personnel using mice).

Supplementary Training Resources – These titles are also available for referencing and include:

- Training in Basic Biomethodology for Laboratory Mice;
- NIH Training in Survival Rodent Surgery;
- Anesthesia of Rats;
- Humane Principles and Procedures in Animal Research;
- The Humane Care and Use of Laboratory Animals;
- Alternatives in Animal Research;
- The Challenges of Animals in Research;
- Resources Today for the Research of Tomorrow;
- Practical Methodology: Humane Handling and Laboratory Techniques for the Mouse;
- Practical Methodology: Humane Handling and Laboratory Techniques for the Rat;

- Practical Methodology: Humane Handling and Laboratory Techniques for the Hamster;

- Practical Methodology: Humane Handling and Laboratory Techniques for the Guinea Pig;

- Practical Methodology: Humane Handling and Laboratory Techniques for the Rabbit;

- Restraint and Handling of the Dog; and

- Working with the Laboratory Dog.

2.0 Animal Health and Care

The Guide states, "Proper management of animal facilities is essential to the welfare of animals, validity of research data, and health and safety of the ARF Personnel. A good animal care program provides a system of housing and care that permits animals to grow, mature, reproduce (where appropriate under the protocol) and maintain good health. Good animal care minimizes variations that can modify an animal's response to experimentation." Animals shall only be housed in facilities or locations approved by IACUC. A location that is housing animals is defined as any area where live animals are kept for 12 or more hours.

2.1 CAGING AND HOUSING OF ANIMALS

2.1.1 Structure – All Animals

Rodents are housed in either standard static microisolator caging or in individually ventilated caging systems. Animal procedures for rodents, such as cage change and experimental manipulation, are recommended to be performed under HEPA filtered laminar flow hoods or biosafety cabinets if available. Mouse rooms are equipped with biosafety cabinets.

The ARF provides cages for animal housing. Principal Investigators should notify the ARF Manager of any unusual or special need for caging; otherwise, animals will be housed per the information below, in accordance with *The Guide*. No cages for mice and small animals may be placed on the floor. All small animal cages must be placed on a securely anchored nonporous surface. Such cages shall not be stacked on top of each other when housing animals. Housing requirements are as follows:

Figure 1: Housing Requirements

Microisolator Type	Cage Size (in.) WxDxH	Sq. In.	Maximum Capacity	Composition	Flooring
Mice:					
Ventilated	5 x 12.1 x 5.6	51.7	3	Polycarbonate	Solid
Ventilated	12.1 x 12.1 x 5.6	112.9	7	Polycarbonate	Solid
Static	7.5 x 11.5 x 5.5	67.0	4	Polycarbonate	Solid
Static	7.5 x 11.5 x 5.5	75.0	5	Polycarbonate	Solid
Static	10.5 x 19 x 6	153.0	10	Polycarbonate	Solid
Rats:					
Static	10.5 x 19 x 8	143.0	2	Polycarbonate	Solid
*Wire Bottom	7 x 10 x 8	70.0	1	Stainless Steel	Wire
*Wire Bottom	16 x 10 x 8	160.0	2	Stainless Steel	Wire
*Wire Bottom	25 x 10 x 8	250.0	3	Stainless Steel	Wire

* Use of suspended wire bottom caging requires prior approval by IACUC based upon

experimental goals.

Guinea pigs – Six animals or less in 29”W x 21”D x 10”H plastic cage unit;

Hamsters/Gerbils – Six animals or less in 10.5”W x 19”D x 6.5”H or 10.25”W x 16.5”D x 6”H polycarbonate static Microisolator caging;

Rabbits – Individually housed in 24”W x 30”D x 15.5”H stainless steel caging utilizing water bottles and “J” type feeders. Racks are located on opposite walls of the room when possible. Flooring is a 1/8” flat stainless steel grid;

Dogs – Primary enclosures consisting of galvanized runs and provided with a coated wire resting panel. Primary enclosures provide at least two times the required floor space of a dog measuring up to 38 inches, as calculated using the formula provided in the Code of Federal Regulations 9CFR1.1 section 3.6. Feed and water are provided in stainless steel receptacles attached to the front of the run;

Swine – Group housed in a room/pen when possible. Individual housing in galvanized runs measuring 24 square feet are utilized for short-term perioperative care in the surgical suite. Water is provided via automatic watering troughs or stainless steel bucket. Feed is provided in heavy weight livestock feeders;

Goats – Group housed in a room/pen when possible. Individual housing in galvanized runs measuring 24 square feet, with coated wire resting panel, are utilized for short-term perioperative care in the surgical suite. Water is provided via automatic watering troughs or stainless steel bucket. Feed is provided in heavy weight livestock feed pans;

Lizards – Housed individually in fiberglass terraria of approximately 100-liter volume with a removable clear plexiglass panel forming one wall. A heat lamp is on five hours per day, and a retreat provided. Water is provided in a heavy glass dish, and food is placed in a stainless steel bowl;

Frogs – Housed in a variety of enclosures depending on the size and number of individuals and the time they will be maintained; glass aquaria, large plastic tubs and specialized stainless steel enclosures are all used. Frogs are provided with a shallow pool of water, dry area and refuge; and

Fish – Larger fish are housed in glass aquaria of 20 to 80 liters, while smaller fish may be housed individually in one-liter aquaria. Water chemistry is appropriate to the species, as is a suitable refuge.

2.1.2 Animal Safety – All Animals

Ventilation and Animal Access – All cages or areas of animal confinement have adequate ventilation. Food and water are provided *ad libitum* unless stated in an approved IACUC protocol;

Inspection – All cages or enclosures are routinely inspected by ARF Personnel or other individuals approved by IACUC and/or the Attending Veterinarian to ensure security of animal confinement as well as animal safety. Under no circumstances may an individual interfere with these inspections;

Social Environment – Every effort is made to house rodents in compatible groups unless it is in conflict with the scientific goals of the approved IACUC protocol. This allows for mutual grooming and other social interactions among cage mates. If possible, the caging of all species is arranged to allow for visual contact between adjacent cages as well as visual contact with room activities. In addition, species-specific environmental enrichment is provided which allows the animals to express normal behavior such as digging, burrowing and gnawing;

Security in the ARF and Animal Holding Rooms – Entry into the Animal Resource Facility is controlled utilizing key access at the elevator level, proximity reader at the Facility level, and designated sub-master keys at the room level. Ingress is limited to one elevator (Criss I freight elevator) and one stairwell (Criss I north stairwell). Exit may be accomplished through one stairwell (Criss I north stairwell) or any of the five elevators located throughout the Facility. Access to the ARF is granted upon verification of IACUC Basic Certification or active in-training status. The proximity readers will be programmed to allow entry only to personnel with IACUC Basic Certification. Personnel who are 'in-training' may not enter the ARF unless accompanied by an individual who has completed all animal-related training in order to gain access to the ARF. All animal rooms remain locked at all times. Each animal room has its own sub-master lock so that each key issued to an Investigator allows entry only into a specific animal room. Keys are issued only for specific rooms for which the PI needs access. In general, the PI is issued only one key for access to the ARF and rooms that must be utilized by all the PI staff. These keys must be kept in a secure place at all times. All ARF keys must be returned to the ARF Manager or ARF Director upon termination of employment. In addition, the proximity readers may be programmed to allow entry to Public Safety, Facilities Management, and personnel whose duties require entrance into the ARF. Emergency contact information, including telephone numbers and/or beeper numbers of the Principal Investigator and other designated project personnel, is located inside each animal room. The ARF Manager is listed as the final contact. If the primary emergency contact personnel cannot be reached, the ARF Manager will be called; and

Animal Security in IACUC-Approved Animal Rooms Outside the ARF – ARF personnel shall adequately secure rooms housing animals to prevent access by unauthorized individuals. Emergency contact information, including telephone numbers and/or beeper numbers of the Principal Investigator and other designated project personnel, shall be posted inside each animal room, with the ARF Manager listed as the final contact. If the primary emergency contact personnel cannot be reached, the ARF Manager will be called.

2.1.3 Environment of Animals Housed in the ARF

Environmental parameters in the ARF are maintained as follows, unless otherwise allowed under an IACUC-approved protocol:

Temperature – Each room where animals are confined is set at a temperature appropriate for the housed species. The temperature of each animal room is monitored and recorded daily. The Principal Investigator will be notified promptly if the temperature deviates greater than ±5 degrees Fahrenheit from either the animal-specific norms or the temperatures designated in the IACUC-approved protocol. Animal rooms are kept at 70±2. Room temperature is monitored and recorded daily by Animal Resource Facility staff. In addition, temperature is controlled and monitored by Facilities Management via a state of the art computerized control system. This system allows for the immediate notification of designated personnel should room temperature fall outside the specified parameters;

Noise – Noise levels are species appropriate. Species that are sensitive to noises are housed as far as possible from species that are more vocal. Animal room doors are kept closed to minimize noise pollution;

Lighting – All animal rooms have timed lighting devices with a 12-hour light and 12-hour dark cycle, unless otherwise required by the species or as allowed under an IACUC-approved protocol;

Pest Control – See Section 4.1.3 under ARF Maintenance; and

Failures in Environmental Control Systems – The ARF HVAC systems are controlled and monitored by Facilities Management via a computerized control system. Designated personnel are immediately notified in the event of failure in the HVAC system. ARF Personnel shall take reasonable and necessary steps to address any failures in environmental control systems to ensure that the animals are maintained at an acceptable temperature and receive an appropriate lighting schedule. The Principal Investigator will be promptly notified of any environmental control system failures affecting his/her animals. The course of action should there be a failure in these systems is outlined in Appendix A.

2.1.4 Environment of Animals Housed in IACUC-Approved Animal Rooms outside the ARF

In those rare situations where IACUC approves housing of animals outdoors, IACUC and the Attending Veterinarian shall ensure that, through the approved protocol, animals are provided appropriate shelter from the weather and are housed in a secure structure.

The Principal Investigator is responsible for maintenance of environmental parameters outside the ARF, unless otherwise allowed under an IACUC-approved protocol, as follows:

- **Temperature** – The room where animals are housed must be maintained at a temperature appropriate for the species. Room temperature is monitored and recorded daily by Research staff. In addition, most Satellite facilities have temperature controlled and monitored by Facilities Management via a state of the art computerized control system. This system allows for the immediate notification of designated personnel should room temperature fall outside the specified parameters;

- **Noise** – Noise levels must be species-appropriate;

- **Lighting** – Species-appropriate light/dark cycles must be established (typically 12 hours light/12 hours dark), unless otherwise allowed under an IACUC-approved protocol;

- **Pest Control** – Adequate pest control must be in place; and

- **Failures in Environmental Control Systems** – Most Satellite Facilities' HVAC systems are controlled and monitored by Facilities Management via a computerized control system. Designated personnel are immediately notified in the event of failure in the HVAC system. The Attending Veterinarian, ARF Manager, and designated ARF Personnel perform random checks of any outside facility, at least once monthly, to ensure compliance with SOP protocols and guidelines. Twice each year, IACUC inspects all facilities where animals are housed as part of its semiannual program and facility inspection, as required by the OLAW.

2.2 RECORDS AND IDENTIFICATION

2.2.1 Records

The ARF Manager is responsible for maintaining a record of all animals in the ARF. These records include noting the acquisition of dogs, cats, guinea pigs, gerbils, hamsters, rabbits, and other animal species (including farm animals) as required by the USDA.

2.2.2 Identification of Animal Rooms and Animals in the ARF

All doors to individual animal rooms have signs describing the species housed and any special precautions that must be taken or considered prior to entry, including protective clothing that may be needed and the type, if any, of hazardous materials in use.

Rodents – Cages of small animals, including mice, rats, gerbils, guinea pigs, and hamsters, are identified via the use of cage cards. The ARF Personnel places pre-printed white standard animal cage cards, as shown below, on each small-animal cage at the time of delivery, containing the following information:

- Name of the Principal Investigator,
- IACUC Protocol Number,
- Date animals were received,
- Animal species,
- Vendor, gender, weight and age of animals upon arrival,
- Number of animals initially placed in the cage (This information will be revised by the Principal Investigator/Research Personnel or the ARF Personnel as animals are permanently removed from or added to the cage), and
- Any other pertinent information.

Figure 2: Cage Card Example

Creighton University				
Investigator name:			**Protocol # :**	
Date Rec'd:	**Date Bred:**	**Date Weaned:**	**Species / Strain:**	
Vendor:	**Weight:**	**Age:**	**Gender:**	**# Housed**
# Euthanized	Date Euthanized		# Remaining	

The Principal Investigator may identify individual animals within a cage as long as such marking is consistent with methods approved in *The Guide*. Approved methods of animal identification include ear notches and tags, tattoos, and subcutaneous transponders. Toe-clipping is only allowed when no other alternative is available. Individuals wishing to toe-clip rodents must obtain IACUC approval for this procedure.

It is the responsibility of both the Principal Investigator and ARF Personnel to record dates of euthanasia and natural deaths on the cage card. When the last animal in a cage is euthanized or otherwise dies, the card is returned to the ARF Office with the date of euthanasia or death. When fewer than all of the animals are euthanized or die, the number of animals noted on the card is revised to reflect the euthanasia date and new number of animals.

Individuals performing procedures or injections on rodents should note the date of such manipulations on the card. This assists the Attending Veterinarian and ARF Personnel in performing their rounds. In addition, group medical records are utilized for rodents or cages of rodents that undergo interventions requiring the use of anesthetic or analgesic

agents. Peri-surgical and peri-anesthetic care is documented on the dark purple Rodent Post-Procedure Monitoring Veterinary Alert Card. If questions arise regarding the information contained on the cage cards, ARF Personnel will notify the Principal Investigator.

