1. VETERINARY MEDICAL CARE

Veterinary medical care is an essential part of an animal care and use program and is composed 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
- 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.

2. 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.

2.1. 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/wmspage.cfm?parm1=16), Jackson Labs (http://www.jax.org/), and Charles River (http://www.criver.com/en-US/Pages/home.aspx). 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 July 1st for Harlan and on January 1st 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, and the ARF Manager will notify the Principal Investigator of the animal's arrival. 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. Creighton University's requirements for commercial vendors are as follows:

Rodents – Only vendors 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 are obtained from only USDA-licensed Class A dealers.

Rabbits – Only specific pathogen free (SPF) rabbits are procured for use at Creighton University; and

Livestock/farm animals – USDA-licensed facilities are utilized for Creighton University 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.

1.1.2 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 noncommercial 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 Policy R&C-ARF-7.5), 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. Costs associated with serological testing are the responsibility of the Principal Investigator and/or the shipping institution;

- 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

- 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 Isolators. The ARF will:

    - Verify that the number of animals requested by the Principal Investigator corresponds to the number approved under the IACUC protocol 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. If a request is disapproved due to health concerns, the Attending Veterinarian has the ultimate authority to determine the suitability of the animals for receipt.

1.1.3 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.

1.1.4 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 an autoclave 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, and be part of an IACUC approved protocol. 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 must 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 – The ARF van must be used to transport mammals externally. (Note: It is an AAALAC requirement that personal vehicles not be used to transport animals.)

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. 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, and as part of an IACUC approved protocol.

1.1.5 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 an autoclavable 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 consult 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.

1.2 Preventive Measures to Protect Animals

1.2.1 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 Policy R&C-ARF-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 clearly understand 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 are sent to a diagnostic laboratory 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 to a diagnostic laboratory for comprehensive testing at seven weeks. 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 Policy R&C-ARF-3.0 section 1.3.3).

In some rare 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 Policy R&C-ARF-7.5). 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) after sacrifice of the animals, as 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 Policy R&C-ARF-3.0 section 1.1.2 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.

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 Policy R&C-ARF-3.0 section 1.2.2.

1.2.2 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. 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 must document this physical examination on the Animal Medical Record Progress Notes. 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 Policy R&C-ARF-3.0 section 1.3.3 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 (see Policy R&C-ARF-7.4). 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 Animal Medical Record Summary. 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 the Animal Medical Record Progress Notes. 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.

1.2.3 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. Charles River, RADIL and the Nebraska State Diagnostic laboratory 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 Policy R&C-ARF-3.0) may contact the Attending Veterinarian by telephone or e-mail. 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. 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 identified by or reported to ARF personnel 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.

1.2.4 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 cell lines can often 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.

1.2.5 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 with these animals 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 these animals may not appear infected. These diseases have the potential to interfere with research, particularly in transgenic, knockout and/or immunodeficient animals.

1.3 Surveillance, Diagnosis, Treatment and Control of Diseases

1.3.1 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). The individual performing rounds notes any concerns regarding a particular room on the rounding sheet. Results of daily rounds are noted on Animal Medical Record Progress Notes for all non-rodent mammals in a SOAP-notes format (see Policy R&C-ARF-7.4), as well as the rounding sheet. 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.

1.3.2 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. Under standard conditions all farm swine are purchased from USDA-licensed herds with a tuberculosis, brucellosis, hog cholera, lawsonii and pseudorabies-free area status. The herd must be USDA Validated Brucellosis-free and Qualified Pseudorabies negative. Yucatan miniswine are vaccinated for leptospirosis, lawsonii 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. Rodents obtained from approved commercial vendors are placed into housing rooms upon receipt.

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.

1.3.3 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 Policy R&C-ARF-3.0 section 1.3.4. Other mouse and rat diseases are handled on a case-by-case basis as directed by the Attending Veterinarian in consultation with the Principal Investigator and/or 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. 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.

Sentinel rats are obtained 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. 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 for some pathogens 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 post exposure. 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.