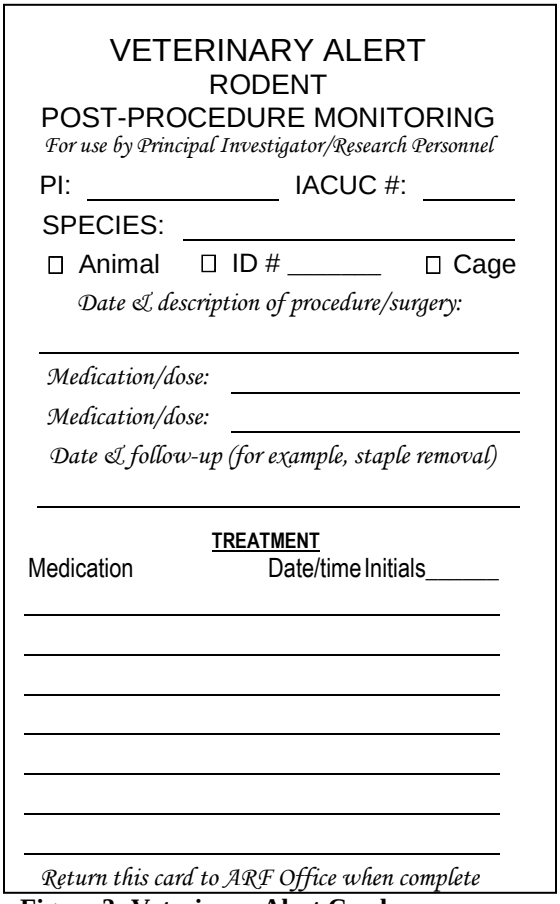
VETERINARY ALERT
RODENT
POST-PROCEDURE MONITORING
For use by Principal Investigator/Research Personnel
PI: _____ IACUC #: _____
SPECIES: _____
☐ Animal ☐ ID # _____ ☐ Cage
Date & description of procedure/surgery:
_____
Medication/dose: _____
Medication/dose: _____
Date & follow-up (for example, staple removal)
_____
TREATMENT
Medication Date/time Initials_____
_____
_____
_____
_____
_____
_____
_____
Return this card to ARF Office when complete

Figure 3: Veterinary Alert Card

Cards are utilized to track animals that are used for breeding purposes as well as the offspring that are generated. This involves a two-card system. The two cards, yellow and blue, are designed to help identify breeding cages as well as track the date of birth and expected weaning date of the pups generated.

The yellow breeding box card is placed on breeding cages in place of the standard white cage card. It contains the identical information as the standard white cage card. This card assists in identifying breeders for the ARF Personnel, Principal Investigators and Research Personnel.

Figure 4: Breeding Box Card

Creighton University *Breeding Box*				
Investigator name:			**Protocol # :**	
Date Rec'd:	**Date Bred:**	**Date Weaned:**	**Species / Strain:**	
Vendor:	**Weight:**	**Age:**	**Gender:**	**# Housed**
# Euthanized	Date Euthanized		# Remaining	

When establishing breeding cages, it is recommended to place no more than one pair (1M/1F) in a small static Microisolator cage. Larger breeding groups may be established (1M/2F, 1M/3F when using large static Microisolator caging or 1M/2F when housing in the Thoren ventilated racks).

The blue card is used to track dates of birth and weaning dates for pups generated by the cage of breeders and is placed behind the yellow cage card so that, if possible, it is visible. The blue card is used *only* to document all pup information to keep the primary cage card easier to read. A summary of the cage card system can be found in Appendix B.1.

Non-Rodent Mammals – Cages of non-rodent mammals including rabbits, dogs, and farm animals are also identified via the use of cage cards containing the same information as described for rodents. In addition, an individual Animal Medical Record (Form Med1, Form Med2) will be maintained on these animals (see Appendix B.2). The Animal Medical Record documents information including the Principal Investigator; IACUC Protocol Number; Species; and animal identification such as USDA animal number and/or tattoo/other identification number as applicable. In addition, all movement to other areas is documented on Form Med1. The Animal Medical Record is utilized to document any deviation from normal in the health of the animal or any procedure, with the exception of a procedure involving anesthesia, which is performed on the animal. Form Med2 is used to record SOAP (subjective, objective, assessment, plan) notes for animals that are experiencing deviations from normal health. In addition, daily rounds are to be recorded on Form Med2. A further, more detailed discussion of the use of Animal Medical Records is found in Appendix B.2.

Large Animals – These may be identified by the following means:

- **Dogs** – Each dog is identified via individual ear tattoo or may be fitted with a collar with an identification tag or number. Individual dogs may also be identified using methods consistent with *The Guide*; and

- **Farm Animals** – Numbered ear tags identify Pigs. Other farm animals may be

identified by numbered ear tag or in another humane manner consistent with *The Guide*.

2.2.3 Identification of Animals and Rooms in IACUC-Approved Animal Rooms outside the ARF

The Principal Investigator is responsible for providing current and accurate information to the ARF Manager to ensure that animal rooms are appropriately identified. This includes, at a minimum, emergency contact information; protective clothing required, if above and beyond that required by the ARF Gowning and De-Gowning Fact Sheet (see Appendix C); identification of any biohazardous material; and any appropriate biohazard markings. The Principal Investigator is responsible for adequately identifying individual animals in the room. Identification methods must be consistent with methods approved in *The Guide*. The Attending Veterinarian and ARF Manager provide oversight and monitoring of this process.

2.3 ANIMAL RESOURCE FACILITY PERSONNEL

Copies of the USDA regulations, the National Institutes of Health (NIH) Policy, and *The Guide* are available to all ARF Personnel. ARF Personnel are required to know and understand these guidelines.

2.3.1 Animal Resource Facility Director

The ARF Director has general responsibility for the overall administrative and fiscal operation of the ARF, including, but not limited to, the following:

- Overseeing the day-to-day activities (including holidays and weekends) to ensure a safe, clean, and efficient environment is provided and maintained for housed animals and users of the ARF;

- Supervising personnel, as appropriate;

- Interacting with the Attending Veterinarian, IACUC, Principal Investigators, and the Office of Research Compliance; and

- Leading the ARF toward higher standards of operation in support of Creighton University's animal research goals and maintaining accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International).

2.3.2 Animal Resource Facility Manager

The ARF has one ARF Manager who reports to the ARF Director. The ARF Manager's duties include, but are not limited to, the following:

- Ensuring that daily rounds are made at random times on weekdays and that weekend and holiday coverage is prescheduled to ensure that all tasks are being performed properly. ARF personnel make rounds in the following order: 1) Large, USDA regulated species; 2) nude mice rooms; 3) non-quarantined rat/mice rooms; 4) quarantined rat/mice rooms; 5) all other non-quarantined animal rooms; and 6) all other quarantined animal rooms. The ARF Manager's observations include bedding changes, evidence of proper feeding and watering, and evidence that personnel, technicians and Principal Investigators use protective clothing. ARF Personnel are randomly observed to ensure animals are handled properly, SOPs are followed, and time spent efficiently. The ARF Manager may appoint a designee to assist in this process;

- Distributing the responsibility for care of animals and maintenance of rooms to specific technicians during each weekday and on weekends and holidays;

- Notifying Principal Investigators if any of their animals are sick, injured, or die;

- Coordinating care of any sick animals by informing technicians of the proper procedures and checking to ensure that the prescribed care is carried out;

- Assigning animal housing space in the ARF;

- Issuing of Animal Procedure Records;

- Provide ARF orientation to new users of the ARF;

- Coordinating the training of technicians, including the administration of the Purina Mills Exams, and providing study materials for AALAS technician certification examinations;

- Working with the Principal Investigator to resolve any issues of noncompliance. All instances of non-compliance are to be reported to the ARF Director. Depending on the nature of the non-compliance, the ARF Manager may also contact the Attending Veterinarian, the IACUC Chair, and/or the Research Compliance Officer. Issues related to inhumane treatment of animals will be reported to all of the above-mentioned individuals;

- Reporting any known lapses in animal care to the Principal Investigator that may compromise the health of the Principal Investigator's animal(s) or the research. These issues include, but are not limited to, loss of or confusion regarding cage cards, lapses in feeding or watering of animals, and failure of or abnormal environmental controls (for example, temperature fluctuations);

- Ensuring that appropriate animal care procedures are followed, including any special instructions for animal care required by a Principal Investigator;

- Ensuring that adequate supplies of clean cages, water bottles, food, and bedding are available for the care of the animals;

- Taking appropriate disciplinary action, in conjunction with the ARF Director, the Attending Veterinarian, and/or the Office of Research Compliance, when Creighton University's Personnel Policy or ARF SOPs are not followed by ARF Personnel;

- Communicating with the Principal Investigators to ensure that the needs of the research protocols are being met; and

- Consulting with the ARF Director and Attending Veterinarian as appropriate.

2.3.3 Animal Care Technicians

The ARF has and will maintain an appropriate number of full-time Animal Care Technicians. Training and certification for Animal Care Technicians includes one or more of the following:

- **Purina Mills, Inc. Exam** – All new ARF Animal Care Technicians must complete the self-study PMI Laboratory Animal Care Course and meet passing criteria within the first 90 days of employment (the probationary period);

- **American Association for Laboratory Animal Science (AALAS) Certifications** – Animal Care Technicians are encouraged to further their knowledge by seeking certification through AALAS. The three levels of certification are Assistant Laboratory Animal Technician (ALAT), Laboratory Animal Technician (LAT) and Laboratory Animal Technologist (LATg). The ARF will pay for one test per year per technician. Furthermore, an incentive program is in effect to recognize and reward the Animal Care Technicians for obtaining AALAS certification. The ARF subscribes to the AALAS Learning Library (ALL). This resource provides training that emphasizes the appropriate handling, care, and use of animals and is designed to assist in studying for AALAS certification. ALL subscriptions include access to the Animal Care and Use Library and The Contemporary Topics Continuing Education Unit Test Library. The Animal Care and Use Library offers courses on certification, regulatory mandates, bioethics, biomethodologies, biosafety and management. The Contemporary Topics Continuing Education Unit Test Library offers the opportunity to earn continuing education units by taking the self-administered test based on the scientific articles in *Contemporary Topics* online. Following completion of a unit, a transcript is provided for documentation. In addition, AALAS training material is available from the ARF;

- **Species-Specific Training** – Animal Care Technicians must be trained in handling the specific species housed in the ARF. The ARF Manager has extensive experience in handling a diverse variety of conventional laboratory

animal species, such as mice, hamsters, rats, guinea pigs, rabbits, dogs, cats, and non-human primates, as well as livestock species, such as swine, calves, and sheep. In addition, species-specific educational videos available from the ARF are used to train technicians as needed. If a new species is brought to the ARF that the ARF Manager or Attending Veterinarian is not trained to work with, the ARF Manager orders the appropriate training video, if available, and arranges for technicians to receive training from someone experienced in handling the species. All ARF personnel handling mice are required to complete microisolator training;

- **CITI Training** – All Animal Care Technicians are required to complete the species-specific modules for all animals housed in the ARF;

- **Animal Occupational Health and Safety Training** – All Animal Care Technicians receive Animal Occupational Health and Safety Training through Creighton University in order to address specific concerns related to working with animals. This program is described in Section 5.0 and in further detail in the Animal Occupational Health and Safety Program; and

- **Tuition Remission** – Full-time employees are eligible to receive tuition remission for up to two Creighton University classes per semester, to be scheduled outside of regular working hours.

Animal Care Technician Responsibilities – Animal Care Technicians' responsibilities include, but are not limited to, the following:

- Observing all animals daily on weekdays and as scheduled on weekends and holidays. Concerns are reported to the ARF Manager, who will contact the Principal Investigator, ARF Director, Attending Veterinarian, Facilities Management, and/or the IACUC Chair as warranted;

- Changing and cleaning animal cages, cleaning ARF rooms, and performing other tasks related to the care of animals as requested by the ARF Manager and/or Principal Investigator;

- Attending technician meetings to communicate special care needs in individual animal rooms;

- Reporting sick, injured or dead animals to the ARF Manager;

- Emptying food and bedding from dirty cages;

- Autoclaving dirty cages as appropriate;

- Cage washing; and

- Reporting possible instances of noncompliance by the Principal

Investigator/Research Personnel or other ARF Personnel to the ARF Manager, Attending Veterinarian, and/or ARF Director. ARF Personnel may report issues of noncompliance anonymously to the Creighton University Research Compliance Hotline at 402-280-3200.

2.4 ANIMAL CARE – GENERAL

Animals are housed on the Creighton University campus in the ARF and IACUC-approved areas outside the ARF. In those rare situations where IACUC approves housing of animals off campus, IACUC and the Attending Veterinarian shall ensure, through the approved protocol that animals are provided appropriate shelter from the weather and are housed in a secure structure.

The Principal Investigator is responsible for maintenance of environmental parameters outside the ARF, unless otherwise allowed under an IACUC-approved protocol, as follows:

- **Temperature** – The room where animals are housed must be maintained at a temperature appropriate for the species. Temperature must be recorded on a daily basis;

- **Noise** – Noise levels must be species-appropriate;

- **Lighting** – Species-appropriate light/dark cycles must be established (typically 12 hours light/12 hours dark), unless otherwise allowed under an IACUC-approved protocol; and

- **Pest Control** – Adequate pest control must be in place.

Failures in Environmental Control Systems – Any failures in environmental control systems must be remedied to ensure the animals have acceptable temperature levels and lighting schedules. ARF Personnel will respond to any environmental control problems observed by ARF Personnel or reported by the Principal Investigator. The Attending Veterinarian, ARF Manager, and designated ARF Personnel make random checks of any outside facility, at least once monthly, to ensure compliance with SOP protocols and guidelines. Twice each year, as required by OLAW, IACUC inspects all facilities where animals are housed as part of its semiannual program and facility inspection.

The Attending Veterinarian makes rounds at the ARF at least once a week to ensure that all animals are in good health and to check for any possible deviations from the animal care protocol. Designated ARF Personnel perform rounds on all animal rooms in the facility on a daily basis, including weekends and holidays.

2.4.1 Care of Animals in IACUC-Approved Animal Rooms outside the Animal Resource Facility

Principal Investigators with animals housed in IACUC-approved animal rooms outside the ARF are responsible for the following:

- Arranging for adequate health checks of animals housed outside the ARF;
- Implementing adequate disease- and parasite-prevention plans for all animals housed outside the ARF;
- Maintaining appropriate environmental parameters (see section 2.1.4); and
- Providing humane animal care in accordance with *The Guide*, including the following:
    - Appropriate feeding and watering schedules,
    - Species-appropriate feed,
    - Appropriate checks of food and water during the weekdays, as well as appropriate weekend and holiday care,
    - Adequate storage and maintenance of animal feed and bedding,
    - Species-specific environmental enrichment,
    - Maintaining records documenting daily temperatures, and
    - Notifying the ARF Manager or Attending Veterinarian of any problems.

2.4.2 Feed and Bedding

General – Animals maintained by the ARF Personnel are fed a complete nutritional diet and provided species-appropriate bedding. Some animals may be fed specialized diets provided by the Principal Investigator, as approved in their IACUC protocol;

Storage – The primary storage area is in a dedicated, climate controlled feed and bedding area. Unopened bags of feed and bedding are stored on raised stainless steel pallets. Feedbags are not to be placed directly on the floor. Bedding is dispensed from a hopper in the clean cage preparation area. Open bags of feed may be stored in appropriately marked containers either *inside* the room for small animals or in designated clean caging preparation rooms and in a designated location *outside* the room for large animals. Feed and bedding containers must remain in small animal rooms unless they are being transported to the cage washer for cleaning. Autoclaved or irradiated rodent chow is available within animal rooms in an autoclaved cage. This container should only be opened in the biosafety hood. All containers with animal feed or bedding are clearly labeled, without abbreviations. Animal Care Personnel are trained to regularly check the mill date and calculate expiration dates of the feed. Animal feed may be used up to six months after the mill date. Expired feed is discarded in the nearest waste container; if feed contains biohazardous materials, it is discarded in a red biohazard waste container;

Transportation – Feed and bedding are either transported to the ARF in the departmental van or delivered to the ARF in a dedicated vendor-owned truck. Feed is transported on a flatbed cart that has been sprayed with Clidox cold sterilizer and wiped dry with clean paper towels by an Animal Care Technician prior to transportation. The outer surface of all feed and bedding bags are disinfected with Clidox prior to entering the facility. The inner surface of the van is disinfected with Clidox after each use. The outside of the van is washed routinely.

Feed – Commercially available laboratory animal diets are obtained from Harlan Teklad. Each diet is selected for its high palatability and consistent formulation to minimize nutritional variables. Special diets, as required to meet the scientific goals of the study, are purchased by the Principal Investigator. The following is the current list of feed products by species:

- **Rodents** – Teklad #2018 Global Rodent Diet, Teklad #2018S Global Rodent Diet (Sterilizable), Teklad #2018 SX Global Rodent Diet (Sterilizable, Extruded); Teklad #2918 Irradiated Global Rodent Diet,
- **Guinea Pig** – Teklad #2040 Guinea Pig Diet,
- **Rabbit** – Teklad #2031 Global High Fiber Rabbit Diet,
- **Dog** – Purina Dog Chow Adult formula, and
- **Swine** – Teklad #8753 Mini Pig Diet;

Bedding – Contact bedding used in the ARF include Harlan Teklad #7097 ¼" Corn Cob Bedding; Teklad #7092 1/8" Corn Cob Bedding; #7907 Irradiated ¼" Corn Cob Bedding; #7990 Irradiated Sani Chip Bedding; #7086 Pelleted Hardwood Bedding; and #7093 Shredded Aspen Bedding. Non-contact bedding includes Harlan Teklad #7086 Pelleted Hardwood Bedding and pan liners; and

Water – The Metropolitan Utilities District, in Omaha, Nebraska, provides water to the ARF. Autoclaved water is provided when requested by the Principal Investigator. All animals except dogs, swine and goats are watered with bottles. Dogs are watered with stainless steel pans, swine and goats are watered in stainless steel receptacles.

2.5 ANIMAL CARE DAILY PROCEDURES

The following animal care procedures apply to the ARF during regular weekdays. This schedule may vary for holidays and weekends, as noted in section 2.5.1. The Principal Investigators are responsible for instructing ARF Personnel in the proper precautions that must be employed to care for their housed animals. The ARF Personnel is responsible for ensuring that these instructions are implemented. ARF Personnel perform all animal care procedures, except in those cases where the Principal Investigator and ARF Director and/or ARF Manager have agreed upon other arrangements.

2.5.1 Weekend and Holiday Care

Designated members of the ARF Personnel report each Saturday, Sunday and weekday holiday, as prescheduled. All animals are observed to ensure they are healthy and clean.

Large Animals (Including Dogs) – Large-animal rooms, including dog runs, are cleaned, and animals are fed and watered; and

Small Animals – Animals are given adequate water and food. Bedding is checked, and cages are replaced if bedding has been excessively wetted.

2.5.2 Daily Care of Farm Animals

Food and Water – Large animals are fed daily and provided with water via a species appropriate receptacle;

Socialization – Animals are placed in social groups as appropriate to the species to allow for physical and social contact;

Environmental Enrichment – Species specific toys such as basketballs and Kong® toys are provided as a means for animals, such as swine, to practice normal rooting behavior; and

Cage Cleaning – Cage floors are sprayed with hot water, washed with disinfectant, and rinsed with clean water daily. Monthly, or as indicated, acidic foam is used to clean and disinfect the runs in use.

2.5.3 Daily Care of Dogs

Food and Water – Water is provided *ad lib* either through an automatic system or through manual watering. Fresh food and water are provided during cleaning of the dog cages;

Socialization – Dogs are housed in primary enclosures consisting of galvanized runs and provided a resting panel. These runs allow them visual, auditory and olfactory contact with other dogs. When compatible, dogs are released together within the room during daily cleaning to provide additional exercise and social interaction;

Environment Enrichment and Exercise – Environmental enrichment devices including, but are not limited to, Nylabones®, Kong® toys, and rawhide chews, are provided to all animals unless otherwise prohibited as outlined in an IACUC-approved protocol. In some instances, a radio may be used to provide environmental enrichment for dogs, per veterinary directives, in the dog room of the ARF. The programming will be contemporary music and/or talking at a normal voice range usually during daytime hours. Creighton University, as required by the Department of Agriculture, APHIS, Animal

Welfare, Standards; Final Rule, published in the *Federal Register*, February 15, 1991, maintains a dog exercise program as follows:

- 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,

- Opportunity for additional exercise will be provided to each dog as follows: Dogs will be individually placed in an open enclosure (run). All animals are allowed to exercise for a minimum of 2 hours per session four days a week. A log will be kept in the animal room indicating how much exercise a dog receives. Dogs which are not candidates for exercise will be so noted by the attending veterinarian,

- Dogs will be removed from their cages and released individually into a run with an area no less than twice the required housing space,

- One or more technicians will physically interact with the dogs during the opportunity for exercise,

- To minimize disease transmission, the exercise pen will be hosed, cleaned and sanitized between occupancy by animals.

Exceptions – Dogs are not exercised under the following conditions: 1) If the Attending Veterinarian deems that exercising will cause the animal to injure itself or other dogs, 2) If the animal is diagnosed with a contagious disease or parasite or treated with any substance that would harm other animals, or 3) If quarantined. All exceptions are reviewed by the Attending Veterinarian after 30 days.

Cage and Run Cleaning – Dog runs are cleaned daily. Run floors are sprayed with hot water, washed with disinfectant, and rinsed with clean water. Monthly, or as indicated, acidic foam is used to clean and disinfect the runs in use. Exercise runs are cleaned with a disinfectant and rinsed with clear water after the dogs have been returned to their cages for the day. The exercise area is disinfected monthly from ceiling to floor with a high-pressure tank using hot water and disinfectant.

2.5.4 Daily Care of Cats

Food and Water – Water and fresh food are provided daily;

Socialization – Cats are group housed whenever possible. If the animals are group housed, cats are placed in cages that provide visual contact with other animals in the room;

Environmental Enrichment/Exercise – Whenever possible, cats are group housed. When group housed, cats are allowed free access inside the animal room and provided

devices on which to climb, play and in which to hide. Species-appropriate enrichment devices, such as balls, are also provided. If individual housing is required, cats are provided resting panels and species-specific enrichment devices, such as balls; and

Cage Cleaning – Group housed cat rooms are cleaned twice per week. Floors are sprayed with hot water, washed with a disinfectant, and rinsed with clean water. The room is disinfected monthly from ceiling to floor with a high-pressure tank using hot water and disinfectant. Litter pans in group housing rooms are cleaned daily. Individually housed cats have their litter pans changed three times per week. Cages are changed every other week.

2.5.5 Daily Care of Rabbits

Food and Water – Fresh water and food are given daily;

Socialization – Rabbits are housed in cage banks that allow them auditory and olfactory contact with other rabbits. When more than one bank of cages is in a room, they are placed, if possible, opposite each other in order to provide visual contact with other rabbits within the room;

Environmental Enrichment – Rabbits are provided with alfalfa blocks for gnawing and species appropriate toys such as dumbbells, stainless steel rattles, canning jar rings and balls; and

Cage Cleaning – Non-contact bedding excreta pans and pan liners are changed twice each week. The rabbits are transferred into clean caging weekly.

2.5.6 Daily Care of Guinea Pigs

Food and Water – Guinea pigs are given food *ad lib* and fresh water daily. Clean water bottles are provided weekly;

Socialization – Guinea pigs are group housed in solid bottom caging on contact bedding. Every effort is made to house guinea pigs in compatible groups unless it is in conflict with the scientific goals of the approved IACUC protocol. This allows for mutual grooming and other social interactions among cage mates; and

Environmental Enrichment – The use of contact bedding in guinea pig caging allows them to express normal behavior such as digging and burrowing. In addition, guinea pigs are provided with species appropriate devices such as shower curtain rings attached to the front of the cage; dumbbells, balls, and chew sticks for gnawing. In addition, cages may also be equipped with transparent plexiglass huts that allow guinea pigs to control further their environment.

Cage Cleaning – Bedding and clean caging is provided twice weekly.

2.5.7 Daily Care of Hamsters and Gerbils

Food, Water, and Bedding – Sterile caging, food, water, and bedding are provided to all hamsters and gerbils. Food and water are provided *ad lib*. Fresh food and water provided twice weekly, and/or as otherwise needed;

Socialization – Hamsters and gerbils are normally housed in solid bottom caging on contact bedding. Every effort is made to house hamsters and gerbils in compatible groups unless it is in conflict with the scientific goals of the approved IACUC protocol. This allows for mutual grooming and other social interactions among cage mates;

Environmental Enrichment – The use of contact bedding in hamster and gerbil caging allows them to express normal behavior such as digging and burrowing. Gerbils are also provided with nesting squares to use in constructing nests. Hamsters are not provided nesting squares due to their tendency to impact them into their cheek pouches;

Cage Cleaning - Bedding and caging are changed twice weekly; and

Handling – Gerbils are handled by grasping with the hand at the base of the tail.

2.5.8 Daily Care of Rats and Mice (Including Nude Mice)

All ARF personnel are trained to recognize overcrowded cages of small animals. Using *The Guide* as a resource, they are able to weigh animals and calculate how many should occupy a given cage. The maximum number of animals per cage is determined by species (see section 2.1.1).

Animal Room Sequence – To protect the health and well-being of rats and mice, rooms are entered by ARF Personnel in the following order: 1) nude mice rooms, 2) non-quarantined rat/mice rooms, and 3) quarantined rat/mice rooms. All procedures and experimental manipulations on mice within the ARF housing areas, including cages changes, are performed under HEPA filtered laminar flow hoods or biosafety cabinet utilizing Microisolator technique. Disinfection, both prior to and after use of Biosafety cabinets is required to decrease the likelihood of cross-contamination between cages and/or Principal Investigators. The disinfection instructions are posted on each Biosafety cabinet;

Food, Water, and Bedding – Sterile caging, food, water, and bedding are provided to all rats and mice. Food and water are provided ad lib. Fresh food and water are provided twice weekly for rats, and once weekly for mice, and/or as otherwise needed. Food and water levels are observed daily and replenished as needed. Clean water bottles are provided during the second cage-change. Food is replenished as needed during the second cage cleaning. Nude mice are provided autoclaved feed and water;

Socialization – Mice and rats are normally housed in solid bottom caging on contact bedding unless in conflict with the scientific goals of an IACUC-approved study. Every

effort is made to house rats and mice in compatible groups unless it is in conflict with the scientific goals of the approved IACUC protocol. This allows for mutual grooming and other social interactions among cage mates;

Environmental Enrichment for Mice – The use of contact bedding in mouse caging allows them to express normal behavior such as digging and burrowing. Mice are also provided with nesting squares to use in constructing nests. In addition, mice may be provided, at the Principal Investigator's request, transparent plexiglass igloos that further allow them to control their environment;

Environmental Enrichment for Rats – The use of contact bedding in rat caging allows them to express normal behavior such as digging and burrowing. Nylabones® are provided to individually housed rats, where not in conflict with the IACUC-approved protocol;

Cage Cleaning – Sterile cages and bedding are provided for all rats and mice housed in solid bottom caging. Bedding and caging are changed twice weekly for both rats and mice which are housed in static microisolator caging. Mice housed in ventilated rack systems are provided bedding and cage changes weekly. Non-contact bedding excreta pans and pan liners are changed twice weekly in the event rats must be housed in suspended wire bottom caging in order to meet the scientific goals of the study;

Handling – Forceps, dipped in 1:5 Clidox are used to handle mice. There are two forceps in each room where mice are housed. One pair is used and returned to its Clidox bath, and then the other is used, alternating between forceps after each use; and

Mice in Behavioral Studies – Because of the rigid handling protocols required by behavioral studies, the Principal Investigator/Research Personnel may be responsible for daily care of animals involved in these studies, including changing cages and cleaning the animal room. Appropriate noise reduction measures may also be needed, as per the protocol.