1.3.4 Quarantine for Diseased Mice

Failure of the Principal Investigator and/or their staff to follow Veterinary directions in quarantine rooms may result in a loss of access to the ARF.

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 Policy R&C-ARF-7.2, 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 manufacturer's 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.

1.4 Other Areas of Veterinary Care

1.4.1 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.

1.4.2 Anesthesia and Analgesia, Surgery and Post-surgical 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, the anesthetics and analgesias used are quite variable. Creighton University's IACUC requires anesthetic choices, pain management choices, dosages, and frequency clearly documented in the protocol. The Attending Veterinarian is 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 and 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:

1. 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);

2. The Principal Investigator is responsible for communicating with the ARF Manager and Attending Veterinarian regarding any special needs that an animal may have;

3. 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;

4. A copy of the SOP must be in the room where the animal is housed;

5. 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;

6. 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 noncompliance may result in the loss of the Principal Investigator's rights to use the data

obtained from such experiments; and

7. 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 and/or Research Personnel 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 the Animal Medical Record Progress Notes. 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 and are listed on the Principal Investigator's protocol (NOTE: the ARF Manager is contacted to release the animal). Once this list is verified, the ARF Manager will be contacted and 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 the Animal Medical Record Progress Notes. 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 the Animal Medical Record Operative Report and Anesthesia Recovery. Any unexpected adverse effects are to be documented within 72 hours of the event. The appropriate form is to be returned to the ARF Manager, who will ensure that the Principal Investigator; Attending Veterinarian; ARF Director; Research and Compliance Officer; Research and Compliance Monitor; IACUC and IACUC Coordinator are notified immediately.

Post-surgery – After survival procedures, all non-rodent mammals must be closely observed for the following 24 to 48, per the IACUC approved protocols hours. Animal health, complications, and treatment must be documented on the Animal Medical Record Post Operative

Evaluation/Treatment 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 Policy R&C-ARF-7.6). 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 the Animal Medical Record Anesthesia Recovery and Post Operative Evaluation/Treatment. 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 ensure that 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 – 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 within 72 hours of the event. This form is to be returned to the ARF Manager, who will ensure that the Principal Investigator; Attending Veterinarian; ARF Director; Research and Compliance Officer; Research and 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. The Animal Medical Record Summary and Progress Notes must be completed and include a presurgical physical within two hours of surgery. Animal Medical

Record Anesthesia and Operative Report must be filled out during pre-surgery and surgery to document animal health and complications. An Anesthesia Recovery Record is completed during the immediate recovery period. Close post-operative monitoring is required and must be documented on Anesthesia Recovery Record until the animal recovers from anesthesia. At a minimum, daily monitoring of each animal is required with SOAP's recorded on the Post-Operative Report 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 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 the Animal Medical Record Progress Notes. 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 without written approval of the Attending Veterinarian. 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 is responsible for determining whether an individual listed on the protocol is qualified for the responsibilities 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 Veterinary Technician's 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. 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 immediately suspend any procedure 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 procedure 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. Ongoing 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 noncompliance 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, their training or experience with regard to the surgery, and details on pre-, intra-, and post-operative monitoring and pain 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 Policy R&C-ARF-7.7) 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 responsible for providing this monitoring and care.

Creighton University has guidelines for survival surgery in rodents (see Policy R&C-ARF-7.8) – The surgery should be performed on a clean, bare surface that is disinfected prior to and following surgery. Hair should be removed from the surgical site by clipper or a depilatory. The surgical area should be treated with an antiseptic scrub followed by an antiseptic solution as per standard surgical preparation. Post-operative care for survival surgeries must be documented on

the Veterinary Alert Rodent Post-Procedure Monitoring card.

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

1.4.3 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 and rats (see Policy R&C-ARF-3.0 section 1.3.3).

1.4.4 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 IACUC 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 humanely relieve 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 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 on the Creighton University IACUC website: (http://www.creighton.edu/researchcompliance/iacuc/about/index.php).