3.0 Veterinary Medical Care

Veterinary medical care is an essential part of an animal care and use program and is comprised of effective programs for:

- Preventive medicine;
- Surveillance, diagnosis, treatment, and control of disease, including zoonoses control;
- Management of protocol-associated disease, disability, or other sequelae;
- Anesthesia and analgesia;
- Surgery and post-surgical care;
- Assessment of animal well-being; and
- Euthanasia.

The Attending Veterinarian is primarily responsible for Creighton University's Veterinary Care Program. Some aspects of the Veterinary Care Program may be conducted by ARF Personnel under the direction of the Attending Veterinarian. Any problems identified in the Veterinary Care Program shall be directed to the Attending Veterinarian. The Attending Veterinarian provides guidance to Principal Investigators and all other personnel involved in the care and use of animals at Creighton to ensure appropriate handling, immobilization, sedation, analgesia, anesthesia, and euthanasia.

3.1 ANIMAL PROCUREMENT AND TRANSPORTATION

All animals authorized for use by IACUC must be ordered through the ARF, unless otherwise allowed under the IACUC approved protocol. Principal Investigators are responsible for ordering animals approved by IACUC from USDA-licensed either commercial or noncommercial vendors, using the procedures set forth below. Only animals ordered and purchased through the ARF may be used in IACUC-approved protocols. The purchase request must be entered into the University's accounting system (Banner) by the Principal Investigator's Department for processing purposes. No animals shall be purchased using a direct pay request (DPR) or credit card. All animals must be used in the protocol for which their use was approved unless they are transferred to another approved protocol or investigator. All animal transfers must be coordinated through the ARF Manager.

3.1.1 Ordering Mammals from Commercial Vendors

Prior to ordering animals, the Principal Investigator should obtain current pricing information. Commercial animal vendors normally utilized by Creighton are Harlan, Taconic, Jackson Labs, and Charles River. Pricing information may be obtained via the Internet from Harlan (http://www.harlan.com/), Taconic (http://www.taconic.com), Jackson Labs (http://jax.org), and Charles River (http://criver.com). Other commercial vendors, as approved under the protocol, may also be used. Shipping and crate costs can be obtained from the Creighton University ARF Office. Annual price increases usually occur on 1 July for Harlan and on 1 January for Taconic, Jackson Labs and Charles River.

Once pricing information for animals, shipping, and crates have been obtained, the Principal Investigator is responsible for making sure the order is placed through the University accounting system (Banner). The IACUC-assigned protocol number must be included in the order. In most cases, animals must be ordered no later than 11:00 a.m. CST Friday to be received the following week. The ARF Manager will contact the Principal Investigator if the requested delivery date is not available.

After the order has been placed in the Banner system, the Animal Resource Office will enter the appropriate information into the ARF database. The request will be verified against the protocol number, species, and the animal numbers available. Once the order is approved against the database, the ARF Manager or other appointed personnel in the ARF will submit the order to the vendor. If the number of animals requested is not available under the protocol, the ARF Manager will promptly notify the Principal Investigator.

Upon arrival, animals will be placed in the appropriate room. Unless the Principal Investigator requests specific caging prior to arrival of the animals, the animals will be housed using the best judgment of the ARF Personnel, who shall rely upon *The Guide* and these SOPs. Housing for species is as follows:

Rodents – Only vendors or institutions that can provide records of an ongoing health surveillance program are utilized. Commercial animal vendors normally used by Creighton University are Harlan, Taconic, Jackson Labs, and Charles River Laboratories. The Attending Veterinarian or designee monitors all health surveillance reports from the commercial vendors listed above;

Dogs – Dogs procured for use at Creighton University approved IACUC protocols are obtained from only USDA-licensed Class A dealers effective January 31, 2002;

Rabbits – Only specific pathogen free (SPF) rabbits are procured for use in Creighton University approved IACUC protocols; and

Livestock/farm animals – USDA-licensed facilities are utilized for Creighton University approved IACUC protocols requiring the use of farm animals, for example the University

of Nebraska-Lincoln Field Research Laboratory located in Mead, Nebraska. Miniature swine will be procured from commercial sources and include S&N Farms.

3.1.2 Ordering Mammals from Noncommercial Vendors

The ARF generally discourages ordering or housing any animals from noncommercial vendors, also referred to as extramural sources, because of the risks involved (for example, possible spread of disease or parasites, potential adverse affects on research protocols). When animals from noncommercial vendors are accepted in the ARF, the procedures set forth in these SOPs must be strictly followed. The Principal Investigator must certify that the animals being obtained from the noncommercial vendor are not available commercially.

If the use of non-commercial animals was not originally approved in the IACUC protocol, the Principal Investigator must submit a letter to both the IACUC chair and Attending Veterinarian describing the need for obtaining the animals from the identified noncommercial vendor. The Principal Investigator will then be provided a copy of the requirements, via e-mail attachment (see Appendix G), which they are then responsible to forward to the Veterinary Personnel at the shipping institution. Prior to ordering animals from a noncommercial vendor, the Principal Investigator must receive written approval from the Attending Veterinarian. To obtain this written notification of approval, the Principal Investigator must obtain the following information and submit to the Attending Veterinarian for review:

- A health certificate from the shipping institution's Attending Veterinarian including a description of the facility (barrier or conventional) and the animal housing conditions (standard Microisolator or ventilated rack; requirement for autoclaved caging/feed/water). A description of the health surveillance program is required detailing the method of sentinel contact; frequency of testing; testing profiles performed. In addition a summary of the health status of the facility over the past 12 months is required, identifying any animal health concerns/problems during that time period and the steps taken to treat or contain the pathogen (if applicable);

- A minimum of the past 12 month period of health surveillance reports (sentinel mice) for the facility, clearly identifying information on the room from which the animals were housed;

- A serum profile for each specific mouse, or cage of mice to be shipped, may be required by the Attending Veterinarian. The mice will need to screened for, at a minimum, SEND, PVM, MHV, TMEV, REO, MPUL, MPV and EDIM. The serology results must be recent (within approximately 30 days of receipt of the animals). It is best to obtain blood samples approximately two weeks prior to shipment of the animals. Alternatively, arrangements may be made, with Veterinary approval, for cage mates of the mice requested to be sent for serology or the cage mates may be sent as extras with the requested shipment. A serology

profile will be performed on these animals upon arrival to the ARF. RADIL or Charles River will perform the serological testing;

- In addition to the above required health status information, the following information must also be provided: the immunological status of the strain or line (if known) and/or any animal housing considerations which are above those routinely provided in a conventional facility; and

- The Creighton University ARF does not normally accept animals known to be positive for pinworms. Animals from a facility known to have a recent outbreak of MHV will not be accepted.

Responsibilities of Attending Veterinarian and ARF – The Attending Veterinarian is responsible for reviewing all required health reports and making the following decision within five working days:

Approval – After all required documentation is received, the Principal Investigator will be provided prompt written notice if the Attending Veterinarian agrees to allow the ARF to accept the animals, providing a period within which the animals will be accepted. This period may be revised depending on weather conditions and/or availability of Quarantine Areas. The ARF will:

- Verify that the number of animals requested by the Principal Investigator corresponds to the number approved under the IACUC protocol number provided by the Principal Investigator;

- Determine whether the species/strain requested by the Principal Investigator matches the animals requested on the approved protocol;

- Determine the availability of Quarantine Areas and arrange for sentinel mice to be placed in the appropriate Quarantine Areas by the arrival day of the new mice; and

- Provide written confirmation to the Principal Investigator verifying the number and species/strain of animals requested by the Principal Investigator.

Upon receipt of the written notice of approval from the Attending Veterinarian and ARF, the Principal Investigator will provide the contact information for the noncommercial vendor to the ARF Manager who will contact the noncommercial vendor to arrange for shipment of the animals.

Disapproval – If the Attending Veterinarian disapproves receipt of the animals requested by the Principal Investigator, written notice will be provided explaining why the animals will not be allowed to enter the ARF. The Principal Investigator has the option of accepting the decision or addressing and correcting as appropriate the areas identified in the written notice and resubmitting the request for the animals.

3.1.3 Ordering Non-Mammals

Commercial Vendors – Principal Investigators ordering non-mammals (for example, fish, reptiles) from commercial vendors must place the order through the Banner system and may be contacted by the ARF Manager for additional information prior to the animals being ordered.

Noncommercial Vendors or Collection – Principal Investigators must obtain approval from the Attending Veterinarian prior to ordering non-mammals from noncommercial vendors or prior to collecting such animals.

3.1.4 Transportation of Animals

A long-term goal of the ARF is to provide a specific pathogen free (SPF) facility for the health of the animals and to support the integrity of research projects involving animals housed at the ARF. Controlling the movement of animals to and from the ARF provides critical support for attaining this goal. In general, animals removed from the facility are not allowed to re-enter. Re-entry of live animals is the exception and as a result, provisions (including training of the investigators and their staff by the ARF Manager) must be made prior to their re-entry.

After animals have been received and housed in either the ARF or other IACUC-approved facilities, Principal Investigators may need to transport their animals to another location, either internally or externally. Internal transportation involves moving animals within the building in which they are housed (for example, transporting animals from the ARF to a laboratory in the complex). External transportation involves moving animals to a location outside the building in which they are housed (for example, transporting animals from the ARF to the surgical suite).

Internal Transportation of Animals – To control exposure to potential pathogens, the ARF and Attending Veterinarian have developed procedures regarding internal transportation of animals. Principal Investigators may transport animals from the ARF to another internal location for euthanasia. Animals must be transported in filter top caging and the caging must be autoclaved prior to return to the ARF. The caging will be sealed in a plastic bag and the outside of the bag sprayed with Clidox immediately prior to entry into the ARF. The caging is then transported to the designated dirty caging storage area. Principal Investigators transporting animals from the ARF to another internal location and planning to return the animals to the ARF should discuss appropriate return procedures with the ARF Manager and the Attending Veterinarian. In this instance, the mice are usually housed in the Community Mouse Room. In some cases, the Attending Veterinarian may require that animals returning to the ARF undergo a Quarantine period. Due to Occupational Health and Safety considerations, Principal Investigators/Research Personnel moving animals out of the facility should use the freight elevator to minimize the transportation of animals on public access elevators, thus reducing allergen exposure to the general population and addressing security concerns.

External Transportation of Animals and Use of the ARF Van – Unless otherwise allowed under an IACUC-approved protocol or by the Attending Veterinarian, the ARF van must be used to transport animals externally.

Anyone who drives the ARF van must have completed Creighton University's vehicle safety training course. The ARF van is washed and disinfected by ARF Personnel after each animal transport, and the ARF Manager will maintain a record of van usage. No mammal shall be transported in a private vehicle without prior written authorization from IACUC or the Attending Veterinarian. Animals transported from the ARF to any external location will not be allowed back into the ARF without the written approval of the Attending Veterinarian.

3.1.5 Transportation Cages

Reusable Transportation Cages – The ARF has reusable transportation cages, available upon request by the Principal Investigator, for transporting any non-rodent mammal ranging in size from rabbits to pigs. If Principal Investigators use ARF cages to transport rodents, caging must be autoclaved prior to return to the ARF. The caging will be sealed in a plastic bag and the outside of the bag sprayed with Clidox immediately prior to entry into the ARF. The caging is then transported to the designated dirty caging storage area The Principal Investigator should check with the ARF Manager on the appropriate manner in which to return non-rodent mammal cages. Arrangements for transporting animals larger than pigs should be described in the IACUC-approved protocol.

3.2 PREVENTIVE MEASURES TO PROTECT ANIMALS

3.2.1 Quarantine Areas

The ARF has established Quarantine procedures to protect the health of incoming animals and animals housed at the ARF. Failure to follow the Quarantine procedures can spread disease or parasites and can have potential adverse affects on research protocols. Noncompliance is a serious breach of ARF procedures and will result in corrective action (see section 6.0).

Commercial source animals are not normally quarantined upon arrival. These animals are included in the quarterly sentinel-testing program. If a question or problem arises regarding the health of a particular shipment, an ordering block is initiated until the issue is resolved. All noncommercial mice are isolated, quarantined and placed in the sentinel-testing program for a minimum of seven weeks. Investigators should contact the ARF Manager to understand clearly the charges they will incur due to the receipt of animals requiring quarantine.

Animals Received from Noncommercial Vendors - Mice entering the ARF which are obtained from any source other than an approved commerical vendor shall be quarantined as detailed below:

The Quarantine period for extramural shipments is a minimum of seven weeks. Sentinel mice are utilized and testing is performed at least twice during the Quarantine period. Sera from two sentinel mice is sent at three weeks into the period to aid in diagnosing pathogens the animals may have been exposed to immediately prior to shipping. Whole animals are sent for comprehensive testing at seven weeks and animals may be released into the general population when satisfactory results have been obtained. A portion of the cost for sentinel testing is charged to the Principal Investigator. The Principal Investigator should contact the ARF Manager to determine these costs for a particular shipment (see section 3.3.3).

In some instances, the Quarantine period can be waived. When animals are procured for tissue collection only, the Attending Veterinarian may allow an investigator to bypass the Quarantine if the animals are to be euthanized within 24 hours of arrival (see Appendix G). In such cases, the Principal Investigator/Research Personnel are not allowed to enter the ARF for a period of time (usually two to three days) determined by the Attending Veterinarian. This is designed to prevent any contamination of the ARF with outside pathogens. Also, all of the criteria outlined in section 3.1.2 must be met.

Dogs and Cats – All dogs and cats that are to be used in IACUC-approved protocols must be procured from Class A commercial vendors, effective January 31, 2002.

All New Arrivals – ARF Personnel shall evaluate the health and, if appropriate, the pathogen status of newly received animals and consult with the Attending Veterinarian on whether or not to quarantine any animals that exhibit unusual behavior or show signs of disease or parasites. The ARF Manager will notify the Principal Investigator of any unusual behavior or signs of disease or parasites and of the possible need for quarantine. Additional responsibilities of the Principal Investigator in the instance of receipt of non-rodent mammals are outlined in 3.2.2.

3.2.2 Stabilization and Separation

ARF Personnel shall provide an appropriate period for physiological, psychological, and nutritional stabilization of animals entering the ARF before they are used in a protocol. Animals are separated by species.

Dogs – Only Class A dogs are permitted in the ARF. The animal will be received into the ARF, general health assessed and a physical examination (minimum Temperature, Pulse and Respiration (TPR); and body weight) will be preformed within 12 hours of receipt by ARF staff and recorded on Animal Medical Record Progress Notes (Form MED2). Any health issues are noted in the animal's medical record and a plan of treatment outlined by the Attending Veterinarian. Within 48 hours of receipt, it is the responsibility of the Principal Investigator to perform a physical examination of all dogs. The Principal Investigator, using Form Med2, must document this physical examination. Dogs require a seven-day acclimation period following receipt into the facility before any USDA Category D or E procedures can be performed on the animal.

Rodents – Rodents are examined by ARF Personnel upon arrival and placed into cages. Mice from noncommercial vendors are placed in quarantine for a minimum of seven weeks with sentinel mice. Refer to section 3.3.3 for further details.

Rabbits – Rabbits are examined by ARF Personnel and placed into cages. Any health abnormalities are reported to the ARF Manager and/or the Attending Veterinarian. Rabbits are obtained from commercial dealers (for example, Harlan, Charles River, Myrtle's Rabbitry) who perform regular health surveillance on their colonies.

Goats/swine –The animal will be received into the ARF, general health assessed and a physical examination (minimum Temperature, Pulse and Respiration (TPR); and body weight) will be preformed within 12 hours of receipt by ARF staff and recorded on Animal Medical Record Progress Notes (Form MED2; Appendix D). Any problems are reported to the ARF Manager and/or the Attending Veterinarian. Goats are immunized for tetanus upon entry into the ARF. All vaccines are to be documented on Form Med1. Within 48 hours of receipt, it is the responsibility of the Principal Investigator to perform a physical examination of all goats or swine. This physical examination must be documented by the Principal Investigator on Form Med2. Goats and swine require a seven day acclimation period following receipt into the facility before any USDA Category D or E procedures can be performed on the animal.

3.2.3 Availability of Attending Veterinarian

The Attending Veterinarian is an employee of Creighton University and is available to research personnel and ARF Personnel to address animal care and/or use issues. The Attending Veterinarian visits the ARF at least one day each week (except during holidays/vacation) or more often as necessary for appropriate animal care. Animal Care Technicians and Research Personnel may contact the ARF Manager or the Attending Veterinarian directly if an animal is in need of veterinary attention. A message (verbal, e-mail, or written) should also be left with the ARF Manager. The Principal Investigator is notified of any veterinary care that is required. Veterinary care is performed either by the Attending Veterinarian or under the direction of the Attending Veterinarian. The treatment plan is recorded on either the Veterinary Alert Card or the animal's medical record. Both Charles River and RADIL are used for diagnostic purposes. Emergency coverage is arranged when the Attending Veterinarian is not available. The Attending Veterinarian maintains a log of activities when physically away from the Creighton University campus.

- Principal Investigators who want to meet personally with the Attending Veterinarian may notify the ARF Manager at ARF@creighton.edu to arrange a meeting in a timely manner;

- Principal Investigators with technical problems (for example, animal treatment concerns, anesthesia uses, or others, as listed in the introduction to section 3.0) may contact the Attending Veterinarian by telephone. If the Attending

Veterinarian is not available, Principal Investigators should either leave a detailed message or state that a message will be sent via e-mail. The ARF Manager and ARF Director should be copied on all correspondence. If the Attending Veterinarian is not on site, the PI should contact the ARF Manager in person. The Attending Veterinarian will also provide input on the development of laboratory-specific SOPs for non-rodent mammal Category D and E procedures; and

- Any animal health issues will be sent immediately to the Attending Veterinarian for instructions and/or possible examination of the animal(s) affected. The ARF Manager will notify the Principal Investigator promptly of any such issues.

3.2.4 Use of Cell Lines *in vivo*

Prior to injecting animals with cultured cells, the ARF requires that the cell lines be tested for animal (for animal cell lines) or human (for human cell lines) pathogens. Information regarding commercially available human cell lines can normally be obtained from the vendor. Animal cell lines can be tested by commercial diagnostic laboratories such as RADIL or Charles River. It is recommended that the Principal Investigator consult the ARF to determine the requirements for their specific circumstance.

3.2.5 Rodents at Home

The ARF performs quarterly testing of the facility using sentinel mice to screen for pathogens that may be found in a given room. One potential source of these infections is rodents purchased at pet stores either as pets or as feeder animals for reptiles. These rodents are frequently infected with such pathogens as mouse hepatitis virus (MHV), Sendai virus (parainfluenza), EDIM (epizootic diarrhea virus of rodents; rotavirus), mouse minute virus (MMV), reovirus-3, mouse parvovirus (MPV), Mycoplasma pulmonis, pinworms, and fur mites. Some of these pathogens are highly contagious. If you are handling rodents obtained from pet stores (or other sources) at home it is recommended that you limit your contact to after working hours or weekends. If rodents are handled at home, do not enter the ARF until showering and changing clothes. Many of these diseases are asymptomatic in healthy rodents and it may not appear that these animals are infected. These diseases have the potential to interfere with research, particularly in transgenic, knockout and/or immunodeficient animals.

3.3 SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASES

3.3.1 Surveillance

ARF Personnel who are trained to recognize signs of illness, injury, or abnormal behavior in animals observe each animal at least once per day (including weekends and holidays). Results of daily rounds are noted on Form Med2 for all non-rodent mammals in a SOAP-notes format (see Appendix B.2). If an animal is found in an unhealthy condition, the ARF Personnel will contact the ARF Manager and/or the Attending Veterinarian. ARF

Personnel also contacts the Principal Investigator/Research Personnel on the project involving the affected animal(s). If the Principal Investigator or other personnel assigned to the protocol involving the affected animal cannot be reached or fail to respond and the animal is in distress beyond the criteria stated in the IACUC-approved protocol, the Attending Veterinarian will make an immediate decision as to the treatment of the animal. If severe breaches of IACUC protocols occur, the Attending Veterinarian will consult with the IACUC Chair and Principal Investigator, if available, to ensure humane treatment of the affected animal(s). If the Principal Investigator or associated project personnel are not immediately available or fail to respond, the ARF Manager will notify the Principal Investigator of the actions taken by the Attending Veterinarian (or designee). The Attending Veterinarian has the authority to make decisions based on health concerns with or without consultation with the IACUC Chair or Principal Investigator.

3.3.2 Vaccine and Disease/Parasite-Control Program – All Animals (excluding mice)

Swine – All Yucatan miniswine are rigorously pre-screened for possible zoonotic diseases prior to arrival in the ARF. All farm swine are purchased from USDA-licensed herds with a tuberculosis, brucellosis, hog cholera, and pseudorabies-free area status. The herd must be USDA Validated Brucellosis-free and Qualified Pseudorabies negative. Yucatan miniswine are vaccinated for leptospirosis and erysipelas, and tested for tuberculosis, brucellosis, pseudorabies and toxoplasmosis; they are also examined for endo-parasites prior to shipment.

Dogs – Dogs are purchased from commercial, Class A USDA-licensed vendors. Creighton University requires the dogs to be tested for heartworm. Dogs must be vaccinated for rabies, distemper, parvovirus, hepatitis, leptospirosis and parainfluenza prior to shipment. The animals are isolated in their housing room for several days to ensure they have acclimated to their new surroundings before moving to another holding room if necessary. The Principal Investigator gives the dogs a complete physical exam within 48 hours of receipt.

Rabbits – Rabbits are purchased from SPF production colonies and are isolated to acclimate to their surroundings and to ensure they are eating and drinking. The animals are assessed for overall good health upon receipt.

Rodents – Rodents are assessed for overall health at the time of arrival. Standard profiles for serology, pathology, and parasitology are required from vendors. The Attending Veterinarian must approve rodent shipments from noncommercial vendors. Before approval for shipment is given, these animals must be shown to be free of pathogenic agents by standard surveillance serology profiles, pathology and parasitology exams. Upon arrival, these animals are placed in Quarantine Areas until additional testing is completed.

Guinea pigs/Hamsters – Guinea pigs and hamsters are purchased from commercial

vendors. Standard profiles for serology, pathology, and parasitology are required from vendors. They are examined on arrival for overall health.

3.3.3 Vaccine and Disease/Parasite-Control Program – Mice and Rats

Sentinel animals monitor the health status of mice and rats used in research projects. This program is essential in assuring that experimental mouse and rat colonies, and the results generated by Principal Investigators, are not adversely affected by pathogens. This allows the Attending Veterinarian to identify diseases and provide appropriate treatment as needed. Specific procedures have been established for treatment of *syphacia* or *aspicularis* (mouse pinworm) as described in section 3.3.4. Other mouse and rat diseases are handled on a case-by-case basis with the Attending Veterinarian, the Principal Investigator, the ARF Personnel.

Sentinel Animals – For established rooms, an overlapping system of sentinel animals is utilized. The goal of the overlapping system is to have sentinel animals available that have been exposed to any potential pathogens for a minimum of six weeks. This allows for re-testing should results indicate a potential concern. Sentinel animals are placed in the rooms approximately every six weeks (quarterly and mid-quarter). One cage of sentinel mice is assigned to 25-35 cages of experimental animals. Infective material may be too dilute to detect pathogens if bedding is pooled from too many cages. This is equivalent to placing a sentinel cage on each side of a rack within a room. A sentinel cage may be used for each Principal Investigator in rooms that house multiple Principal Investigators' mice. This assists in diagnosing specific affected areas should testing indicate a pathogen is present. CD-1 outbred mice are used in all mouse rooms and mount strong antibody responses in the presence of murine pathogens. Female mice at approximately three to five weeks of age are ordered from Charles River Laboratories. A small amount of soiled bedding (approximately one tablespoon) is taken from each cage at bedding change and added, over a layer of clean bedding, to the corresponding sentinel cage. Sentinel rats are obtained from Charles River Laboratories.

After a specified time of exposure, generally 90 days, all sentinel animals (or serum samples) are shipped overnight to either Charles River Diagnostic Laboratories or the University of Missouri-Columbia Research Animal Diagnostic Laboratory (RADIL) for Comprehensive Health Monitoring and/or serology. The results of these tests are a good indication as to the general health status of the animal colony. At a minimum Comprehensive Health Monitoring includes:

- **Pathology** – A complete post-mortem examination of the animal along with histological evaluation of gross abnormalities;

- **Parasitology** – Examination of the pelage and skin for ectoparasites and screening of the gastrointestinal tract for protozoa and helminths;

- **Microbiology** – Respiratory and enteric cultures are screened for bacterial species that are primary pathogens and/or important opportunistic pathogens (Pasteurella

pneumotropica, Mycoplasma pulmonis, Salmonella spp.);

- **PCR** – Molecular Diagnostic Infectious Disease PCR may be performed including M. pulmonis PCR;

- **Serology** (mouse) – MHV, MVM, MPV, Sendai, Mycoplasma pulmonis, TMEV (GDVII), EDIM, PVM, Reo3; and

- **Serology (rats)** – Screening (by a commercial provider) will be performed for: Ciliary associated bacillus; Kilham rat virus; H1 Virus; Mycoplasma pulmonas; Lymphocytic choriomenigitis virus; Parvovirus; Pneumonia virus of mice; Sialodacryoadenitis virus; Sendai virus; Reovirus.

The Quarantine period for extramural shipments is a minimum of seven weeks in duration. Four female CD-1 mice (approximately three to five weeks of age) are ordered from Charles River Laboratories. Soiled bedding is taken from the shipping container upon receipt and is added, over a layer of clean bedding, to the sentinel cage. During all subsequent cage changes, a small amount of soiled bedding (approximately one tablespoon) is taken from each cage and added to the sentinel cage as described above.

Testing is performed at least twice during the Quarantine period. Serum from two sentinels is sent at three weeks to aid in diagnosing pathogens the animals may have been exposed to immediately prior to shipping. The serum is screened, at a minumum, for the pathogens listed above under Serology. Whole animals are sent for Comprehensive Health Monitoring (Pathology, Parasitology, Microbiology, and Serology as decribed previously) at seven weeks. The health report must be approved by the Attending Veterinarian prior to release into the general population. The ARF Manager will notify the Principal Investigator as soon as the sentinel health report is determined to be satisfactory and the animals are moved into their permanent housing area.

3.3.4 Quarantine Areas for Diseased Mice

Pinworms – Oxyuridae (*Syphacia muris, S. obveleta,* and *Aspicularis tetraptena*) are common pathogens of rats and mice. Oxyurids are not highly pathogenic, but they have been associated with decreased growth rate, rectal prolapse and intestinal impaction. More severe effects have been found in immunocompromised rodents. Alterations in research using rodents infested with pinworms have been reported. Mice with symptoms or are diagnosed with pinworms shall be quarantined under the following procedures:

- Affected mice will be quarantined in their room until sentinel animals have returned a clean health report. No mouse will be moved to another room within the ARF unless approved by the Attending Veterinarian. Mice should be euthanized in the room. Mice should not be removed from the room as pinworms are extremely contagious and can contaminate the environment thereby perpetuating/spreading infection. Strict microisolator technique should be followed;

- All dirty caging will be autoclaved prior to removal of soiled bedding and subsequent cage-washing;

- As per the Creighton University ARF Gowning and De-gowning Fact Sheet (see Appendix C), all gowning materials must be removed immediately inside the door to the room (prior to exiting), specifically:

    - De-gown at the door immediately prior to exiting the room. Before leaving the animal room, all PPE must be removed and discarded in the trash receptacle located just inside the door to the room. DO NOT step into the hallway with any article of personal protective equipment, and
    - These rooms should be the last rooms entered during the course of the day. It is recommended that you do not enter/reenter a clean animal room after having entered Quarantine Areas;

- Harlan Teklad 2018S Global 18% Sterilizable Rodent Diet with Fenbendazole will be fed according to manufacturers recommendations. The medicated diet will be fed on alternating weeks for 21 days of medication in nonbreeding populations. This treatment schedule will be repeated in breeding populations in two weeks; and

- After the treatment has been completed, a minimum of four sentinel mice will be exposed to dirty bedding for a minimum of 30 days and sent for necropsy and parasitology. If positive results are received, the colony will be re-treated as described above and testing repeated. This schedule will be in effect until it is determined that the pinworm has been eradicated.

Fur mites – Fur mites are an ectoparasite that is spread by direct contact of animals with an infected animal. Fur mites are diagnosed by plucking hairs from the animal and examining the shaft for eggs. In a euthanized animal, cooling the animal results in the mites crawling up the hair. In dark-colored animals, the coat will appear to have white specks. If one animal is diagnosed with fur mites, the cage is presumed to be infested. Treatment options include dusting the animals with pyrethrin, ivermectin in the drinking water, utilizing dichlorovous pest strips in the cages, and utilizing Frontline-treated nestlets placed in the caging. Colonies known to have reoccurring fur mite infestation are treated prophylactically every six months as described above.

Other Diseases – Other diseases will be handled on a case-by-case basis as determined by the Attending Veterinarian. Mouse Hepatitis Virus (MHV) is extremely contagious and rooms testing positive for MHV may be subject to euthanasia.

3.4 OTHER AREAS OF VETERINARY CARE

3.4.1 Management of Protocol-Associated Disease, Disability or Other Sequelae

The Principal Investigator is responsible for management of protocol-associated diseases, disability, or other sequelae. The Attending Veterinarian is available as a resource to the Principal Investigator/Research Personnel for any questions related to protocol-associated diseases, disability or other sequelae.

3.4.2 Anesthesia and Analgesia, Surgery and Postsurgical Care

Individuals qualified and experienced in such procedures conduct anesthesia and analgesia, surgery, and post-surgical care according to the protocol approved by IACUC. The Attending Veterinarian provides oversight to surgery programs and post surgical care. The Principal Investigator/Research Personnel are responsible for post surgical care in accordance with their protocol. The ARF Manager will contact the Attending Veterinarian if problems arise.

In addition to an approved IACUC protocol, each laboratory must maintain species-specific SOPs, which provides detailed systematic practices for the specific procedure being proposed. Examples of these SOPs may be obtained from the ARF Manager. The Attending Veterinarian must approve these SOPs before the IACUC will approve an animal protocol.

Due to the wide variety of protocols and species used in the ARF, anesthetics and pain management is quite variable. Creighton University's IACUC requires anesthetic choices, pain management choices, dosages, and frequency clearly documented in the protocol. The ARF Manager and Attending Veterinarian are available for consultation. In addition, texts such as *Formulary for Laboratory Animals*, by C. Terrance Hawk and Steven L. Leary, and *Laboratory Animal Medicine*, by J. Fox, L.C. Anderson, F.M. Loew, and F.W. Quimby are available for consultation in the ARF. Please refer to the Research Compliance Website (http://www2.creighton.edu/researchcompliance/iacuc/about/index.php) to access the Minnesota Formulary, pain assessment charts, guidelines for aseptic surgery and other relevant information regarding the use of animals at Creighton University. Below are listed the standard agents used by species. Contact the Attending Veterinarian for more information if required.

Anesthetics:

- **Swine** – Injectable: Ketamine HCL/ Medetomidine; Telazol/Ketamine HCl – Inhalation: Isoflurane;

- **Dogs** – Injectable: Acepromazine/ Glycopyrrolate/ Butorphanol (premedication/tranquilization)/ Propofol (induction); Ketamine/ Diazepam –

Inhalation: Isoflurane;

- **Rabbits** – Injectable: Ketamine/Xylazine, Ketamine/Xylazine/Acepromazine – Inhalation: Isofluane;

- **Goats** – Injectable: Ketamine/Xylazine – Inhalation: Halothane; and

- **Rodents** – Injectable: Ketamine/Xylazine, Pentobarbital, Avertin – Inhalation: Isoflurane.

Analgesia:

- **Swine** – Buprenorphine, Butorphanol, Carprofen, Fentanyl patches;

- **Dog** – Buprenorphine, Tylenol with codeine, Butorphanol, Carprofen;

- **Rabbit** – Buprenorphine, Butorphanol, Tylenol with codeine elixir, carprofen;

- **Goat** – Buprenorphine; and

- **Rodents** – Buprenorphine, Tylenol with codeine elixir, aspirin, carprofen

Non-pharmacologic means to moderate pain or distress may include decrease in the numbers of animals in each cage, provision of additional bedding materials, and provision of food and/or water sources on the cage floor for easy access by the animal.

Non-rodent category D and E procedures (Includes survival and non-survival surgery) – The responsibilities for Principal Investigator and Research Personnel associated with non-rodent mammal category D and E procedures are as follows:

The major responsibility for animal protection and monitoring during and after Non-Rodent Mammal Procedures lies with the Principal Investigator, as is true for all use of live animals. This means that:

- The Principal Investigator is responsible for all actions taken by their Research Personnel. It is the responsibility of the Principal Investigator to ensure that all Research Personnel are familiar with the IACUC-approved protocol, and their role(s);

- The Principal Investigator is responsible for communicating with the ARF Manager regarding any special needs that an animal may have;

- The Principal Investigator must provide an accurate list of individuals and their specific roles on the protocol to the ARF Manager. The Principal Investigator is responsible for providing a contact list so that a Research Personnel member is

available at all times to deal with animal complications;

- A copy of the SOP should be in the room where the animal is housed;

- All Medical Record forms must be completed to document interventions, monitoring, care, complications, and treatment throughout the protocol. Monitoring and treatment must be documented utilizing the appropriate forms, in accordance with the SOPs of the ARF, federal regulatory agencies, and the Principal Investigator's IACUC-approved protocol;

- Any instances where a Principal Investigator or Research Personnel member fails to follow IACUC approved procedures or IACUC policies may result in suspension of a protocol, suspension or loss of all animal research privileges, or other action in accordance with IACUC or ARF policies and procedures. The most serious cases of non-compliance may result in the loss of the Principal Investigator's rights to use the data obtained from such experiments; and

- Failure to document completely all animal care/observations is considered an infraction by the USDA and the Creighton University IACUC. Failure to document completely animal care may result in suspension of a protocol and/or the privilege of a Principal Investigator to perform animal research.

Pre-Surgery – Upon arrival of a non-rodent mammal, it is the responsibility of the Principal Investigator to ensure that an individual listed on the protocol observes the animal within 48 hours of arrival. During this period, the Principal Investigator (or their designee) should perform a complete physical examination of the animal. These observations should be recorded on Form Med2. At least two working days prior to the scheduled procedure, it is the responsibility of the Principal Investigator to submit the names of all individuals who will be involved in the specific procedure to the IACUC Coordinator. The IACUC Coordinator or designee will verify that the individuals listed have received the appropriate training, are listed on the Principal Investigator's protocol, and contact the ARF Manager. Once this list is verified, the animal will be released to the Principal Investigator by the ARF. Inclusion of any individuals not trained or listed on the IACUC-approved protocol may result in a delay in the scheduled procedure. A physical exam must be performed on the animal within two hours of the start of the procedure by the Principal Investigator or designated Research Personnel. This exam must be documented on Form Med2. This exam should include a minimum of temperature, pulse and respiration of the non-rodent mammal.

Surgery – Individuals performing survival procedures must be knowledgeable about aseptic surgical techniques and have adequate training and skill to conduct the procedure without causing undue intra- and post-operative distress to the animal. All survival procedures on large animals must be conducted in IACUC-approved surgical facilities. All individuals involved in the procedure (including the monitoring of vital signs) must be trained, competent, and be approved to perform their tasks by the IACUC and the Attending Veterinarian. Anesthesia, surgery, and anesthesia recovery must be

documented on Forms Vet1, 2, and 3 (see Appendix D). Any unexpected adverse effects are to be documented on the Animal Incident Form (Med3) within 72 hours of the event (see Appendix D). This form is to be returned to the ARF Manager, who will ensure that the Principal Investigator; Attending Veterinarian; ARF Director; Research Compliance Officer; Research Compliance Monitor; IACUC and IACUC Coordinator are notified immediately.

Post-surgery – After survival procedures, all non-rodent mammals should be closely observed for the following 24 to 48 hours. Animal health, complications, and treatment must be documented on Forms Vet4a and 4b for the 10 days following invasive procedures. All observations must be documented in accordance with the SOPs of the ARF, federal regulatory agencies, and the relevant IACUC-approved protocol. Failure to document all care/observations is considered an infraction by both the USDA and the Creighton University IACUC. Failure to provide or document appropriate animal care will result in suspension of the protocol and possible suspension of all privileges to use animals in research. Depending on the nature of the procedure performed, this window of time may be widened to ensure the well-being of the animal. Animals which do not exhibit normal behavior (for example, eating, drinking, activity) within 48 hours may be experiencing procedure-related infections/complications and require further evaluation. It is the responsibility of the Principal Investigator to notify the ARF Manager and Attending Veterinarian of these potential complications. All incision sites must be observed daily for 10 days following surgery or until healed, whichever comes first. Adequate levels of antibiotics should be present at the time of invasive procedures to help prevent infections. The choice of post-procedure antibiotic should be listed on the IACUC-approved protocol.

It is the policy of Creighton University that all animals undergoing major invasive procedures must be given analgesic agents for at least the initial 24-48 hours post-procedure. Continuance or withdrawal of analgesics after this time should be based on the Pain Assessment Protocol (see Appendix B.3). The choice of analgesic depends on the species and severity of the manipulation. The dose, route of administration, and the frequency of administration of the analgesic must be listed on the IACUC-approved protocol and documented on Forms Vet3, 4a and 4b. If analgesics would interfere with the experimental design, the need to withhold analgesia must be justified in the protocol application and approved by the IACUC.

It is the responsibility of the Principal Investigator to make sure all animal care personnel are aware of the health condition of all Category D and E animals under their care and whom to notify in case of an emergency.

Notification and Record Keeping –Unexpected or atypical complications (including but not limited to animal death under anesthesia) that were not anticipated or identified in the approved protocol are to be documented on the Animal Incident Form (Form Med3) within 72 hours of the event (see Appendix D). This form is to be returned to the ARF Manager, who will ensure that the Principal Investigator; Attending Veterinarian; ARF Director; Research Compliance Officer; Research Compliance Monitor; IACUC and

IACUC Coordinator are notified immediately. Postmortem examinations may be performed at the discretion of the Attending Veterinarian.

It is the responsibility of the Principal Investigator to maintain accurate records on all procedures and peri-operative care. Forms Med1 and 2 must be completed and include a presurgical physical within two hours of surgery. Forms Vet1 and 2 (Appendix D) must be filled out during pre-surgery and surgery to document animal health and complications. Form Vet3 is completed during the immediate recovery. Close post-operative monitoring is required and must be documented on Form Vet3 until the animal recovers from anesthesia. At a minimum, daily monitoring of each animal is required with SOAP's recorded on Forms Vet4a and 4b until the end of the post-operative period (defined as, when sutures are removed and the surgical wounds are properly healed), unless indicated by the condition of the animal or the IACUC-approved protocol. The originals of these forms are to become part of the animal's medical record. Examples of conditions that may require two or more daily observations include administration of pain medications or antibiotics and bandage changes. All records must be kept in the animal's housing area so that they are readily available to the personnel involved in post-surgical monitoring, the ARF Personnel, Attending Veterinarian, the IACUC and federal regulatory officials.

ARF Monitoring and Oversight

Pre-surgery – Upon arrival in the facility, it is the responsibility of the ARF Personnel to note the general condition of the animal on Form Med2. The Principal Investigator and the ARF Manager should be notified immediately of any potential problems. This does not substitute for the complete physical examination that the Principal Investigator is required to perform within 48 hours of receipt. It is the responsibility of the ARF Personnel to prepare an animal for the procedure per the IACUC-approved protocol. It is the responsibility of the Principal Investigator to provide the appropriate information (such as the time to remove food and water from the animal) to the ARF Manager.

The ARF will not release any animals for procedures until the IACUC Coordinator or designee has verified the individuals involved in the procedure have appropriate training, and has contacted the ARF Manager. No animals will be released to the Principal Investigator prior to the approved acclimation period. The ARF Personnel will move the animal to the surgical suite in a manner consistent with federal guidelines.

Post-surgery – Once an animal has been returned to the ARF, ARF Personnel will perform daily rounds on the animals. It is the responsibility of the ARF Personnel to notify the Principal Investigator and/or Attending Veterinarian of any health concern. In the event of an emergency the Principal Investigator or Research Personnel should be contacted immediately, as well as the Attending Veterinarian. The Principal Investigator still maintains the responsibilities outlined above.

Attending Veterinarian Monitoring and Oversight

The Attending Veterinarian is initially responsible for approving the technical aspects of the submitted protocol. In addition, the Attending Veterinarian will also review any changes or deviations from the General standard operating procedures (SOPs) for the identified species that are Category D or E. These changes or deviations must be clearly identified and defined with the IACUC protocol. If the protocol requires major changes from the General SOP then the investigator must submit a Lab SOP. The Attending Veterinarian will also be responsible for determining whether particular personnel listed on the protocol are qualified for the responsibilities that they are assigned. The Attending Veterinarian may employ a number of means to determine whether an individual is qualified to perform the surgical or post-surgical duties assigned to them. It is desired that the individual be a graduate of an AVMA (American Veterinary Medical Association) approved program and holds a valid state license. If an individual does not meet this standard, the Attending Veterinarian will use other means to determine their qualifications. These include (but are not limited to), meeting with the individual, observing the individual perform the technique, previous experience, or administering an examination. If an individual does not agree with the assessment of the Attending Veterinarian, the individual may appeal the decision to the IACUC and the Institutional Official. The final decision rests with the Institutional Official.

The degree of involvement of the Attending Veterinarian pre- and post-surgery is determined by the individual project. The Attending Veterinarian will consider a variety of factors including the experience of the surgeon, the proposed procedure, the species involved, and the potential post-operative care required by the animal. At a minimum, on-going veterinary monitoring will consist of regular review of pre- intra- and post-surgical documentation (Forms Med1 and 2, Vet1-4). Animals will be observed to ensure that they are receiving adequate post-surgical care. The Attending Veterinarian (or designee) is available for consultation when planning for post-operative care of animals, as well as for post-surgical emergencies. It is the prerogative of the Attending Veterinarian to suspend immediately any protocol if there are unanticipated complications and/or if said complications are not handled in what is deemed a humane, proper manner.

Upon receiving an Animal Incident Report form, the Attending Veterinarian determines whether the adverse event requires immediate suspension of the protocol or an emergency IACUC meeting.

IACUC Monitoring and Oversight

The IACUC evaluates proposed procedures and pre- and post-operative care during the review of the submitted protocol. On going monitoring and oversight occurs during the semi-annual inspection process, when animal facilities (including surgical and recovery areas) are inspected. The IACUC also reviews information obtained from the Attending Veterinarian and the ARF Personnel, and may act upon information obtained from the Attending Veterinarian and the ARF Personnel through adverse event reporting. The IACUC will review all Animal Incident Reports on a monthly basis. Any member of the

IACUC may monitor procedures at any time. Any instances of non-compliance may result in suspension of a protocol, or suspension or loss of all animal research privileges or other action in accordance with the IACUC Policies and Procedures. The most serious cases of noncompliance may result in the loss of the investigators rights to use the data.

Research personnel are encouraged to consult with the Attending Veterinarian or other experts regarding the proposed surgical procedures prior to submission of an IACUC Protocol Application for Animal Use Form. The IACUC Protocol Application for Animal Use Form requires information on various aspects of the surgical procedure and allows reviewers (including the Attending Veterinarian) to address any outstanding questions regarding training, equipment, supplies, and care of animals. Surgeries are performed in procedure rooms/labs (rodents) or in the surgical suite (large animals). The IACUC Protocol Application for Animal Use Form must specify who is performing the surgeries, what their training or experience is with regard to the surgery, and details on pre-, intra-, and post-operative monitoring and management of animals. It is the standard of animal care to administer analgesics following any surgical procedure, unless otherwise approved by the IACUC.

Survival Surgery – Creighton University has guidelines (see Appendix F.1) covering the use of aseptic technique for survival surgeries. Briefly, the guidelines state that instruments must be sterilized, the surgical site on the animal must be appropriately prepared (for example, removal of hair and disinfection of site), and the surgeon must be appropriately dressed (facemask, surgical gloves, bonnet at minimum).

Principal Investigators are responsible for ensuring and providing sterilized instruments and protective clothing. An autoclave is available in the ARF should the Principal Investigator not have access to another means of sterilization. An autoclave is also available in the surgical suite.

Details of post-operative monitoring and care are described in each approved IACUC Protocol Application for Animal Use Form. The Investigator must list the types and frequency of monitoring and care that will be provided, as well as the person who is responsible for providing this monitoring and care.

Creighton University has guidelines for survival surgery in rodents (see Appendix F.2) – The surgery should be performed on a clean, bare surface that is disinfected prior to and following surgery. The surface should be covered by a clean drape. Hair should be removed from the surgical site by clipper or a depilatory. Treat the surgical area with an antiseptic scrub followed by an antiseptic solution as per standard surgical preparation. Post-operative care for survival surgeries must be consistent with that outlined above, and documented on the Veterinary Alert Rodent Post-Procedure Monitoring card, see Figure 3.

All precision vaporizers must be certified annually. It is the responsibility of the Principal Investigator to arrange for this. Annual certification will be verified during the Semi-annual IACUC inspections.

3.4.3 Assessment of Animal Well-Being

The Attending Veterinarian establishes appropriate mechanisms to ensure adequate assessment and monitoring of animal well-being. The Attending Veterinarian routinely monitors animals housed in the ARF. The Attending Veterinarian may delegate that responsibility to qualified individuals for animals housed outside the ARF. Quarterly testing is performed on sentinel mice (see section 3.3.3).

3.4.4 Euthanasia

Animals must be properly euthanized using the techniques for the species as established by the American Veterinary Medical Association (AVMA), unless otherwise approved under the protocol. The Principal Investigator is responsible for conducting or arranging for the euthanasia of animals under the protocol. If the Attending Veterinarian determines that any animal must be euthanized outside the protocol to relieve humanely the animal of undue pain and suffering, the ARF Manager will immediately notify the Principal Investigator. It is the responsibility of the Principal Investigator to arrange for euthanasia of the animal(s) within the period required by the Attending Veterinarian. If the Principal Investigator fails to euthanize the animal(s) in a timely manner, the ARF will arrange to euthanize the animal(s). The Principal Investigator will be charged for any costs associated with euthanizing the animal(s). Acceptable methods of euthanasia are listed AVMA Guidelines for the Euthanasia of Animals: 2013 Edition: (https://www.avma.org/kb/policies/documents/euthanasia.pdf).

4.0 ARF Maintenance

4.1 CLEANING AND SANITATION

ARF Personnel are responsible for all routine maintenance of the ARF, which includes cleaning and polishing floors, managing the storage areas, cleaning restrooms and offices, laundering uniforms, and any other tasks that do not require intervention by Creighton University Facilities Management.

4.1.1 Sanitation

Frequency of Contact and Non-Contact Bedding Changes:

- **Rodent Polycarbonate Shoe Box Cages (Static Microisolator Caging) –** Contact bedding in static microisolator caging is changed twice weekly unless otherwise requested by the Principal Investigator or required by the nature of the protocol (for example, diabetic animals);

- **Rodent Individually-Ventilated Caging Systems –** Contact bedding in mice caging is changed weekly unless otherwise requested by the Principal Investigator or required by the nature of the protocol (for example, diabetic animals);

- **Rodent Suspended Cages –** Non-contact bedding excreta pans and pan liners are changed twice each week unless otherwise requested by the Principal Investigator;

- **Guinea Pig Cages –** Contact bedding in guinea pig cages are changed twice weekly unless otherwise requested by the Principal Investigator;

- **Rabbit Cage Pans –** Non-contact bedding excreta pans and pan liners are changed twice each week unless otherwise requested by the Principal Investigator;

- **Dog and Swine Pens –** Pens are hosed daily including weekends and holidays; and

- **Goat Pens/Room –** Fecal material is removed from contact bedding daily.

Dirty Cage/Soiled Bedding Locations – After soiled cages are replaced and excreta pans are removed from the animal cages, the cages/pans are transported to the dirty cage processing area adjacent to the cage washer. The soiled bedding is emptied into a HEPA Filtered bedding dump station. When potentially infectious agents are present (either as part of the experimental design or due to infection of the colony), cages are autoclaved prior to emptying to inactivate any infectious agents.

Cleaning and Disinfection of Primary Enclosures – Washing/Sanitizing Frequency:

- **Rodent Polycarbonate Shoe Box Cages (Static Microisolator Caging) –** Mouse, rat, guinea pig, hamster and gerbil caging is changed and sanitized twice weekly unless otherwise requested by the Principal Investigator or required by the nature of the protocol (for example, diabetic animals). Hamster caging is sprayed with a 10% bleach solution prior to transport to the dirty cage processing area;

- **Rodent Individually-Ventilated Caging Systems (Solid Bottom Caging) –** Mouse caging is changed and sanitized weekly unless otherwise requested by the Principal Investigator or required by the nature of the protocol (for example, diabetic animals);

- **Suspended Wire Bottom/Slotted Floors –** Rabbit caging is changed and sanitized on a weekly basis. Rat suspended caging is changed and sanitized at least every two weeks;

- **Cage Tops –** All cage tops, including wire-bar lids and filter tops, and feeders are changed at least once every two weeks;

- **Cage Racks and Shelves –** All mobile cage racks and shelves are sanitized through the cage washer at least quarterly;

- **Cage Pans Under Suspended Cages –** Excreta pans for all wire bottom caging is changed and sanitized twice each week at the time of litter or pan liner change;

- **Playpens, Floor Pens, Stalls, etc. –** All dog and swine runs are sanitized daily including weekends and holidays. Goat pens are disinfected at least three times each week; and

- **Cage Washing/Sanitizing Procedures –** The ARF utilizes two cage washers, one cage/rack washer and one tunnel washer. The cage/rack washer is programmed to maintain a wash cycle of five minutes, followed by a guaranteed 180°F rinse cycle for three minutes. Steris Corporation is under contract to provide preventative maintenance services for this cage washer. The tunnel washer is also programmed for a 180°F-rinse cycle. Sterile caging set-ups (cage with bedding, nestlet, feed, wire bar lid and filter top) and water bottles are provided. Getinge Corporation is under contract to provide preventative maintenance services for the tunnel washer and the autoclave.

Cleaning and Disinfection of Secondary Enclosures:

- **Animal Room Cleaning Frequency, Procedures, Methods and Agents** – Animal room floors are swept and mopped on cage change days, or more often if necessary. All small animal rooms are cleaned and sanitized at least every three months or as the rooms become empty. The rooms are washed with a high-

pressure sprayer including walls, ceilings, and floors. The floors of rooms containing large animals housed in pens or runs are cleaned of excreta, washed and disinfected daily;

- **Corridor and Support Area Cleaning** – The corridors, dirty cage processing area and receiving area are swept and mopped daily. Procedure rooms are swept and mopped after each use, or at a minimum, weekly. The feed and bedding storage room is mopped weekly. Other support areas are cleaned monthly or more often if needed. Corridors are stripped, mechanically scrubbed and sealed at least twice a year. Animal rooms are stripped, scrubbed and sealed as they become available. The Cage Wash area is hosed daily and cleaned with a mechanical scrubber at least monthly;

- **Implements** – Mop heads are sanitized every two weeks or more frequently if needed. Mop buckets are rinsed after each use and placed in the cage washer every two weeks or more frequently if needed; and

- **Separation of Cleaning Implements by Room** – Cleaning implements are not shared between animal rooms. Each animal room has dedicated cleaning equipment including a broom, dustpan, mop and mop bucket.

Sanitation of Cage Equipment:

- **Procedures and Frequency for Feeders** – Wire bar lids, which serve as feeders in shoebox caging, and hanging cage feeders from suspended rat caging are changed and sanitized in the cage washer every two weeks. J-type hanging feeders associated with guinea pig and rabbit caging are changed and sanitized in the cage washer weekly. Other types of feeders such as bowls and feed pans are washed daily and sanitized weekly;

- **Procedures and Frequency for Watering Devices** – Water bottles are changed and sanitized weekly. Bottles and sipper tubes are sanitized in the cage washer. Water bowls and troughs are washed daily and sanitized weekly; and

- **Procedures and Frequency for Enrichment Devices** – Enrichment devices including Nylabones, balls, lids, and toys are changed and sanitized weekly or more often if the need arises.

Sanitation of Transport Cages, Equipment, and Vehicles – Cages and portable kennels used for transporting large animals to the surgical suite are sanitized in the cage washer after each use. ARF carts designated for transporting caging, feed and bedding between animal rooms and both clean and dirty cage processing areas are sanitized after each use. In addition, the ARF van is sanitized after every use.

Cage Sanitation Monitoring – The proper function of the cage washer, as determined by monitoring the water temperature of the final rinse cycle, is used to assess its

effectiveness. Indicator strips allow for immediate visual verification that the critical final temperature (180°F) is met.

Cage sterilization is monitored by several means. Each load is monitored using physical indicators – both a Castle® control card and a steam sterilization integrator. Integrators provide immediate visual indication that critical parameters for sterilization have been met including steam pressure, temperature and time. The printer tape is examined at the completion of each load and verified to have reached the appropriate temperature for the appropriate time. Furthermore, Castle biological indicator test, ampules containing both Bacillus stearothermophilus and Bacillus subtilis var. niger spores, is run weekly. A Castle Sterilizer Mechanical Air Removal Test Pack ®, (S.M.A.R.T. Pack) designed to produce the challenge that is presented by the BOWIE and DICK towel pack, is also run on a weekly basis.

Periodically, the Animal ARF Manager or ARF Personnel will perform microbiological monitoring to ensure appropriate sanitation and disinfection of the ARF utilizing RODAC (Replicate Organism Detection and Counting) plates. Within twelve hours of sanitation, the surface of the plate will be pressed against the sample surface. The plate is marked with the source of the sample and the plate incubated at 37°C for 24 hours. Colonies are counted and plates disposed of as Biohazardous waste.

Interpretation of the results is as follows:

- 0-5 colonies: excellent;
- 6-15 colonies: good
- 16-30 colonies: borderline
- 31-50 colonies: poor
- >50 colonies: unacceptable

4.1.2 Waste Disposal Methods

Soiled Bedding and Refuse – All excreta from dogs, swine and goats are either flushed into the city sewage system daily or disposed of in plastic bags into the dumpster. Conventional waste from small animal caging is transported immediately to the dirty cage processing area where it is emptied into a HEPA filtered bedding dump station. **Caging and bedding may be autoclaved prior to dumping in special circumstances when determined to be potentially infectious to either animals or humans.** The dump station's receptacle consists of a 30-gallon Rubber Maid container lined with a heavy-duty plastic bag. The bags are filled to approximately 30-40 lbs then tied off and placed in a Rubber Maid trash cart. As the trash cart is filled (minimum of once per day) it is taken to the dock area where it is emptied into the dumpster. A facility contractor transports the dumpster to a sanitary landfill.

Animal Carcasses – All animal carcasses are placed into a plastic or Ziploc bag and placed inside a lined biohazard container that is stored in dedicated carcass freezers in the ARF. All containers must be labeled with the Principal Investigator's name, room number where animals originated and number of animals placed in container, and date of euthanasia. Stericycle Inc. of Lincoln, Nebraska, Creighton University's contracted biohazard waste disposal firm, picks up the containers monthly for incineration, or more often if needed.

Hazardous Wastes – All potentially infectious waste is placed into a biohazard container lined with a red biohazard bag. When the container is filled, it is transported to a dedicated storage area within the ARF prior to removal by a contracted biohazard waste disposal firm or by specific arrangement made by Creighton University's Environmental Health Services. All potentially biohazardous waste (for example, waste containing carcinogens, etc.) is handled on a case-by-case basis in consultation with Creighton University Environmental Health Services.

4.1.3 Pest Control

Program – Creighton University has a pest and vermin control program maintained by a commercial pest control company, Presto-X Company. Bait gel is used throughout the ARF for control of roaches. ARF personnel report any sightings of pests to the ARF Manager and Facilities Management who, in turn, contact Presto-X. Rodent monitoring is accomplished by the use of live mousetraps located throughout the facility. These traps are checked weekly by ARF Personnel.

5.0 Animal Occupational Health and Safety – Animal Resource Facility Personnel

The Public Health Service Policy on Humane Care and Use of Laboratory Animals and *The Guide* identify the need for an occupational health and safety program for all personnel who work in laboratory animal facilities or have regular animal contact at work. The emphasis of the ARF training program is preventing workplace illness and injury, including providing for early diagnosis and treatment when an injury or illness occurs in ARF Personnel, which supplements the university's Animal Occupational Health and Safety Program for all individuals working with or around animals.

5.1 PROTECTIVE CLOTHING

Animal Rooms and Animal Handling – To maintain the best quality environment for animals and individuals working with animals, the ARF requires that protective clothing be worn any time in animal rooms (see Appendix C) or when handling animals in the ARF. This applies to all animals, both in and out of Quarantine Areas.

Personal Protective Equipment/Work Clothing Provided – Protective clothing including gloves, gowns, shoe covers, masks and head covers are provided to all personnel (research and animal care) who enter animal rooms within the ARF. All animal care personnel are also issued uniforms or scrubs for use while working within the ARF. Animal care personnel while working in the ARF should not wear street clothes. This clothing is washed within the ARF and does not leave the premises.

Small-Animal Rooms – At a minimum, protective clothing must include a gown, mask, shoe covers, head cover, and latex or vinyl gloves. Shoe covers must be put on as a person enters the room to minimize the spread of contaminants into and out of each room. Additional protective clothing may be required by the Attending Veterinarian or per the protocol. If so, this information shall be posted on the animal-room door.

Large-Animal Rooms – At a minimum, protective clothing must include a laboratory coat, mask, and latex or vinyl gloves. Additional protective clothing may be required by the Attending Veterinarian or per the protocol. If so, this information shall be posted on the animal-room door.

De-gowning/Clean Rooms – All gowning materials must be removed when you exit an animal room. Shoe covers must be removed *immediately after* stepping out of the room and *before* walking down the hallway. All other gowning materials must be removed at the nearest hallway trash receptacle. DO NOT enter any other room, including the clean cage storage room, wearing dirty gowning materials. DO NOT re-enter any animal room, for any reason, without first donning fresh gowning materials.

De-gowning/Quarantine or Housing Areas with Biohazards – Under special housing conditions, including Quarantine Areas and rooms where hazardous materials and/or

pathogens are being administered, slightly different de-gowning procedures are required in order to assure potential pathogens are not brought out into the common hallway. All gowning materials must be removed *prior to* exiting, immediately inside the door to the room.

5.2 PHYSICALS AND IMMUNIZATIONS

5.2.1 Physicals

All ARF Personnel must have a complete physical examination prior to working with animals. The ARF Manager arranges for physical examinations with a Creighton University Physician for ARF Personnel, as required.

5.2.2 Tetanus Shot

All ARF Personnel working with animals must have a current tetanus shot. The date of immunization must be confirmed by the Animal Occupational Health and Safety Nurse. Tetanus immunizations must be updated every 10 years or following known injury or upon advice of a physician.

5.2.3 Immunizations for Zoonoses

Zoonoses are animal diseases that can be transmitted to humans through regular contact, animal bites, or scratches. The Animal Occupational Health and Safety Nurse is responsible for ensuring that appropriate immunizations are obtained based on ARF Personnel interaction and contact with certain animals housed in the ARF. The ARF Manager and/or the Attending Veterinarian will advise the Animal Occupational Health and Safety Nurse of potential zoonoses if new species are introduced into the ARF. Titers for rabies, Q-fever, and other infectious agents involved in research are obtained as necessary.

5.2.4 Other Requirements

ARF Personnel shall also meet any additional physical examination or immunization requirements set forth under Creighton University's Animal Occupational Health and Safety program for individuals working with animals.

5.2.5 Costs Covered

The ARF shall pay for the cost of required physical examinations and any medical laboratory work and immunizations required for ARF Personnel.

5.3 MEDICAL CONDITIONS

5.3.1 Allergies

ARF Personnel may develop allergies to some animals while working in the ARF. Depending on the severity of the allergy, ARF Personnel will be provided appropriate protective clothing, reassigned to work with other species, or otherwise accommodated. Non-latex gloves are provided should a worker be sensitive to latex.

5.3.2 Pregnancy

Because cats can transmit toxoplasmosis to humans, a disease that may cause complications during pregnancy, any technician who becomes pregnant is encouraged to notify the ARF Manager or ARF Director in a timely manner to minimize exposure to cats during the pregnancy. The ARF Manager and ARF Director shall treat such information as confidential and institute appropriate safety precautions.

5.4 PROTECTION

5.4.1 Uniforms

For health and safety reasons, uniforms are provided to ARF Personnel for wear during working hours. These uniforms are kept in the ARF. All uniforms are laundered in the ARF washer and dryer. ARF Personnel share the responsibility of laundering uniforms.

5.4.2 Safety Equipment

Protective Clothing – The ARF provides protective disposable garments for individuals entering all animal housing areas. These include head cover, gloves (latex or vinyl), gown, mask and shoe covers (see section 5.1 and Appendix C).

Noisy Environments – ARF Personnel are provided earplugs when working in noisy environments.

Other Safety Items – Some tasks require safety items such as goggles, rain suits, aprons, and rubber boots. These and other required safety items are provided at no cost to the ARF Personnel by the ARF, as needed.

5.5 PERSONAL HYGIENE

5.5.1 Hygiene Materials

The ARF provides soap, shampoo, and towels for required showering. ARF personnel are provided a reasonable selection of soaps and shampoos from which to choose, including hypoallergenic products.

5.5.2 Routine Showers

All ARF Personnel members are required to shower after completing daily change out schedule, prior to performing clean caging preparation. Other intermittent showers may be taken, as the workload requires.

5.5.3 Quarantine Areas

ARF Personnel must shower immediately after cleaning or working in any Quarantine Areas and change into a new uniform before working in other areas of the ARF.

5.6 SAFETY

5.6.1 Accidents and Injuries

All accidents and/or injuries must be reported to the ARF Manager immediately. If this is not possible, the technician should contact the ARF Director or other departmental official. A Creighton University Incident Report Form (HR-24) must be completed and filed with Creighton University's Risk Management as soon as possible after the incident. If an injury is life threatening, contact Public Safety at 402-280-2911. Treatment should be sought at the nearest emergency room. If an injury is non-life-threatening, employees should contact Risk Management to arrange for medical evaluation at the provider of their choice

Animal Bites and Scratches – Animal bites and scratches should be reported immediately to the ARF Manager and a Creighton University Incident Report Form (HR-24) filed with Risk Management. The affected employee shall be referred to a physician for treatment, with consideration of possible tetanus prophylaxis and antibiotic treatment. Depending on the species, the Attending Veterinarian will be contacted to determine if the treating physician should consider rabies prophylaxis.

Records – The ARF Manager shall maintain copies of all reports of work-related accidents and injuries involving ARF Personnel.

5.6.2 Emergencies

Contact Creighton University Public Safety at 402-280-2911 to report any emergency.

5.6.3 Security

ARF Keys - ARF Personnel are issued appropriate keys to access animal rooms in the ARF. These keys must be kept in a secure place at all times. All ARF keys, including card keys, must be returned to the ARF Manager or ARF Director upon termination of employment.

ARF Access - In addition to ARF Personnel, only individuals listed on an IACUC-

approved protocol are allowed access to the ARF. Access is controlled by card keys that are activated or inactivated by the ARF Manager. Persons without keyed access must obtain permission from the Attending Veterinarian, ARF Director, ARF Manager, or IACUC Chair to enter the ARF. Persons not recognized by ARF Personnel will be questioned to ascertain whether they are authorized to access the ARF. Any suspicious person(s) or activity should be immediately reported to Creighton Public Safety at 402-280-2911. ARF Personnel should contact Public Safety if needed.

Access of Facilities Personnel to the Animal Rooms – All Creighton-employed Facilities personnel who may perform work in the ARF participate in the Animal Occupational Health and Safety Program. Facilities personnel are also instructed that if it is necessary to enter a room housing animals, only ARF personnel are permitted to move racks and/or caging housing animals. Protective clothing is required of all individuals entering an animal room.

Access of Contractors to the Animal Rooms – In some instances, it may be necessary for non-Creighton employees to enter animal rooms. In these circumstances, ARF personnel will escort the contractor to the room. The contractor will determine whether it is necessary for caging, rack and/or animals to be moved in order to complete the required work. Only ARF personnel are authorized to move animal caging, racks and/or animals. This policy will be explained to the contractor prior to entry into the animal room. Individuals must wear the same protective clothing required of Creighton personnel to enter the animal room.

5.7 EDUCATION AND TRAINING

The ARF Manager is responsible for ensuring that all ARF Personnel are fully aware of the application and training procedures set forth in these SOPs and of any potential hazards involved in their work, including how to recognize, prevent, and treat occupational hazards. The ARF Manager may seek input from the Attending Veterinarian and the University's Environmental Health and Safety Officer in meeting this responsibility. The ARF Manager shall maintain written records of such training for each ARF Personnel member.

In addition, Creighton University's Education Program for the Use of Animals in Research and Teaching includes a component on Animal Occupational Health and Safety Program that is required for all personnel who work in laboratory animal facilities or have substantial animal contact at Creighton. For additional information, please see the Education Program section in the *Investigators' Manual for the Care and Use of Animals in Research.*

All ARF Personnel are required to participate in the Animal Occupational Health and Safety Program. Information regarding this program can be found at http://www2.creighton.edu/researchcompliance/iacuc/aohsp/index.php.

Use of Agents Hazardous to Humans – It is the responsibility of the Principal

Investigator to identify all agents hazardous to humans or the environment that may occur because of their research. All animals that may be treated or infected with potentially hazardous agents must be disposed of in an appropriate manner. The Principal Investigator is advised to consult with the ARF, Environmental Health and Safety, and the Attending Veterinarian prior to submitting a protocol to the IACUC. It is the responsibility of the Principal Investigator to provide the ARF with written assessment of the risks and proper handling procedures.

6.0 Corrective Action

The ability to work with animals is a responsibility, and the actions of an individual have the potential to affect the ability of all researchers at Creighton University to engage in animal research. While the IACUC is ultimately responsible for Creighton University's animal care program, various individuals are involved in the corrective action process if these SOPs are violated. Corrective action is based on University Policy and is dependent on the individual's status (for example, staff, faculty, or student) and the seriousness of the violation. All individuals working with animals are expected to be familiar with the SOPs of the IACUC and the ARF, as well as the IACUC rules and regulations. Corrective action can include, but is not limited to, the following:

- Additional education and/or training;
- Verbal warning;
- Written warning;
- Suspension of protocol (where animal health or safety is compromised as a result of misconduct by the Principal Investigator/Research Personnel);
- Suspension of access to the ARF; and
- Termination of association with the University.

The ARF Director is ultimately responsible for the conduct of ARF Personnel. Disciplinary action is based on Creighton University's progressive disciplinary policy for staff. Any conduct that results in danger to any animal will be reported to the Attending Veterinarian and IACUC, which may recommend additional corrective action as necessary and appropriate.

Principal Investigators are responsible for their own conduct and the conduct of animal study personnel. Noncompliance or misconduct of Research Personnel will be reported to the Principal Investigator for appropriate disciplinary action. In addition, the ARF and IACUC may also deem it appropriate, depending on the seriousness of the incident, to take disciplinary action against the individual.

Principal Investigator noncompliance will be handled and reported as set forth throughout these SOPs and may include participation of the Office of Research and Compliance and/or IACUC. Noncompliance or misconduct of any research personnel, including the Principal Investigator that results in inhumane treatment of any animal will be reported to the Attending Veterinarian and IACUC, which may suspend the Principal Investigator's protocol pursuant to IACUC Policies and Procedures.

Any individual who violates the IACUC or ARF disciplinary actions will be immediately

barred from entering the ARF. Any disciplinary action taken against an individual by the ARF may be appealed as listed below:

- **The ARF Director** – An individual may submit, in writing, the reason that they feel that the action taken by the ARF was not appropriate. The decision may then be appealed to the IACUC; and

- **IACUC** – Any disagreement with the decision of the IACUC can be appealed, in writing, to the Institutional Official , who is the final arbiter of the matter